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The potential of plant ingredients in diets of juvenile lumpfish (*Cyclopterus lumpus*)

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FACULTY OF BIOSCIENCES AND AQUACULTURE

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Florence Chandima Perera Willora Arachchilage

A thesis for the degree of
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Preface

This thesis is submitted in fulfilment of the requirements for the degree of Philosophiae Doctor (PhD) at the Faculty of Bioscience and Aquaculture (FBA), Nord University (Nord), Bodø, Norway. The presented original research was performed as part of the Stipendiatprogram Nord with financial support from Nordland county and Innovation Norway (2016/119025).

The project team consisted of the following members:

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Ørjan Hagen: Associate Professor, FBA, Nord University: Primary supervisor

Mette Sørensen: Professor, FBA, Nord University: Co-supervisor

Florence Chandima Perera Willora Arachchilage

Bodø, 2nd October 2020

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List of abbreviations

ANFs:	Anti-nutritional factors
AI:	Anterior intestine
BW:	Body weight
DI:	Distal intestine
DHA:	Docosahexaenoic acid
EPA:	Eicosapentaenoic acid
FA:	Fatty acid
FM:	Fishmeal
FO:	Fish oil
HIS:	Hepato-somatic index
LAP:	Leucine-amino peptidase
MI:	Mid intestine
PP:	Plant protein
PPC:	Pea protein concentrate
PC:	Pyloric caeca
PUFA:	Polyunsaturated fatty acids
RO:	Rapeseed oil
SGR:	Specific growth rate
SPC:	Soy protein concentrate
TAP:	Total alkaline protease
TRP:	Trypsin

Abstract

Lumpfish (*Cyclopterus lumpus*) is the most widely used species in biological delousing of Atlantic salmon (*Salmo salar*). From 2012 to 2019, the production of lumpfish has increased from thousands of individuals to more than 40 million individuals in Norway alone. This suggests that lumpfish has a promising future as a cleaner fish. Future feeds for farmed species are expected to become less reliant on marine-based ingredients. Plant feedstuffs are commonly used in commercial feed production as alternatives to marine ingredients. In this context, the present thesis mainly focuses on generating knowledge on the ability of lumpfish to utilize plant protein (PP) and plant oil ingredients in their diet. Specific objectives were also addressed; 1) dietary effect on growth performance, somatic indices, chemical composition and muscle fiber growth and 2) effect of dietary PP on digestive enzyme activities and gut health. Two feeding experiments were conducted; in the first experiment, juvenile lumpfish were fed PP mixture of soy and pea protein concentrates (1:1 ratio), replacing fishmeal at 25%, 50% and 75% in the diets. In the second experiment, juvenile lumpfish were fed rapeseed oil that replaced marine oil at 25%, 50% and 100%, and the feeds contained fishmeal and a mixture of soy protein concentrate and pea protein concentrate at a ratio of 0.50: 0.25: 0.25.

The results showed that incorporation of plant protein concentrates at 50% had no negative effects on growth, hepatosomatic index (HSI) and specific growth rate (SGR), whereas slightly increased body protein and decreased body lipid levels was observed. The highest incorporation of plant proteins (75%) in the diet reduced the growth and altered the mucosal fold height in the anterior intestine. Also, an increase in the number of goblet cells in distal intestine was observed at the end of the experimental period. Muscle fiber cellularity and activities of digestive enzymes such as total alkaline protease, trypsin and leucine amino peptidase in different intestinal segments and the pyloric caeca were not affected by increased levels of PP. Inclusion of rapeseed oil at 25% and 50% in juvenile lumpfish diets had no negative effect on growth parameters, SGR, condition indices, whole

body chemical composition or fatty acid profile in liver and whole body. The diet supplemented with 100% rapeseed oil reduced the growth performance and condition factor, but it increased the HSI and crude lipid in the whole body and liver. At the end of the experiment, tissue fatty acids clearly reflected the dietary fatty acid composition. Overall, the present thesis indicates that fishmeal and marine oil can be successfully replaced with plant protein concentrates and rapeseed oil, respectively in juvenile lumpfish diets.

Abstract in Norwegian – Sammendrag på norsk

Rognkjeks (*Cyclopterus lumpus*) er den mest brukte rensefiskarten til biologisk avlusning av Atlantisk laks (*Salmo salar*). Fra 2012 til 2019 økte produksjon av rognkjeks fra mindre enn to millioner individer til mer enn 40 millioner i Norge alene, noe som indikerer at rognkjeks har en lys fremtid som rensefisk. Fremtidens fôr til arter i oppdrett er forventet å være mindre avhengig av ingredienser fra det marine miljø. Planter som fôringrediens er mye brukt nå til dags som et alternativ til å erstatte marine ingredienser. I denne sammenheng har denne Ph.d. avhandlingen hatt som hovedfokus å generere ny kunnskap vedrørende rognkjeksens evne til å nyttiggjøre seg av plante protein (PP) og planteolje som fôringredienser. Hovedmålene var å studere; 1) effekten av diet på tilvekst, somakist indeks, kjemisk sammensetning og muskelfiber utvikling og 2) effekten av PP i dietten på aktiviteten av fordøyelsesenzymer og tarmhelse. To fôrforsøk ble gjennomført, i det første forsøket ble juvenil rognkjeks fôret en PP miks av soya og erteproteinkonsentrat (1:1 ratio), som erstattet 25%, 50% eller 75% av fiskemel. I det andre forsøket ble juvenile rognkjeks fôret med et fôr hvor rapsolje erstattet fiskeolje (25%, 50% eller 100%), samt fiskemel, soya- og erteprotein konsentrat ved et 0.50:0.25:0.25 ratio.

Resultatene viser at inklusjon av planteproteinkonsentrat opp til 50% ikke hadde noen negative effekt på vekst, hepatosomatisk index (HSI) and spesifikk tilvekst (SGR), men en liten økning i kroppsprotein og en nedgang i kroppsfett ble observer. Den høyeste inklusjon av PP (75%) dietten ga en redusert tilvekst og en liten endring i tarmfold høyde i fremre del av tarmen i tillegg til en liten økning i antallet av slimceller ved slutten av forsøket. Antallet muskelfiber og aktiviteten av fordøyelsesenzymene total alkaline protease, trypsin and leucine amino peptidase i ulike tarmsegmenter og pylorus blindsekkene viste ingen forskjeller som følge av økt nivå av PP. Inklusjon av 25% og 50% rapsolje i fôret til juvenile rognkjeks ga ingen negativ effekt på tilvekst, SGR, kondisjonsfaktor og kjemisk sammensetning i helkropp eller fettsyre profil i lever og helkropp. 100% rapsolje i dietten ga derimot redusert tilvekst og kondisjonsfaktor, og en økning i HIS og fett i helkropp og lever. I slutten av forsøket var fettsyreprofilen i fôret reflekterte rognkjeksens i vev.

Alt i alt, så viser denne Ph.d. avhandlingen at fiskemel og marineoljer i fôr til juvenil rognkjeks kan bli erstattes av planteprotein konsentrat og rapsolje.

1. Introduction

1.1 Global Salmon farming

Atlantic salmon (*Salmo salar*) is one of the most successfully farmed aquaculture species, and salmonid (wild and farmed) production contributes to 4.4% of the global seafood supply, enabling the industry to feed millions of people in Europe, the United States, Japan, East and South Asia (FAO, 2018a). In Norway, Atlantic salmon is the largest export species and in 2019, a total of 1.3 million tonnes originated from the 9 salmon producing regions, mainly from Nordland (300.000), Trøndelag (200.000), Romsdal (186.000) and Troms (176.000) (Norwegian Directorate of Fisheries, 2020a). Regarding the global production of Atlantic salmon the share of top five countries was 97.6% in 2018; Norway 55.3%, Chile 25.4%, Scotland 7.6%, Canada 6% and Faeroe Islands 3.3% (Iversen et al., 2020) **(Figure 1)**.

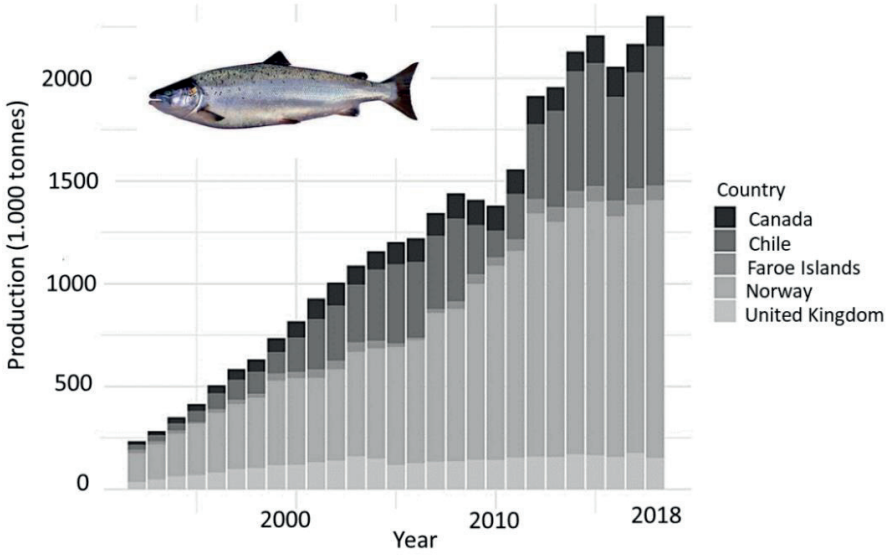

Figure 1. *Top five Atlantic salmon producing countries. Source: Kontali Analyse AS; in Iversen et al. (2020).*

Increased production-associated concerns include losses due to various factors such as premature death from diseases and escape from fish pens (Abolofia et al., 2017). One of the greatest disease challenges currently limiting production within the global Atlantic salmon industry is sea lice infestation (Torrissen et al., 2011, 2013). Both increased knowledge with respect to sea lice and effective control measures are very important for the future growth of the salmon farming industry.

1.2 Sea lice

Sea lice (Copepoda, Caligidae) are the most common ectoparasites found on many marine finfish species (Costello, 2006: Pike and Wadsworth., 1999). Mass infestation by *Lepeophtheirus salmonis* and various other species of caligid copepods poses a significant threat to the global salmonid farming industry (Igboeli et al., 2014); causing annual losses of over € 464 million to Norwegian farming sector alone (Nofima, 2016a), and limiting the growth of the Norwegian aquaculture industry. The southern hemisphere is principally affected by *Caligus rogercresseyi* (Boxshall, 1974), which significantly impacts the salmon farming sector in Chile, while *Lepeophtheirus salmonis* is the most prevalent caligid species in the waters of Europe and Canada, followed by *Caligus elongatus* (Nordmann), which affects both salmonid and non-salmonid species (Hemmingsen et al., 2020). Taken together, these parasites compromise fish welfare and incur extra production costs to salmon farms worldwide. There are 8 stages in the life cycle of caligid species; both *Caligus* and *Lepeophtheirus* species have two naupliar and one copepodid stage before the adult stage, besides the 4 chalimus stages in the case of the former and 2 chalimus and 2 pre-adult stages in the latter (Maran et al., 2013). During the chalimus (post-copepodid) stage, *Lepeophtheirus and Caligus* attach via front filaments feed on the mucus, skin, and blood of the host species (Costello, 2006). During the adult stages, they will be fully mobile and moves around on the host, grazing on blood and mucus (Hamre et al., 2013). Infestations by the sea lice may lead to reduced growth performance, and increased risks of secondary

infections, osmoregulatory imbalance, immunosuppression and mortalities (Grant et al., 2016; Grimnes and Jakobsen, 1996).

1.2.1 Sea lice management

Salmon farmers in different parts of the world have been relying on chemotherapeutants to control ectoparasites; preferred methods are bath treatments (hydrogen peroxide and organophosphates) and oral (feed) treatments (Burridge et al., 2010). Most of these anti-sea lice agents are potential threats to the environment, and there is fear of bioaccumulation and the chemicals can endanger the lives of aquatic organisms in or near aquaculture sites (Haya et al., 2001). Treatment-resistant lice in Europe and America necessitates the use of novel methods to tackle the problem because the aforementioned strategies are less effective today (Aaen et al., 2015). Norwegian salmon farmers have been employing non-chemical methods, including mechanical treatments such as the high-pressure washing and thermolyzing techniques for some time (Overton et al., 2019). These techniques are now being adopted by farmers in other salmon producing regions such as Canada, Chile, and Scotland. Although these treatments are highly effective in removing mobile lice and have little or no impact on the environment, these processes are stressful for the fish and can lead to elevated post-treatment mortality compared to the use of chemotherapeutants (Overton et al., 2019). Consequently, in recent times the focus has been directed towards alternative control strategies, including the use of physical barriers (plankton shielding skirts), non-chemical baths and sea louse predators, known as 'cleaner fish' (McEwan et al., 2019).

1.2.2 Cleaner fish

Cleaning symbiosis can be defined as the association between organisms of diverse taxa, wherein cleaning organisms clean cooperative host organisms by feeding on ectoparasites, diseased and injured tissues and unwanted food particles (Feder, 1966). Although cleaner fish have been used by salmon farming industry in Norway, UK and Ireland, more recently

they have been introduced in Iceland, the Faroes, Canada and Chile (Haugland et al., 2020). Different species of cleaner fish are preferred across these countries (**Table 1**), and the key cleaners are the lumpfish (*Cyclopterus lumpus*) and wrasse species namely the juvenile ballan wrasse (*Labrus bergylta*), goldsinny wrasse (*Ctenolabrus rupestris*), rock cook (*Centrolabrus exoletus*), and corkwing wrasse (*Symphodus melops*) (Skiftesvik et al., 2013). Since 2012 there has been a rapid increase in the use of farmed lumpfish (Powell et al., 2018a, b) compared to farmed and wild-caught wrasse (**Figure 2**), largely because their production cycle is less complicated and almost 60% shorter than that of ballan wrasse (*Labrus bergylta*). While lumpfish attains the deployment size of 15–30 g within six to eight months (Jonassen et al., 2018), ballan wrasse require 18 months to reach their deployment size of 40–50g (**Figure 3**) (Helland et al., 2014).

Table 1. *Overview of species of cleaner fishes that are deployed in different salmon producing countries.*

Species common name	Norway	UK	Ireland	Iceland	Faroes	Canada
Lumpfish 	X	X	X	X	X	X
Ballan wrasse 	X	X	X			
Goldsinny 	X	X	X			
Rock cook 		X	X			
Corkwing 	X	X	X			
Cuckoo 		X	X			

Adopted from Haugland et al. (2020). Wrasse photo credits Bilal et al (2016).

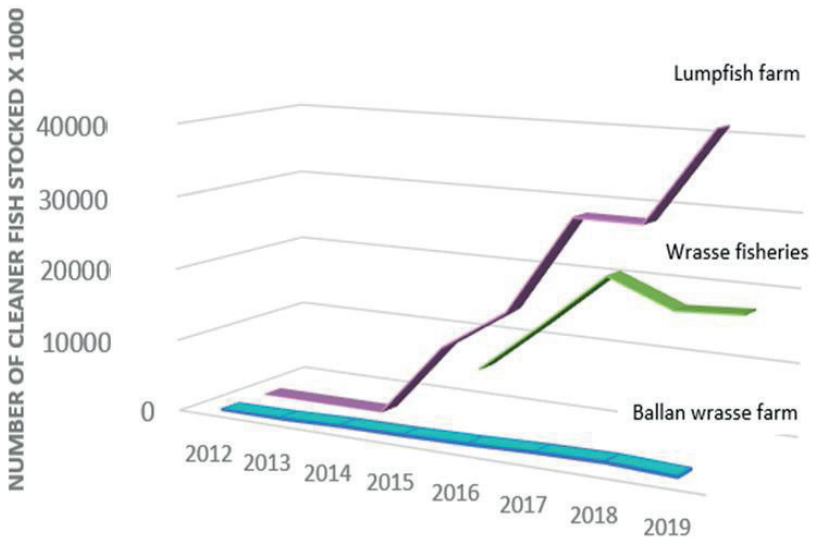

***Figure 2**. Increase in the number of cleaner fishes deployed for removing sea lice from Atlantic salmon and rainbow trout. The data for the period 2012-2019 was obtained from Norwegian Directory of Fisheries (2020a).*

Another advantage of using lumpfish is that they are tolerant to a wide temperature range, and hence, are capable of cleaning salmon at temperatures less than 4^{o}C (Nytrø et al., 2014). Additionally, their robustness ensures good survival rates during hatching and sea transfer (Alarcón et al., 2016). Ballan wrasse, on the other hand, survives in a lower temperature range, and when temperatures fall below 6°C their grazing efficiency decreases (Sayer and Reader, 1996). In 2019, Norwegian salmon and rainbow trout farmers deployed almost 60.5 million farmed and wild cleaner fish (Norwegian Directorate of Fisheries, 2020b). The majority (approximately 42.4 million) were lumpfish, which have become the country's second largest aquaculture species in terms of numbers.

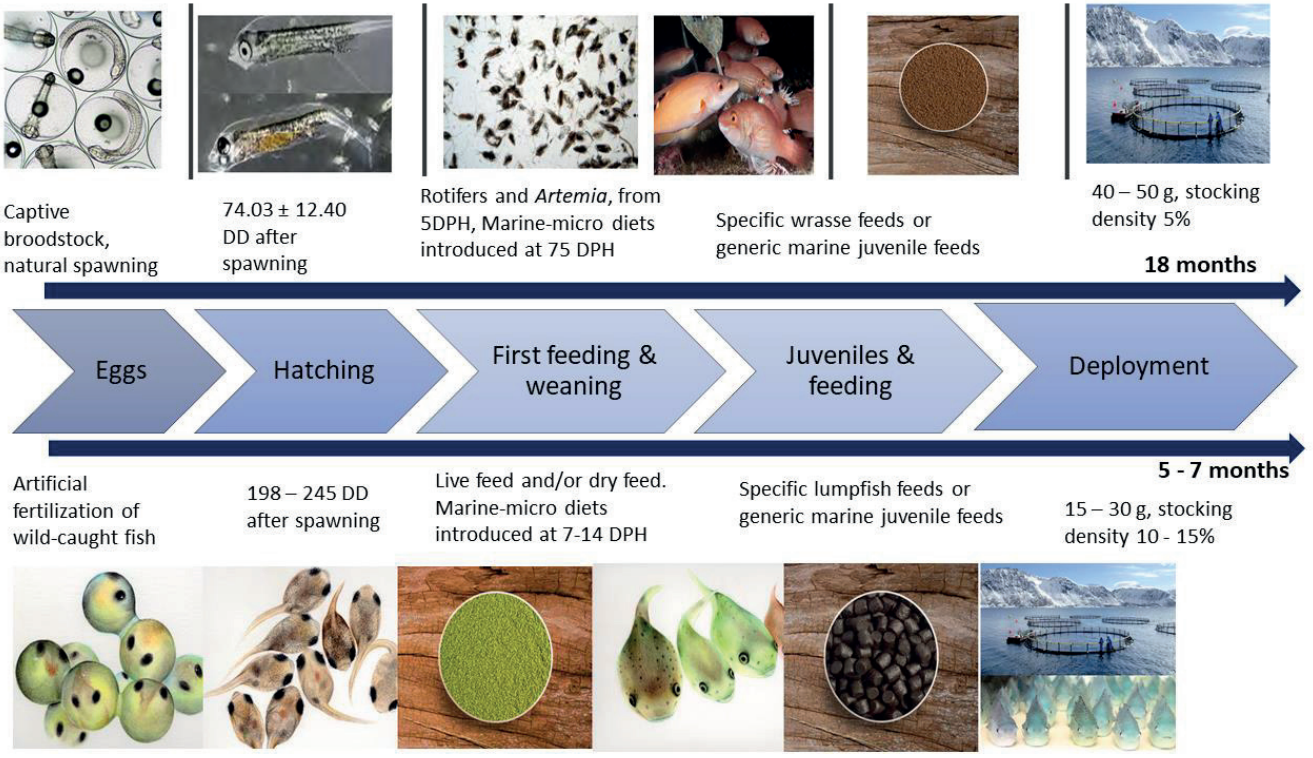

Figure 3. *Commercial production timelines for ballan wrasse and lumpfish.*

DD = degree days, DPH = days post-hatch. Modified version of the illustration in Brooker et al. (2018). Image credits: Skretting AS and web sources.

1.3 Lumpfish

Lumpfish is a bony fish (class: Osteichthyes, infraclass: Teleostei) belonging to the order Scorpaeniformes, family Cyclopteridae. Several unique features of lumpfish make it morphologically distinct from other cleaner fish species and they are the only species of the genus *Cyclopterus*. In Europe they are commonly referred to as lumpsucker, but are also known as stone biter, sea chicken and fat fish (Davenport, 1985). Lumpfish are sub-arctic species, which are distributed in the boreal region of the east and west North Atlantic coasts. The geographic distribution allows this species to thrive in most of eastern Canada, Iceland, the southern part of Greenland and the Faroes, Norway and countries nearby the North Sea, mainly France, the UK and Ireland (Holst, 1993).

Davenport (1985), provided a detailed generic and specific explanation of adult lumpfish. In brief, the lumpfish has a small head with a body length double that of its depth, and its body is compressed anteriorly and posteriorly. The first dorsal fin has a significant crest with large compressed tubercles. The each side of the body has three longitudinal rows, each delineated by compressed tubercles. On the ventral surface is a wide circular sucker disc musculature, and because of this feature the fish is known as lumpsucker (**Figure 4**). Its pigmentation can be described by a great variety of tints from blue, bluish-grey, to greenish and brownish; the young ones take the color of the surroundings, and during the breeding season the males are vividly color compared to females (Johannesson, 2006). Furthermore, sexual dimorphism is typical for this species and females are generally larger than males. Females may have a total length of 61 cm and weigh up to 9.1 kg, while male lumpfish can grow to 35.6-38.1 cm and 1.4-2.7 kg in length and weight, respectively (Davenport, 1985; Stevenson and Baird, 1988).

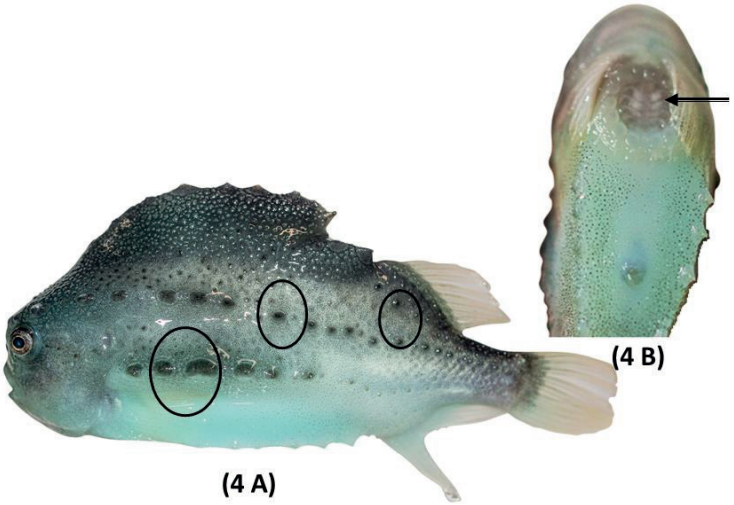

Figure 4. *External features of lumpfish, Cyclopterus lumpus (nearly 90 g and 15 cm). Area inside the circles shows the body tubercles (4A); ventral view showing the suction disc, which is a modification of the pelvic fin (4B). Photo: Florence Willora.*

Lumpfish is a bathypelagic or semi pelagic fish species (Hedeholm et al., 2014). The fish has traits that suggest a benthic modus vivendi, e.g. its circular shape, the presence of a suction disc and the absence of a swim bladder. The presence of a substantial quantity of gelatinous tissue (approximately 18% of body weight), reduced muscle density, low-density of ovarian fluids and the presence of a cartilaginous skeleton help the fish to maintain buoyancy; an essential characteristic for adaptation to the pelagic environment (Davenport and Kjorsvik 1986; Hedeholm et al., 2014).

Historically, the fish has been commercially exploited; the roe from the females are harvested, sorted, smoked or dried and then sold as lumpfish caviar (Kennedy et al., 2018). Canada is the main producer of this delicacy (35% of global production), followed by Iceland (31%) and Norway (15%); around 4 million kg are produced globally (Johannesson, 2006). Although the roe is a gourmet seafood, the other parts of the fish is regarded as “low value” (Sumaila et al., 2007). However, because of its delousing ability, in certain countries (e.g. Iceland, Norway and the UK) it is a valued fish (Powell et al., 2018b).

1.4 Lumpfish aquaculture

The commercial production and use of lumpfish as cleaner fish in salmon sea-pens are fairly new practices. The fish is mainly produced in Norway, and in 2018 lumpfish reached 28.9 million, followed by the UK with approximately 6 million fish and Iceland with 3 million. Canada and Ireland produced relatively few; only 1.3 and 0.1 million fish, respectively (Haugland et al., 2020). Currently in Norway, 25 companies have permission to farm lumpfish along the Norwegian coast, from Agder in the south to Tromsø in the north, and the production has increased 500% compared to 2012 (Norwegian Directorate of Fisheries, 2020c). The majority of brood-fish is fished from the wild, normally between September and June in Norway and capture fisheries is still the main supplier of lumpfish eggs to both Norway- and UK- based hatcheries (Pountney et al., 2020). However, wild male lumpfish should be regarded as a limited resource because the usual practice is to collect milt after post-mortem due to difficulties in stripping (Norðberg et al., 2015). Furthermore, the amount of milt that could be obtained from wild fish is very low. Hence, cryopreservation of lumpfish milt is essential. In addition, with the increasing demand, establishment of a breeding program for lumpfish should be given top priority. Now reliable methods are established for long-term storage of spermatozoa (Norðberg et al., 2015). As the first in the field, AquaGen (Norway) has started a breeding program to develop and improve the lumpfish broodstock with certain genetic qualities.

For the captive population, fertilization can be undertaken employing the "dry method", i.e., mixing eggs and sperms (the eggs are relatively large in size, 2 – 2.6 mm) and activating the sperm by adding seawater (Powell et al., 2018b). Hatching generally requires 198 – 245 degree days, but the process will not take place at temperatures below 4°C (Collins, 1976). At 10°C, lumpfish eggs take approximately 279° days for hatching with lowest mortalities (4.7%) (Imsland et al., 2019a). The time of hatching at 10°C, lumpfish are around 4.3 mg in weight and 5.3 mm in length (Imsland et al., 2019a). Newly hatched larvae have a fully developed digestive system, and within few days after hatching they start feeding. The larvae are born with the characteristic sucker disk, with which they can attach to surfaces immediately (Powell et al., 2018b). When held in tanks, small juvenile lumpfish prefers to cling to a smooth, vertical surface in order to maximize growth. However, excessive stocking density and insufficient surface area for attachment may result in

tail biting and cannibalism (Jonassen et al., 2018). Depending on the husbandry conditions, lumpfish will attain a deployment size of about 6 cm (10 g) in around 4 months (Vestsvik, 2013). Once lumpfish are ready for deployment they are transported by road or directly by boat to the farming location, where they are deployed into the salmon cages (Jonassen et al., 2018). Prior to transfer of the fish into the sea cages, they are usually examined to check for any damage or behavioral variations that could indicate poor health status.

Many experiments have proven that lumpfish are suitable grazers of sea lice attached on salmon in small-scale sea-pens (Imsland et al., 2014a, b; Imsland et al., 2015b). Lumpfish of average size of 54 g that were deployed at 10 or 15% stocking densities was found to significantly lower the average numbers of pre-adult, mature males and female stages of *L. salmonis*. Other studies have investigated the effect of 4, 6 and 8% lumpfish density on the sea lice population in large-scale salmon farming; while the 6% group lowered the chalimus stage load, 6 and 8% treatment groups significantly lowered the mature female lice population compared to the control group (Imsland et al., 2018). The same study also demonstrated that lumpfish can reduce the *C. elongatus* population on salmon, which suggests that the introduction of lumpfish may assist in reducing the levels of this parasite also in industrial-scale sea-based farming. Another study found that smaller lumpfish (20-30 g) are more effective delousers than larger lumpfish because the lice consumption rate of smaller juveniles (introduced at 23 g) is 30% greater than that of larger juveniles (introduced at 114 g) (Imsland et al., 2016a). Hence, it is better to focus on strains of lumpfish that grow more slowly; this strategy will help in extending the active grazing period (Powell, 2018b).

1.5 Lumpfish gut, food and feeding habits

The alimentary canal of fishes interacts with the environment. The intestine in lumpfish is a long tube which is located ventrally to the air bladder and the liver is seen overlying the upper part of the intestine. All the regions of the intestine have similar morphology (**Figure 5**, Willora et al., unpublished). In adult fish, the length of the highly coiled gut is nearly twice the body length. Pyloric caeca are finger-like extensions located in the proximal part of the intestine (Davenport, 1985). The lumpfish larvae and adults have small, sharp conical teeth. The teeth are arranged in bended rows, and their size increases from front to back and towards the distal ends of the jaws (Voskoboinikova and Kudryavtseva, 2014).

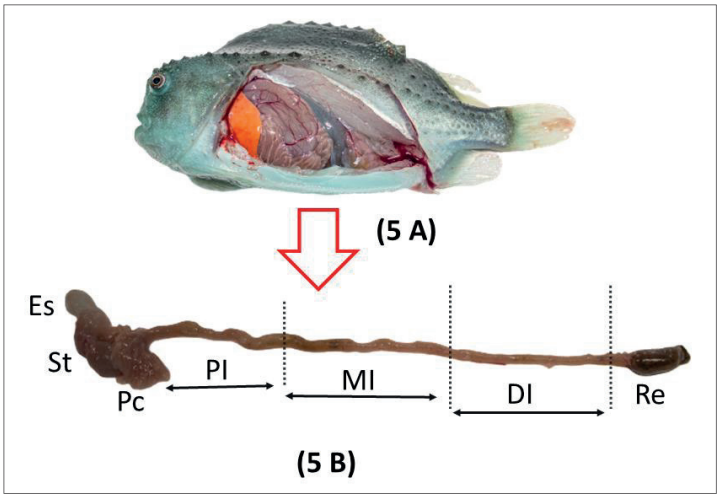

Figure 5. *Internal organs of lumpfish, Cyclopterus lumpus. A) Ventral view showing the gastrointestinal tract in the abdominal cavity. Note the highly coiled intestine. B) Gastrointestinal tract of juvenile lumpfish. Es: Esophagus, St: stomach, Pc: pyloric caeca, PI: proximal intestine, MI: mid intestine, DI: distal intestine Re: rectum. Photo: Florence Willora.*

There is very little literature on the food preferences of the larval, juvenile and adult lumpfish. Based on earlier studies that examined the gut content of juvenile lumpfish, it can be inferred that the juveniles feed on surface plankton and weed-associated invertebrate fauna. On the other hand, adults have diverse food choices; it is reported that they prey on small crustaceans (mysids,

amphipods, euphausids, isopods, decapod zoeae), ctenophores, polychaetes, seagrass, sea weeds, insects, small fish and fish eggs (Davenport 1985). This strongly suggests that lumpfish are omnivores.

In commercial fish farming, during the early development stages of the fish they are either fed with live feed, *Artemia nauplii,* for a brief period before the transition to dry feed, or are directly fed on formulated starter feeds (Saraiva et al., 2019). Three weeks post hatching, larvae in either circular tanks (Powell et al., 2018a) or shallow raceways (Nytrø et al., 2014) are offered immobile dry feed pellets (<800 µm). Lumpfish weighing 4 g can ingest 1 - 1.5 mm sized pellets (see Fig. 2). The feeding habits of lumpfish have been investigated after they have been deployed together with salmon in large net pens. Lumpfish of weight 54 g are opportunistic feeders that feed on any available food; salmon pellets, crustaceans, mussels and sea lice were found in their stomach (Imsland et al., 2015a). Imsland et al. (2016 a, b) reported that food preferences of lumpfish in sea-pens are depend on genetic provenance, size, and the cohabiting species.

Supplementary feeding is essential to satisfy the nutritional requirements of the fish and to ensure the welfare of the cleaner fish in sea-pens (Leqrelq 2015; Imsland et al., 2018). Delivery of pelleted feeds into the hideouts or around the pen edges instigated lumpfish to gather around the edges, and this tactic reduced their sea lice grazing potential (Imsland et al. 2018, 2019b). Reliance on the offered feed may increase the growth rates, but will reduce the cleaning efficacy of lumpfish and increases the risk of cataract development (Imsland et al., 2019b). Such problems can be kept in check by offering feed blocks to lumpfish; although lumpfish readily accepted the feed blocks they had lower growth and the strategy reduced the incidence of cataract (Imsland et al., 2019b, 2020).

To date not much is known about the nutritional requirements of lumpfish. Commercial fish feed producers offer lumpfish feeds, namely Otohime (Marubeni Nisshin Feed Co, Ltd), Pro-Start, Pro-Wean, Grower, SYMBIO (Biomar AS) and Gemma products (Skretting AS). The ingredient composition of these commercial products is unknown, but they are largely based on fishmeal (FM) and fish oil (FO), e.g. Otohime EP0 (for juvenile stage 3-25 g) and EP1 (20-50 g). The main protein sources seem to be fishmeal and krill meal, respectively and fish oil appears to be the

main lipid source. Research on lumpfish nutrition is progressing rapidly in Norway. An ongoing project, clean-feed, aims to delineate the optimal composition of primary and micro-nutrients, e.g. minerals and vitamin requirements for lumpfish (Nofima, 2017).

1.6 The shift from marine to plant-based ingredients in aquafeeds

Aquaculture is an important sector with diverse activities, which includes the farming of a wide variety of species. About 70% of fish and crustacean aquaculture have adopted either semi-intensive or intensive production systems that demand formulated feeds (Tacon and Metian, 2015). Herbivorous species can consume 100% plant-based feeds made from crops and other food and agricultural byproducts. Carnivorous species are better adapted to protein and lipids as part of their diet (Fry et al., 2016). According to the 2019 Alltech Global Feed Survey, aquaculture industry produced 40 million tons of feed in 2018, a 12% increase compared to 2015.

Marine ingredients, such as FM and FO were originally used as basic dietary ingredients to represent the protein and lipid fraction in aquafeeds; this is because they were readily available and inexpensive. Approximately 20 million tons of raw materials such as whole fish/crustaceans, wild and farmed by products are used to produce 5 million tons of FM and 1 million ton of FO per year (IFFO, 2018). Fishmeal is recognized as a "nutrient-rich" feed ingredient (Lunger et al., 2006) because it contains high quality protein with an amino acid profile that meets the nutrient requirements of farmed aquatic species. Moreover, the FM contains digestible proteins and is palatable, and these characteristics make it the preferred feed ingredient for the finfish and shellfish farming industry (Hardy, 2010). Fishmeal that is currently used in Norwegian salmon feeds is mainly produced from forage fishes (11.7%) such as anchoveta, capelin, sprat, blue whiting and sand-eel, and trimmings (2.8%) (Aas et al., 2019). Fishmeal also supply significant amount of long chain omega 3 polyunsaturated fatty acids (n-3 PUFA), cholesterol (6% of FM lipid), phospholipids (17-27% of FM lipid), 2-5% phosphorous (Storebakken et al., 2015; Tocher et al., 2008), and vitamin B-complex especially cobalamine (B12), niacin, choline, pantothenic acid, and riboflavin; bioavailability of all these nutrients are higher than those of plant protein ingredients. Furthermore, several low molecular weight nitrogen substances (e.g. taurine, hydroxyproline,

creatinine, histidine-related peptidase, nucleotides and free amino acids) are known to positively affect feed intake, fish growth and health (Aksnes et al., 2006; Kousoulaki et al., 2009).

Fish oil is an important ingredient in fish feed as farmed fishes require at least one percent of marine fish oil in their feed (Nasopoulou and Zabetakis, 2012). Fish oil is the main source of metabolic energy and is rich in triglycerides with sufficient amounts of essential n-3 PUFA, particularly docosahexaenoic acid (DHA) (22:6n-3) and eicosapentaenoic acid (EPA) (20:5n-3) that positively influence growth, development, and general well-being of humans (Nasopoulou and Zabetakis, 2012; Pickova and Mørkøre, 2007). Fish oil also supplies lipid-soluble vitamin E carotenoids that have antioxidant properties (Pickova and Mørkøre, 2007). Other marine alternative oils containing n-3 LC-PUFAs are those derived from marine invertebrates such as krill (*Euphausia superba*), amphipods, copepods and meso-pelagic species (Olsen, 2011). Krill meal and krill oil products for aquaculture feeds contain oil rich in EPA, DHA, phospholipids and antioxidants such as carotenoids (including astaxanthin) (Virtue et al., 1995). Norwegian salmon feeds contain 10.4% FO, of which 7.8% is produced from forage fish and 2.6% is from trimmings (Aas et al., 2019).

Fishmeal and FO are still considered as essential feed components, and administering them through feeds is the most practical way of providing nutrients to farmed animals. Considering the limited marine ingredients for long-term aquafeed production, aquafeed manufactures should identify and evaluate alternate protein and oil sources (Gatlin et al., 2007; Naylor et al., 2009; Tacon et al., 2011). Intense research efforts, over the last three decades, have helped in reducing the reliance on the limited marine sources; aquafeed industry has identified a broad range of "second generation feed ingredients". These include plant-based meals and protein concentrates produced from oilseeds, grains, pulses and legumes and also high quality animal by-products (e.g., poultry meals, bone meals , blood and feather meals etc.); these components are now used as complete or partial substitutes for FM (**Table 2**) (Tibbetts, 2018).

Table 2. *List of common feed ingredients, their crude protein (%) and respective inclusion levels in compound feeds employed in finfish aquaculture*

Feed ingredients	Inclusion level in compound aquafeed [%]	Crude protein %	References
Plant protein meal			
Soy protein concentrate	20-56	67-72	Hertrampf and Pascual, 2012;ARRINA, 2015
Pea protein concentrate	<30	76-78	VKM,2009*; ARRINA, 2015
Potato protein concentrate	20	90-96	VKM,2009*; Hertrampf and Pascual, 2012
Corn gluten meal	2–40	60-62	Tacon, 2011; ARRINA, 2015
Soybean meal	10-36	36-47	VKM,2009*; Hertrampf and Pascual, 2012
Wheat gluten meal	29	79-82	VKM,2009*; ARRINA, 2015
Rapeseed/canola meal	2–40	32-36	Tacon, 2011; ARRINA, 2015
Cotton seed meal	30	30-50	VKM,2009*; Świątkiewicz et al. 2016
Groundnut/peanut meal	5-61%	40-50	Hertrampf and Pascual, 2012; Batal et al.2005
Sunflower seed meal	20-28	25-50	VKM,2009*; Hertrampf and Pascual, 2012
Linseed meal	2-7	30-40	Hertrampf and Pascual, 2012
Palm kernel meal	3-10	15-18	Hertrampf and Pascual, 2012
Faba bean meal	3-5	27.5	Aas et al. 2019; Ouraji et al. 2013
Mustard oil cake	20-40	≈ 40	Hertrampf and Pascual, 2012

Asterisk indicates that the ingredients are present in the diets for Atlantic salmon, Atlantic cod and rainbow trout. All other dietary inclusion levels correspond to the diets in general for herbivorous and carnivorous species.

The percentages of plant-derived ingredients in Atlantic salmon feeds have increased significantly; from 1990 to 2016 (**Figure 6**; Aas et al., 2019; Ytrestøyl et al., 2015). Among the plant-based sources is soybean (*Glycine max*), which is one of the most important oilseed crops. Global production of soybean has increased 14-fold since 1961 to over 348 million metric tons in 2018 (FAO, 2018b). About one-fourth of the produced soybeans is converted into meals and cakes for use within the animal feed industry. In aquatic feeds, soybean meal (SBM), soy protein concentrate (SPC) and soy protein isolate are all regarded as feasible protein alternatives, and

they can be used to replace FM protein in the feeds for farmed finfish and crustaceans (Gatlin et al., 2007). However, these ingredients have their limitations.

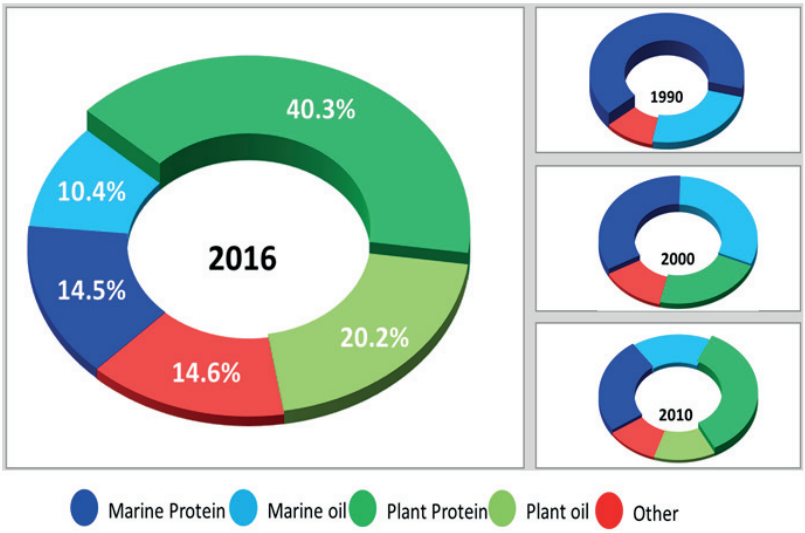

Figure 6. *Illustration of the shift in dependence on marine sources. Comparison of the inclusion levels (% of feed) of ingredients that originated from plant and marine sources; 2016 vs previous years. 1990: 65.4% marine protein (MP), 24% marine oil (MO), 10.5% other ingredients. 2000: 33.5% MP, 31.1% MO, 22.2% plant protein (PP), 13.2% other ingredients. 2010: 24.8% MP, 16.6% MO, 35.5% PP, 12.5% plant oil, 10.6% other ingredients. Illustration: Florence Willora, Nord University; the pie-charts were created with the data from Aas et al. (2019).*

Compared to marine ingredients, plant ingredients are less palatable and nutritionally inferior to the nutrient requirements of the fish. Their limited incorporation levels in fish feeds could be mainly attributed to their high non-starch polysaccharide content and anti-nutritional factors, which may negatively affect feed utilization, growth performance and fish health (Francis et al., 2001; Refstie et al., 2005). Some studies have reported that fish growth, feed efficiency, and feed intake can be improved by choosing protein ingredients of different origin (Agbo et al., 2015; Borgeson et al., 2006). Mixing of several raw materials can yield a better, nutrient-balanced diet; the raw materials can have greater potential to replace a higher proportion of fishmeal compared to single ingredients at higher inclusion levels, and the prepared diet can improve growth performance (Zhang et al., 2012). Thus, a plant protein combination is more advantageous than any individual plant

protein that is considered as an FM replacer. Many plant protein ingredients are routinely used in commercial Norwegian salmon feeds, which consist of 14.5% FM, 19% SPC followed by 9% wheat gluten, 3.6% corn gluten and 3.4% faba beans. Along with them there is a small quantity of plant proteins; from PPC (1.3%), sunflower meal (1.1%) and sunflower protein (0.5%) (Aas et al., 2019; Ytrestøyl et al., 2015). The main legume protein among them, SPC, has been studied widely than PPC. However, advantages of both these feed ingredients are low cost and their high crude protein content compared to the unprocessed plant ingredients (Zhang et al., 2012). In addition, protein concentrates are more refined products than their meals (SBM and pea meal) because soluble carbohydrates are removed during processing and antinutritional factors are reduced or inactivated (Francis et al., 2001).

Similar to the strategy adopted to substitute FM, terrestrial animal fats and plant-based oils have been used extensively to replace FO in the feeds of farmed fish species (Aas et al., 2019; Tibbets, 2018). In general, a good substitute for FO in aquafeeds needs to be palatable, highly digestible, and a good source of energy, and minimally modify tissue fatty acid profiles of the farmed fish. Plant oils from rapeseed (canola), olive oil, rice bran, sesame that are rich in monounsaturated fatty acids (MUFAs), and n-3 PUFAs-rich linseed oil are viewed as good candidates to replace FO in aquafeeds (**Table 3).**

Table 3. *Concentrations (g/100g fatty acids) of selected fatty acids in plant oils commonly employed in aquafeeds.*

Fatty acids	Oil source									
	Rapeseed	Olive	Rice bran	Sesame	Soybean	Safflower	Sunflower	Cotton-seed	Corn	Linseed
Palmitic (16:0)	4.4	9.5	16.4	9.9	7-12	2-10	3-10	17-29	8-19	6.1
Palmitoleic (16:1 n-7)	0.3	0.5	0.3	0.3	< 0.5	< 0.5	< 1.0	0.5-1.5	< 0.5	0.1
Stearic (18:0)	1.7	3	2.1	5.2	2-5	1-10	1-10	1-4	0.5-4	5.5
Oleic (18:1 n-9)	59.2	81	43.8	41.2	19-30	7-42	14-65	13-44	19-50	20.6
Linoleic (18 :2 n-6)	19	0.5	34	43.3	48-58	55-81	20-75	33-58	34-62	16.3
α-Linolenic (18 :2 n-3)	8.6	0.5	1.1	0.2	4-10	< 1	<0.7	0.1-2	< 2	49.7
EPA (20:5 n-3)	-		-	-	-	-	-	-	-	-
DHA (22:6 n-3)	-	1.6				< 1	-	-	-	-

Sources: Adapted from (Turchini et al. (2010) and ARRINA, (2016).

Concerning the FO replacers in formulated aquafeeds, RO is by far the most abundantly studied and currently utilized MUFA-rich oil. Based on the production volume, RO is the third largest oilseed crop in the world (Turchini et al., 2010). Currently around 27 million metric tons of RO are produced globally, which is nearly 20-fold more than FO production (Turchini et al., 2010). In Norwegian salmon feeds, RO together with camelina oil accounts for 19.8% and linseed oil accounts for 0.3% compared to 10.4% of FO (Aas et al., 2019). Over the last three decades, researchers conducted many feeding trials to evaluate the possible effects of FO replacement with RO; they indicated that partial replacement of FO with plant oils may not compromise the growth of Atlantic salmon (Bell et al., 2001, 2003; Rosenlund et al., 2001; Torstensen et al., 2004). Nevertheless, the fatty acids in the oil is reflected in the flesh of the fish. With plant oils, most commonly RO, the flesh will have low levels of n-3 LC-PUFAs such as EPA (20:5n-3) and, DHA (22:6n-3) as well as high levels of 18C fatty acids, namely oleic acid (18:1n-9), linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3). Diets with higher n-6 to n-3 ratios can negatively affect the fillet and organ fatty acids composition of Atlantic salmon (Bell et al., 2002; Torstensen et al., 2004). The tolerance of lumpfish to plant feed ingredients has not yet been reported. The extent to which plant ingredients (protein and lipids) can replace marine ingredients without reducing growth and affecting the welfare of the fish should be evaluated thoroughly to support the nascent lumpfish farming industry and to ensure its future growth.

1.7 Fish muscle development and growth

Fish growth primarily depends on the accretion of muscle tissues, which is the main edible portion (Johnston et al., 2011; Periago et al., 2005). Mainly there are three muscle fiber types, which are classified according to their color and properties (Luther et al., 1995). Fast (white), muscle fibers are the most abundant fibers, and they are associated with burst swimming (Luther et al., 1995) and make up to 70-90% of total muscle mass depending on the species (Love, 1988). Thus, changes in body mass are largely related to the changes in fast muscle fiber growth. The second muscle fiber type, slow (red) fibers, are recruited for continuous swimming and the quantity of slow fibers are estimated to be 1-30% of the total muscle mass (Bone, 1978). The third type is intermediate (pink) fibers, which are located between white and red fibers and has

intermediate contractile and biochemical properties compared to fast and slow muscle fibers (Bone, 1978).

Muscle growth is a delicate, dynamic process involving both the recruitment of new muscle fibers (hyperplasia) and the growth of existing fibers (hypertrophy) (Rowlerson and Veggetti, 2001). Teleost muscle growth can be classified into three phases; 1) embryonic 2) stratified hyperplasia and 3) mosaic hyperplasia (Rescan, 2005). Embryonic phase is quite complex and involves the formation of embryonic muscle fibers and a population of undifferentiated myogenic progenitor cells (MPCs). The second phase of myogenesis begins with the late embryo stage contributing to the increase in size and development of the somite/myotome by recruitment of new muscle fibers in distinct growth zones, a process called stratified hyperplasia. In most species that attain a large final size, stratified hyperplasia is followed by a third growth phase called mosaic hyperplastic, a phase that leads to the formation of new fibers scattered throughout the whole myotome, giving it a mosaic appearance in the muscle cross section (Rowlerson and Veggetti, 2001). Common for both the stratified and mosaic hyperplastic growth phase is the activation of a distinct population of myogenic progenitor cells and their eventual fusion to create new fibers on the surface of existing fibers either in growth zones or across the myotome. Mosaic hyperplasia can start as early as first feeding and can continue well into the adult stage, resulting in a large increase in the total fiber number in all muscle layers, particularly that of fast muscle (Rowlerson and Veggetti, 2001). Overall, earlier developmental stages are dominated by hyperplastic growth, which results in rapid body mass increase. However, in most cases, mosaic hyperplasia seems to stop when the fish has reached at size of about 44% of their maximum total length (Weatherly et al., 1988). Recruitment of new fibers stops once the fish attains the maximum muscle fiber number, but the recruitment can continue during muscle injuries (Rowlerson et al., 1997). Further muscle growth by hypertrophy also takes place after hyperplastic growth has ceased and until the individual fiber has reached its maximum size; normally the diameter of the fibers will be between 100-300 mm for fast muscle (Rowlerson and Veggetti, 2001).

Muscle growth is species specific (Weatherly et al., 1988) and both hypertrophy and hyperplasia is influenced by several biological factors such as sex (Hagen et al., 2006), diploidy/triploidy (Johnston et al., 1999) and some environmental factors such as egg incubation temperature (Johnston et al., 2000) and photoperiod (Johnston et al., 2003). In addition, nutrition plays a key role in muscle development. Hence, it is important to evaluate and understand the dietary effects on fish muscle growth and development. Protein is the basic component of fish feed, and several studies have shown that muscle cellularity is modulated by dietary protein-level (Alami-Durante et al., 2010; Bjørnevik et al., 2003; Knutsen et al., 2019; Silva et al., 2009). However, there are not many studies that have reported the changes in muscle growth by altering the dietary protein content. There is little or no commercial value for the flesh of farmed lumpfish, but studies including muscle fiber growth and development are crucial to a holistic understanding of the effects of different experimental diets.

2. Objectives

The overall objective of this PhD thesis was to generate new knowledge about the capacity of lumpfish to utilize plant-derived feed ingredients, without compromising fish robustness and health. This new knowledge can be used to develop new feed formulations and reduce the dependency on marine based feed ingredients in feeds for juvenile lumpfish. The specific objectives of this thesis are

1. Investigate the partial replacement of fishmeal by a blend of soy and pea protein concentrates in the diets of lumpfish; effects on i) lumpfish growth performance and somatic indices, ii) chemical composition, iii) fast muscle fiber cellularity

2. Investigate the total and partial replacement of fish oil by rapeseed oil in the diets of lumpfish; effects on i) lumpfish growth performance and somatic indices, ii) Chemical and fatty acid composition in feeds, whole body, muscle and liver

3. Investigate the partial replacement of fishmeal by plant protein mixtures in lumpfish diets; effect on i) digestive enzyme activities ii) histological alterations in the digestive tract

The present thesis is based on the results from two feeding experiments. Briefly, in the first experiment fishmeal was replaced by soy and pea protein concentrates. Key findings of this study were published in Aquaculture Reports (Paper I) and the remaining results of digestive enzymes and histology are presented in the third manuscript (Paper III). The second experiment investigated the replacement of marine oil by rapeseed oil and the effects on growth, somatic indices, chemical composition of whole body, muscle and liver. A manuscript based on the findings are submitted to Aquaculture Reports (paper II) (**Figure 7).**

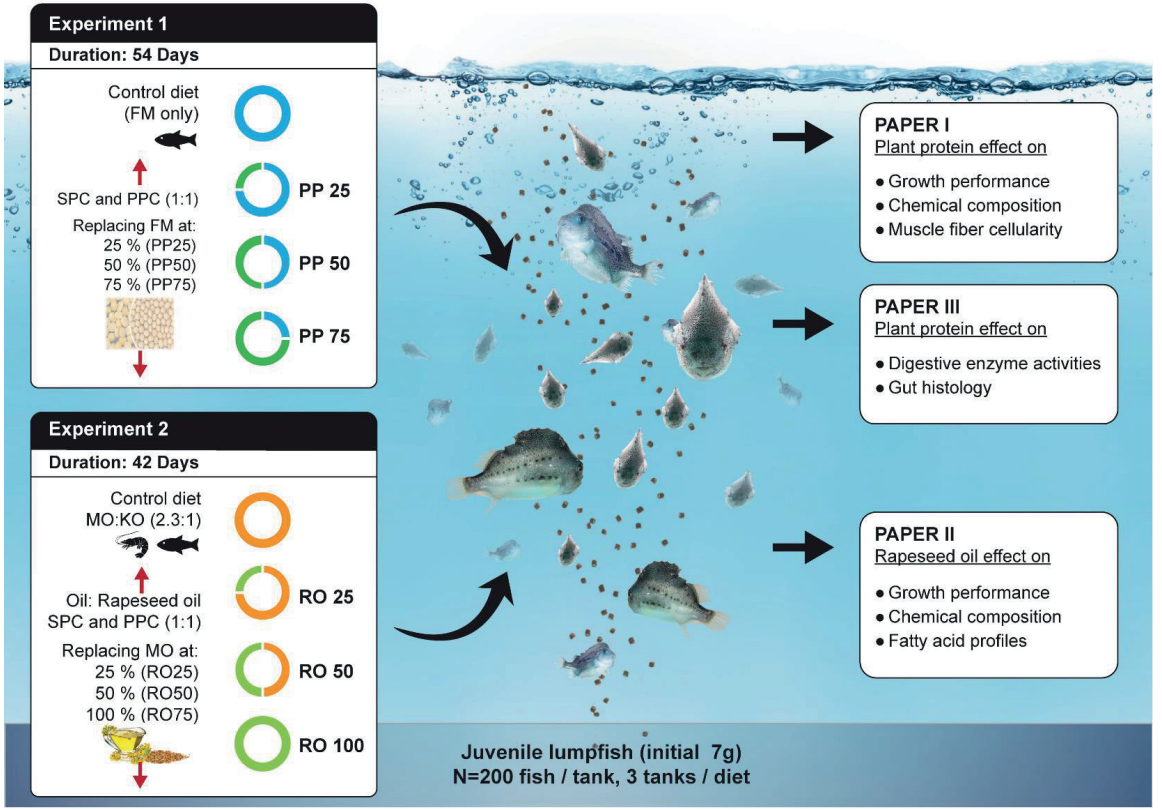

Figure 7. *Graphical illustration of the experimental design and study parameters in the present thesis work. Pie chart color scheme, blue: fishmeal (FM), dark green: plant proteins; SPC and PPC: soy and pea protein concentrates. orange: marine oil + krill oil (MO, KO), light green: rapeseed oil. Illustration: Florence Willora, Nord University.*

3. Summary of papers: Main findings

Paper I

Growth performance, fast muscle development and chemical composition of juvenile lumpfish (*Cyclopterus lumpus*) fed diets incorporating soy and pea protein concentrates
Aquaculture Reports 17 (2020) 100352

In this paper juvenile lumpfish were provided either diets containing fishmeal as the primary protein source or diets with a blend of plant protein concentrates (SPC and PPC) that replaced 25%, 50% and 75% of the fishmeal. At the end of the 54 days feeding trial, better growth and reduced growth performances were observed for the fish fed 50% and 75% of the plant protein concentrates, respectively. Chemical composition of whole body was affected by the diets; fish fed 50% SPC and PPC had higher crude protein content and lower crude lipid compared to the control group. Our study showed that muscle fiber growth of lumpfish was dominated by hyperplasia; however, the diets did not affect the muscle fiber growth.

Paper II

Total replacement of marine oil by rapeseed oil in plant protein rich diets of juvenile lumpfish (*Cyclopterus lumpus*): effects on growth performance, chemical and fatty acid composition
Manuscript submitted to Aquaculture reports on 20 August 2020

In this paper we investigated the effects of rapeseed oil as a marine oil replacer in the diets for juvenile lumpfish on growth, organosomatic indices and chemical composition of whole body and muscle. Four experimental diets were produced with ingredients from similar protein sources; 50:50 fishmeal and a blend of plant protein concentrates (SPC and PPC 1:1 ratio). The diets differed in lipid source; marine oil was used in the control diet (CO) while rapeseed oil replaced 25%, 50% or 100% of the marine oil in the three experimental diets. Fish were fed the experimental diets for 42 days. The results showed that the 50% oil replacement had no effect on growth parameters, specific growth rate, condition indices, whole body chemical composition and liver and whole body fatty acid (FA) profiles. Full

replacement of MO (100% RO) reduced the growth, while hepatosomatic index, and crude lipid content in whole body and liver of the fish were increased. Rapeseed oil in the diets affected the whole body, muscle and liver FA profiles; saturated FAs and polyunsaturated FAs decreased while MUFA and total n-6 FA increased in fish fed increasing amount of rapeseed oil.

Paper III

Replacement of fishmeal with plant protein in the diets for juvenile lumpfish (*Cyclopterus lumpus*): effects on the digestive enzymes and microscopic structure of the digestive tract

Manuscript

The aim of the study was to investigate the intestinal morphology and digestive enzyme activity in juvenile lumpfish fed fishmeal diet (control) or diets with plant protein concentrates (SPC and PPC, 1:1 ratio). The plant protein concentrates were employed to replace 25%, 50% and 75% of fishmeal in the diets. We investigated the alterations in mucosal fold height, thickness of tunica muscularis, number of goblet cells and width of laminar propria in the anterior and distal intestine of lumpfish. Furthermore, we determined the total alkaline protease, leucine amino peptidase and trypsin in different sections of the intestine and pyloric caeca. The fish fed 75% plant concentrates had some morphological alterations; reduced mucosal fold height ($p=0.06$) and increased number of goblet cells ($p=0.07$) in the AI and DI respectively. Dietary plant protein inclusion did not alter the enzyme activities, whereas differences were found only between the sampling locations.

4. General Discussion

Lumpfish is the second largest farmed species in Norwegian aquaculture, but it is still considered as a new species. Health and welfare of this species in aquaculture can be ensured only by generating in depth knowledge of lumpfish biology, artificial breeding and development of fish feeds for juvenile and grow-out phases. Such information will ascertain a continuous supply of disease-free robust larvae. Little is known about the nutrient requirement of this aquatic species, and no studies have addressed the effects of utilization of diets low in marine ingredients on the growth and survival of the fish. Marine ingredients are to a large extent replaced with plant ingredients in diets for carnivorous fish (Aas et al., 2019; Sørensen et al., 2011; Ytrestøyl et al., 2015). Plant ingredients differ from marine ingredients, in terms of their nutritional value. When introducing ingredients which are not in their natural diet, it is important to assess implications on growth, survival and health-related aspects for successful replacement of marine ingredients.

4.1 Growth performance and muscle cellularity

Tolerance to plant protein ingredients is dependent on the species of the farmed organism, their growth status, rearing conditions and more specifically the quality of the protein ingredients, in terms of digestibility and presence of ANFs (Gatlin et al., 2007; NRC, 2011). To our knowledge there are no publications that have investigated the effects of plant ingredients on the growth of lumpfish. The results from the present study showed that growth was not affected when plant protein ingredients replaced up to 50% of the fishmeal (**Paper I)**. Correspondingly, our rapeseed oil (RO) experiment showed no growth differences when fish were fed with marine oil in the control diet compared to fish fed diets where 50% of the marine oil was replaced with rapeseed oil. The protein in the RO experimental diet was mainly derived from fish meal, pea protein concentrate and soy protein concentrate (0.5:0.25:0.25). The growth rates observed in the PP study was similar to the growth rates of the RO study. Both experiments were conducted under similar experimental conditions and feeding rate of 2.5 % BW^{-1}. The fish in the PP study grew from an average weight of 7g to 40g; an overall 6-fold weight gain and nearly 2-fold gain in length

and height during the 54-days feeding trial with an average specific growth rate (SGR) of 3.1 % to 3.5 % per day. The fish in the RO study grew from an average initial weight of 7g to 37g; overall 5-fold weight gain and nearly 2-fold gain in length and height during the 42-days feeding trial, with the average SGR ranging from 3.5 % to 3.8 % per day (**Figure 8**). These values are comparably higher than the results reported in Imsland et al. (2020). In the latter study, lumpfish were fed with commercial feed containing 52 % crude protein and 15 % crude lipid grew from 52 g to 152 g (3-fold weight gain during 147 days); however the fish was larger and the experimental conditions and feeding rate (1.5 BW^{-1}) were not similar to our study.

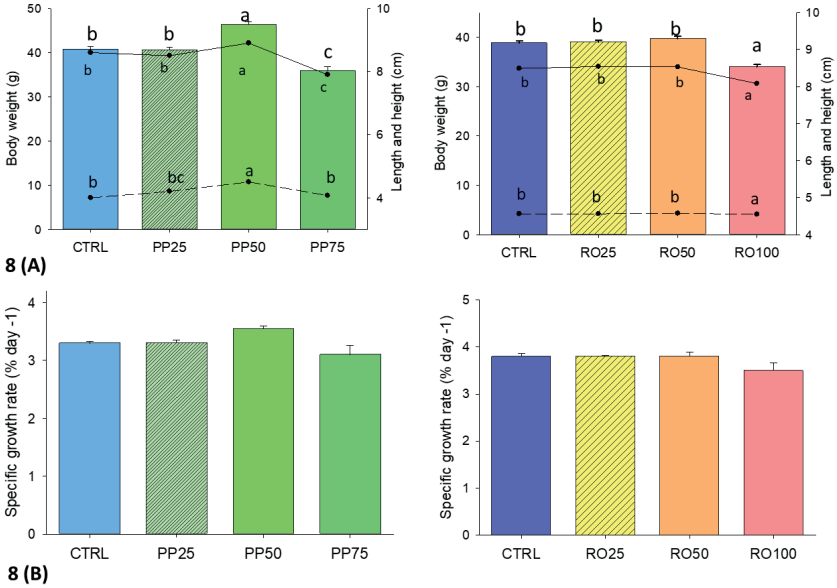

Figure 8. *(A) Body weight (bars), body length (solid line) and body height (dashed line) described in paper 1 (left panel) and paper 2 (right panel). (B) Specific growth rate of the juvenile lumpfish fed different experimental diets in the two experiments at the end of the feeding trial. Note:* ***Paper I****: control diet (CTRL), blend of SPC and PPC at 25% (PP25), 50% (PP50) and 75% (PP75).* ***Paper II****: Fish oil-based control diet (CTRL), rapeseed oil at 25% (RO25), 50% (RO50) and 100 (RO100). Different lowercase letters denote significant differences (P<0.05) between groups.*

As a monogastric organism, fish require a well-balanced mixture of essential and non-essential amino acids (NRC, 2011). Feed ingredients are chosen based on their protein level and quality, functionality, availability and affordability, and these raw materials can act as substitutes for finite feed components (Tacon and Metian, 2015). Feed ingredients originate from different sources and their amino acid profiles vary considerably. Mixtures of plant protein ingredients have great potential to replace FM and meet the nutrient requirements of fishes (Zhang et al., 2012). However, 75% replacement of fish meal with a mix of plant proteins resulted in a 12% reduced growth compared to the control diet **(Paper I).** The lower weight gain may be explained by reduced mucosal fold height and increased goblet cells found in fish fed 75% diet (**Paper III**). Nutrient digestibility was not analyzed in any of the experiments, but the highest incorporation of plant protein concentrates might have affected it (Deng et al., 2006; Mohd Faudzi et al., 2018). Other studies have reported that reduced feed utilization as a result of replacement of fishmeal with soybean meal (Bowyer et al., 2012; Øverland et al., 2009) and SPC in combination with algal meal (García-Ortega et al., 2016), were associated with digestibility. Studies have also reported reduced protein digestibility as well as growth at 100% SPC in feed for Japanese flounder (*Paralichthys olivaceus*; Deng et al., 2006), and 30%-60% SPC in feed for hybrid grouper (Mohd Faudzi et al., 2018). The essential amino acids were balanced by adding synthetic amino acids, and amino acid deficiency is not likely to explain the differences in weight gain. Feed intake was not recorded in the experiment, but palatability may also have been affected at the highest incorporation of plant ingredients in **paper I** and **paper II**. Lumpfish fed with 100% RO had 11.8% lower weight compared to those fed the control diet **(Paper II)**, probably due to lack of n-3 HUFA **(Paper II)**. With regards to essential fatty acids, it has been reported that higher dietary EPA:DHA ratio is positively correlated with growth performance of ballan wrasse (Kousoulaki et al., 2015). However, it must be noted that reduced growth rates are advantageous for lumpfish in order to prolong their grazing time on sea lice (Powell et al, 2018a,b). In contrast, selection for rapid growth is the common strategy within selective breeding among the aquaculture species produced for human consumption.

Condition factors are used for comparing the 'condition', 'fitness', or 'well-being' of fish, based on the assumption that heavier fish of a given length are in better condition (Froese, 2006). Condition factor of the fish in both experiments **(Paper I and Paper II)** were determined by Fulton's condition factor (K, relation between length and weight) and the new factor, B (relation between length, weight and height) proposed by Richter et al., (2000). Our results indicated a higher Fulton's K- value for the group that had the lowest growth performance (PP75 in **Paper I** and RO100 in **Paper II**). Therefore, condition factor B was preferred over K in the present study due to its better description of the relationship between condition of fish and growth performance.

At the termination of the experiment, a slightly higher hepatosomatic index (HSI) was observed for the fish fed RO100 **(Paper II)**. The relationship between HSI and dietary plant proteins or oil does not have a predetermined pattern. Previous studies reported higher HSI when FO was completely replaced with plant oils (Bowyer et al., 2012; Fountoulaki et al., 2009; Mu et al., 2020; Piedecausa et al., 2007; Sun et al., 2011). This can be explained by the high percentage of 18:2 n-6 and lower levels of EPA and DHA in fish fed RO100, which can result in a reduced proportion of n-3:n-6 that would stimulate liver lipid accumulation (Kjær et al., 2008; Piedecausa et al., 2007; Reis et al., 2014). At the end of the experiment, HSI slightly increased with the incorporation of plant proteins **(Paper I)**, and the result corroborated with higher whole body lipid in the fish fed PP75. In higher vertebrates, dietary protein level and source is known to affect lipid deposition (Aoyama et al., 2000). There is growing evidence that lipid metabolism in fish could be modulated by dietary protein level and source (Dias et al., 2005).

In **paper I** muscle fiber cellularity was evaluated in juvenile lumpfish fed plant protein diets compared to control. To our knowledge, these are novel findings as there are no previously published literature on muscle fiber cellularity in lumpfish. In the experiment conducted for **paper I,** fish grew from 7 g to 40 g during 54 days of the experimental period, and their dominant muscle fibers were of size < 30 µm, indicating that the recently recruited muscle fibers were formed through hyperplastic growth. Previous research has shown that

fish species that experience long periods of hyperplasia reach larger body sizes than those that grow primarily through hypertrophy (Rowlerson et al., 1995; Veggetti et al., 1990). According to Weatherly et al. (1988), recruitment of new muscle fibers (hyperplastic growth) stops at a size of nearly 44% of the final body length in several teleost fish species. The maximum body length of lumpfish males and females differ; males are reported to be 35-38 cm while females can grow up to 61cm. It might be that lumpfish displays the same sexual dimorphism in muscle fiber growth pattern as reported for Atlantic halibut, where males stop recruiting fast fibers at a smaller body size than females (Hagen et al., 2006). Our fish had a final mean standard length of only 8 cm and the experiment was executed for a relatively short window and this only gives a snapshot of the muscle fiber growth pattern of this species. The fact that lumpfish has a body shape and swimming behavior that is unique compared to that of most other studied species makes it hard to discuss and relate to existing literature on fish muscle fiber development and growth. It might be that the muscle fiber growth dynamics differ substantially at a later juvenile or adult stage. However, our results show that hyperplasia makes a significant contribution to muscle fiber growth, which could be attributed to the observed increase at this body size. At the end of the experiment, there was no evidence that hyperplasia has stopped and this suggests that mosaic hyperplasia still contributes to muscle growth beyond this size. It might be that lumpfish have different phases like the Atlantic cod (*Gadus morhua*), a cold water bathopelagic species; in this fish growth up to 35 cm was predominated by hyperplasia followed by a phase (35-75 cm), when hypertrophy also contributed substantially (Greer-Walker, 1970). Similarly, juvenile spotted wolffish (*Anarhichas minor*), of 27 cm, was reported to have more muscle fibers, in the range 40-80 µm and 80-120 µm, confirming hyperplastic growth (Knutsen et al., 2019). However, more research is needed to get a better understanding of muscle growth of the cold water, pelagic/semi-demersal lumpfish.

It can be hypothesized that differences in fish growth has an impact on muscle fiber cellularity. Morphometric measurements of mean muscle fiber diameters showed that fish fed PP75 had significantly smaller muscle fibers ranging from 50-70 µm compared to fish

fed control diet. However, the fiber population analysed by probability density distributions did not differ among diets, suggesting that plant protein inclusion did not have any effect on the fast muscle fiber size distribution or fiber number.

4.2 Chemical composition

The values of protein, lipid and ash content of whole body (60.6%, 21.1%, 1.6%) in **paper I**, was nearly in same range as those found in **paper II** (62.8%, 18.6%, 1.5%) (**Table 4)**. Fish fed PP50 had higher crude protein and lower crude lipid content compared to the control group **(Paper I)**. Whole body protein and body lipid were found to be similar for the RO25 and RO50 groups compared to the control diet-fed group. In contrast, RO100 group had the lowest body protein with higher crude lipid compared to the control group.

The liver chemical composition (only analysed for **Paper II)**, showed a greater level of crude lipid in RO100 suggesting a link between the increased HSI observed at the end of the feeding trial and lipid deposition. High deposition of lipid can be explained by 1) low levels of EPA and DHA and/or 2) high levels of oleic and linoleic acids which stimulate the lipid accumulation in the liver. In general, variations in the chemical composition of body and tissues in aquatic species depend on internal factors such as age, gender, size as well as external factors such as water quality, season and geographical area; however the key determinant is the diet (Shearer, 1994). Since external and internal factors were similar, variations in chemical composition of whole body and liver could conclusively be due to the inclusion of plant ingredients in their diets.

Fishes such as lumpfish, with an omnivorous feeding habit, are likely to utilize dietary vegetable oils in a more efficient manner. In agreement with our findings in **paper II**, a complete (100%) replacement of marine oil by RO has also shown to reduce growth in other marine species fed diets based on 100% vegetable oil (Mu et al., 2018; Torrecillas et al., 2017). The main reason for reduced growth is related to the EPA + DHA requirements, which differ among species; recommended levels are 0.5-1.0% for juvenile Atlantic salmon, 1% for

European seabass, 1.4% for Japanese flounder, and 0.5-1% for red drum (NRC, 2011). However, the essential FA requirement for lumpfish is not known. The lack of growth differences found for lumpfish fed control, RO25 and RO50 diets suggest that the requirement for EPA + DHA may possibly be in the range 2.6-1.3%. Diets in which fish oil was fully replaced with rapeseed oil had provided only 0.46% of EPA + DHA; the result was a significant growth reduction.

The fatty acids in RO used in the experiment described in **paper II** were dominated by the monounsaturated fatty acid oleic acid (C18:1,n-9), and polyunsaturated fatty acids such as linoleic acid (C18:2, n-6) and alpha-linolenic acid (C18:3, n-3). The results of **paper II** showed that fatty acid composition of whole body, liver and muscle clearly were affected by dietary fatty acids. Similar to the results reported for fish fed RO (Bell et al., 2001; Sun et al., 2011) or RO blend together with other vegetable oils (Pereira et al., 2019), in the present study, inclusion of RO lowered the EPA + DHA content in the liver and muscle of lumpfish, whereas oleic, linoleic and alpha linolenic acids were gradually increased in the tissues of fish fed increasing dietary RO. The fish fed RO100 had distinctly lower EPA + DHA in the muscles, which is an aberration commonly associated with the incorporation of plant oils in fish diets (Tocher, 2015). Fish fillets are the main edible portion and lower EPA and DHA compromises their nutritional value for humans. However, currently lumpfish are produced mainly as a cleaner fish, but might have the potential as a food fish is some East-Asian countries (Nofima, 2016b). The substitution of fish oil by plant oils in aquafeeds is an economically feasible solution to tackle the finite ingredient issues. However, future lumpfish diets should be formulated by considering the fatty acid composition of potential feed ingredients.

Table 4. *Chemical composition (dry matter %) of the experimental diets, whole body and liver of lumpfish.*

	Paper I				Paper II			
	CTRL	PP25	PP50	PP75	CTRL	RO25	RO50	RO100
Experimental diets								
Crude protein	51.1	52.1	52.5	52.4	52.9	53.7	54.0	53.9
Crude lipid	14.9	14.8	14.4	14.5	14.3	14.8	14.8	15.1
Ash	11.3	9.7	8.5	6.2	8.5	8.6	8.6	8.7
Energy	20.5	20.8	20.8	20.8	20.8	21.0	21.3	21.7
Whole body								
Crude protein	60.6^{b}	61.1^{ab}	62.2^{a}	61.1^{ab}	62.8^{a}	62.2^{ab}	61.8^{ab}	61.5^{b}
Crude lipid	21.1^{a}	19.2^{b}	18.9^{b}	20.1^{ab}	18.6^{a}	18.6^{a}	19.7^{ab}	20.5^{b}
Ash	1.62	1.63	1.66	1.62	1.52^{ab}	1.54^{ab}	1.44^{b}	1.54^{ab}
Liver								
Crude protein			NT		24.8^{a}	23.5^{ab}	22.4^{b}	21.7^{b}
Crude lipid			NT		69.2^{a}	69.7^{a}	73.8^{b}	77.4^{c}

*Note: Data is presented in **Papers I** and **Paper II**; Paper 1: Fish meal based control diet (CTRL), blend of SPC and PPC at 25% (PP25), 50% (PP50) and 75% (PP75). Paper II: Fish oil based control diet (CTRL), rapeseed oil at 25% (RO25), 50% (RO50) and 100 (RO100). NT: not tested*

4.3 Gut health and digestive enzymatic activity

Intestinal histomorphology was assessed in order to confirm the suitability of plant protein concentrates as dietary ingredients in juvenile lumpfish. The results of **paper III** indicated the PP that replaced 25% and 50% of FM did not alter the histomorphology of the anterior (AI) and distal intestine (DI). The 75% replacement of FM with PP tended ($p=0.06$) to reduce the height of the mucosal folds in the AI. Shortened mucosal folds gives the connotation of less surface area to absorb nutrients, and may explain the reduced growth in fish fed the highest incorporation of PP **(Paper I)**. Moreover, a trend of increase in the number of goblet cells ($p=0.07$) was observed in the DI of fish fed the highest incorporation of PP compared to the control diet. These alterations indicate an onset of intestinal inflammation.

Alterations in microscopic structure of intestine due to dietary plant ingredients differ between species. Carnivorous species like Atlantic salmon have short intestine, and therefore are not well-equipped with appropriate features that are essential for digest plant

materials. Soybean meal induced enteritis in the distal intestine of Atlantic salmon develop within short time, starting with morphological changes at day 5 of soybean meal feeding and the condition progresses to a fully developed inflammation at day 21 (Baeverfjord and Krogdahl, 1996). Long-term studies that reported soybean meal-induced enteritis have revealed that inflammation will persist as long as fish is fed the diets with ingredients that cause histopathological changes (Refstie et al., 2005; Sanden et al., 2005).

Number of goblet cells in the AI was higher than those in the DI, irrespective of the fed diet **(Paper III).** This observation contradicts the normal trend in distribution of goblet cells in teleost intestinal tissues. In general, the number of goblet cells increase from the anterior to the posterior segments in the intestine as well as in the rectum (Machado et al., 2013). Mucin synthesis and secretion from goblet cells is stimulated during the acute phase of intestinal infections, but chronic infection reduces the number of goblet cells (Kim and Ho,. 2010). Overall, as no major microscopic changes was observed in our study, the less number of goblet cells in the DI was assumed to be a species-specific feature of lumpfish, similar to the Giurine goby*, Rhinogobius giurinus* (Hur et al., 2016).

Digestive enzyme activity is an indicator of digestive capacity and nutritional status (Engrola et al., 2007). Several factors such as the dietary source, quality and concentration of dietary nutrients (Santigosa et al., 2008), and fish developmental stages (Sahlmann et al., 2015) are known to modulate the intestinal enzymatic profile. In **paper III**, the enzyme activity of total alkaline protease (TAP), leucine amino peptidase (LAP) and trypsin (TRP), in the different intestinal segments (anterior, mid and distal intestine) as well as pyloric caeca (PC) were evaluated in fish fed the four experimental diets reported in **paper I**. The PP inclusion had no significant effect on the different enzymes, but TRP activity was numerically higher in fish fed 50% PP in the diet. The elevated TRP activity coincided with the higher growth in fish fed PP50 **(Paper I)**. Trypsin has dual roles, acting as a key enzyme in protein digestion and activating other proteases. Taken together, high TRP activity may

have improved the protein digestion and amino acid absorption, thereby promoting good growth.

Activities of the intestinal protease TRP was higher in the mid intestine than in the distal intestine. This observation corroborates with the findings reported in other published literature that the anterior segment seemed to be the main site for nutrient digestion and absorption compared to most of the posterior sections (Bakke et al., 2010). We suggest that future lumpfish nutritional studies include other digestive enzymes, namely proteases, amylase and lipases; to provide a more fundamental understanding of the digestive system and potential to utilize main groups of nutrients in the diet. Detailed knowledge of the digestive system of lumpfish is important to improve feed formulations.

5. Conclusions

The main aim of this PhD thesis was to explore the potential to employ plant feed ingredients in juvenile lumpfish diets. To our knowledge, no such research has been conducted on this species and the information acquired may benefit future lumpfish aquaculture. Based on the two studies carried out for this PhD project, following conclusions were made:

- Juvenile lumpfish readily accepted the tested plant protein concentrates and rapeseed oil as feed ingredients in their diets.
- Feeding of soy and pea protein concentrates at 50% did not affect fish growth, whereas increasing the level to 75% in the diet reduced the growth during the 54 days feeding trial.
    - Fish fed 50% plant protein diets had the highest body protein and lowest lipid levels.
    - The muscle fiber growth dynamics of juvenile lumpfish was dominated by hyperplasia.
    - Dietary inclusion of plant proteins did not affect the fish muscle fiber cellularity and digestive enzyme activities of total alkaline protease, trypsin and leucine amino peptidase.
    - Plant protein concentrates in diets slightly altered the mucosal fold height in the anterior intestine and number of goblet cells in the distal intestine, based on the observation from the 75% fish meal replacement study.
- Marine oil can be successfully replaced with 50% of rapeseed oil in juvenile lumpfish diets for a time period of 42 days without affecting fish growth, while 100% rapeseed oil can reduce the growth.
    - Rapeseed oil increased the HSI and lipid content of the liver and whole body.
    - Tissue fatty acids in whole body, muscles and liver mirrored the dietary fatty acid compositions.

- Overall, the results of both studies suggested 50% of fish meal and 50% of marine oil can simultaneously be replaced with blends of soy and pea protein concentrates and rapeseed oil, respectively in the diets for lumpfish juveniles.

6. Future Perspectives

Today's commercial feeds used for lumpfish is largely marine based. The present thesis suggesting that plant protein concentrates and rapeseed oil are good choices for future aquafeeds of lumpfish, and can substitute 50% of marine-based ingredients without compromising fish growth or welfare. Given the global needs for fishmeal and fish oil for aquaculture, there is an increasing demand for more insight on the potential of alternative sources in aquafeeds. Thus, future research should also aim to identify the possible use of low cost commonly available fishmeal and fish oil alternatives. Plant protein mixtures over a single plant protein ingredient have been and will probably continue to be the timely choice when replacing fishmeal. Finding of right plant protein sources and their inclusion levels to formulate feed are challenging. Apart from the plant proteins there are other protein sources; terrestrial animal and fisheries by products, single cell proteins (bacteria, yeast or microalgae), insect meal represents future sustainable sources.

Similar to the fishmeal replacers there has been an ongoing challenge in the aquafeed industry to reducing the dependency of marine oil sources. There are variety of crops, producing almost 200 million tonnes of plant oils yearly, but the only problem is that none of the plants can produce the n-3 LC-PUFA, e.g. EPA and DHA (Tocher et al., 2019). In this respect, research are more tended to focus on novel alternatives, such as oil from transgenic plants and the inclusion of micro and macro-algae originated ingredients. However, the use of transgenic products as feed ingredients is considered non ethical by a large percentage of the population and the latter therefore seams more prominent. However, it is important to evaluate and understand the effects of novel ingredients on feed intake, nutrient utilization, physiological mechanisms in it regulation and metabolic pathways involved in fish growth and welfare.

As a new species, lumpfish have a huge knowledge gap concerning it's nutritional needs and the following aspects should be addressed in order to bridge the gap.

1. Macro- and micro - nutrient requirements are not known for lumpfish, however, there are currently efforts underway to establish basic nutritional requirements.

2. Knowledge on digestive system, digestive secretions and digestibility properties are still unknown.

3. The biosynthesis capabilities of LC-PUFA in lumpfish is still largely unknown.

4. Further research is needed on how ANFs in plant sources effect on fish health and welfare.

Lumpfish aquaculture is facing several challenges and some of them can be overcome by nutritional research. Studies have found that lice eating ability of lumpfish decreases with increasing size of lumpfish (Imsland et al., 2016a). Hence, future diets should needed to formulated to make sure that the nutritional needs are covered, but at the same time not fully utilize the growth potential to maintain stable or slow growth. Recent reports have suggested an association between cataracts and sub-optimal nutrition (Imsland et al., 2019c). Thus, future nutritional studies require greater consideration in regard to produce healthy and robust lumpfish.

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Paper I

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Growth performance, fast muscle development and chemical composition of juvenile lumpfish (*Cyclopterus lumpus*) fed diets incorporating soy and pea protein concentrates

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ABSTRACT

Lumpfish (*Cyclopterus lumpus*) are widely applied as biological delousers in open net-pen farming of Atlantic salmon. As a species new to farming it is necessary to obtain a comprehensive understanding of the capacity of lumpfish to utilize plant derived feed ingredients. A feeding trial lasting for 54 days was conducted to investigate the effects of replacing fishmeal (FM) with a mix of soy protein concentrate (SPC) and pea protein concentrate (PPC) on growth, body chemical composition, and fast muscle fiber cellularity in juvenile lumpfish. Four isonitrogenous and isoenergetic diets (52 % crude protein and 14 % crude lipid) were formulated; a FM based diet was used as control (CTRL), and three experimental diets containing SPC and PPC (equal proportions of 1:1), replacing FM on weight basis at 25 % (PP25) 50 % (PP50) and 75 % (PP75). The fish grew from approximately 6.9 g to an average weight of 40.2 g in 54 days. Fish fed PP50 had significantly higher body weight, length and height compared to the other dietary groups. The whole body crude protein content of fish fed PP50 was significantly higher compared to the CTRL diet, while crude lipids were lower than those on CTRL and PP25 diets. Ash and dry matter did not differ among groups. Probability density functions showed no differences in fast muscle fiber size distributions amongst feeding groups. A higher percentage of smaller fibers in all feeding groups indicated hyperplasia was the dominant mechanism of muscle growth during the experimental period. These results suggest that a mixture of SPC and PPC can replace up to 50 % of FM in diets for juvenile lumpfish without any adverse effects on growth, chemical composition and fast muscle fiber cellularity.

1. Introduction

Two species of caligid copepods, salmon louse (*Lepeophtherius salmonis,* Krøyer) and sea louse (*Caligus elongatus*) are a significant threat to farmed and wild Atlantic salmon (*Salmo salar*). Challenges associated with salmon lice have been reported since 1970 in Norway (Heuch et al., 2005), and have become the main issue for growth and expansion of Norwegian salmon production. Chemotherapeutants, such as bath treatments (hydrogen peroxide and organophosphates) or in-feed treatments (emamectin benzoate), have been used heavily to control these ectoparasites (Burridge et al., 2010). The negative impacts imposed by chemical treatments has driven the industry to use a wider selection of preventive and environmentally friendly alternatives (Powell et al., 2018). Consequently, use of alternative control strategies such as physical barriers, non-chemical baths and sea louse predators (cleaner fish) are increasing (McEwan et al., 2019).

Lumpfish, also known as lumpsucker (*Cyclopterus lumpus*), have little economic value as a food species other than use as a source of roe which is processed and sold as a substitute for caviar in fisheries across the North Atlantic regions (Davenport, 1985). Interest in the commercial production of farmed lumpfish in Norway began in 2011 (Imsland et al., 2014a), as a alternative solution to the sea-lice infestation issue. Lumpfish display cleaning symbiosis; where organisms clean cooperative host organisms, partly feeding on ectoparasites, diseased and injured tissues, and unwanted food particles (Feder, 1966). Studies performed to date have confirmed that lumpfish can be efficient delousers, reducing the mature female lice levels by 93%–97% when cocultured with farmed salmon, at a stocking density of 10%–15%

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(Imsland et al., 2014a, b; Imsland et al., 2014c). The commercial use of lumpfish for delousing has resulted in a rapid increase in their production, reaching 28.9 million fish in 2018 (Norwegian Directorate of Fisheries, 2018) making lumpfish the second largest aquaculture species in Norway. Despite increased production and usage, there is lack of published literature investigating their capacity to utilize commercially and commonly used terrestrial feed ingredients in aquafeeds.

The fish feed industry is increasing the use of plant derived ingredients (Aas et al., 2019; Ytrestøyl et al., 2015) and SPC have come to dominate feeds for Atlantic salmon, accounting for 19 % of the total feed ingredients used (Aas et al., 2019). PPC has also shown great potential as a feed ingredient for carnivorous species (Øverland et al., 2009; Zhang et al., 2012), and is currently used in limited amounts (1.3 %) in Norwegian aquafeeds (Aas et al., 2019). Incorporating plant proteins in fish feeds makes them a feasible, sustainable and cost-effective substitute to FM (Tacon and Metian, 2008). However, complete replacement of FM is still a challenge due to the imbalanced essential amino acid profile, poor palatability and presence of anti-nutritional factors (ANF's) in plant ingredients (Colburn et al., 2012; Drew et al., 2007; Urbano et al., 2000). One way to overcome the limitations of individual ingredients is to use a mixture of plant protein containing ingredients.

Even though lumpfish are not farmed for food, studies conducted on their muscle growth and development are crucial to elucidate feed effects. In most teleost fish species striated muscle predominants, are composed mainly of fast muscle fibers constituting more than 70 % of the total body mass (Sänger and Stoiber, 2001). Thus, changes in body mass are largely attributable to changes in fast muscle fiber growth, which are a consequence of variations in muscle hypertrophy (expansion in fiber diameter) and/or hyperplasia (recruitment of new muscle fibers) (Alami-Durante et al., 2010a). Muscle cellularity, the relative contributions of hypertrophy and hyperplasia to muscle growth, is affected by several factors such as egg incubation temperature (Johnston et al., 2000) and length of the photoperiod (Johnston et al., 2003). Protein is the basic component of fish feed and its level has been reported to influence fish muscle cellularity in several species (Alami-Durante et al., 2010a; Bjørnevik et al., 2003; Knutsen et al., 2019; Silva et al., 2009b). To our knowledge, the effects of plant protein ingredients on muscle development and growth of lumpfish have not been reported. Therefore, the aim of the present study was to investigate the effect of replacing FM with a mixture of SPC and PPC in feeds for juvenile lumpfish, on growth performance, fast muscle development, and whole body chemical composition.

2. Materials and methods

This feeding experiment was approved by the Ethics and Animal welfare committee at Nord University, following the Norwegian animal welfare act (LOV-2009-06-19-97).

2.1. Lumpfish and experimental set up

Juvenile lumpfish of 4 g average weight were obtained from Mørkvedbukta AS, Bodø, Norway. The fish were randomly allocated into 12 indoor rearing tanks (500 L), with 208 fish per tank, at the research station of Nord University, Bodø, Norway. Fish were acclimated to laboratory conditions for 2 weeks prior to the experiment, during which time they were fed a commercial diet (Gemma Silk, Skretting, Stavanger, Norway). Light intensity was controlled by four florescent lamps (24 h) (Grunda Viktor work lamps, 38 W, luminous flux1350 lm) facing upward. Throughout the experimental period light was dimmed to provide an illumination regime similar to that of commercial rearing practice. Fish were provided with seawater from Saltenfjorden, at 250 m depth, with a stable salinity (34‰) through a flow-through water system. Water flow rate was kept constant at 500 L/h. The temperature (7.6 ± 0.9 °C) and dissolved oxygen (86.7 ± 0.11 %) of the rearing water was monitored daily.

Table 1
Ingredient composition of the experimental diets (g 100g^{-1} diet).

Ingredients	CTRL	PP25	PP50	PP75
Fish meal [1]	58.00	43.50	29.00	14.50
Soy protein concentrate [2]	0.00	7.20	14.45	21.67
Pea protein concentrate [3]	0.00	7.20	14.45	21.67
CPSP 90 [4]	2.50	2.50	2.50	2.50
Krill meal [5]	5.00	5.00	5.00	5.00
Wheat gluten [6]	7.00	7.00	7.00	7.00
Wheat meal [7]	10.00	9.16	6.95	4.59
Pea starch [8]	5.35	5.35	5.35	5.41
Fish oil [9]	7.00	7.00	7.00	7.00
Krill oil [10]	1.50	2.25	3.05	3.85
Vitamin & Mineral Premix [11]	1.00	1.00	1.00	1.00
Lutavit E50 [12]	0.05	0.05	0.05	0.05
Antioxidant powder [13]	0.20	0.20	0.20	0.20
Sodium propionate [14]	0.10	0.10	0.10	0.10
MCP [15]	0.00	0.00	0.98	2.10
Carophyll Pink [16]	0.05	0.05	0.05	0.05
Nucleotides [17]	0.50	0.50	0.50	0.50
Garlic extract [18]	0.50	0.50	0.50	0.50
L-Histidine [19]	0.25	0.25	0.25	0.25
L-Tryptophan [20]	0.00	0.09	0.17	0.26
DL-Methionine [21]	0.00	0.00	0.35	0.70
L-Taurine [22]	1.00	1.10	1.10	1.10

CTRL: Control, PP25: 25 % of SPC and PPC inclusion, PP50: 50 % of SPC and PPC inclusion, PP75: 75 % of SPC and PPC inclusion.

[1] NORVIK LT 70 : 70.3 % crude protein (CP) 5.8 % crude fat (CF) (Sopropêche, France).

[2] Soycomil : 63 % CP, 0.8 % CF (ADM, The Netherlands).

[3] Lysamine GPS: 78 % CP, 0.9 % CF (Roquette Frères, France).

[4] Soluble fish protein hydrolysate: 82.6 % CP, 9.6 % CF (Sopropêche, France).

[5] 61.1% CP, 17.4 % CF (Aker Biomarine, Norway).

[6] VITAL: 83.7 % CP, 1.6 % CF, (Roquette, Frères, France).

[7] 10.2% CP; 1.2 % CF (Casa Lanchinha, Portugal).

[8] NASTAR 90 % starch, (Cosucra, Belgium).

[9] (SAVINOR UTS, Portugal).

[10] (Aker Biomarine, Norway).

[11] Vitamins (IU or mg kg-^{1} diet): DL-alpha tocopherol acetate, 100 mg; sodium menadione bisulphate, 25 mg; retinyl acetate, 20000 IU; DL-cholecalciferol, 2000 IU; thiamin, 30 mg; riboflavin, 30 mg; pyridoxine, 20 mg; cyanocobalamin, 0.1 mg; nicotinic acid, 200 mg; folic acid, 15 mg; ascorbic acid, 1000 mg; inositol, 500 mg; biotin, 3 mg; calcium panthotenate, 100 mg; choline chloride, 1000 mg, betaine, 500 mg. Minerals (g or mg kg-1 diet): cobalt carbonate, 0.65 mg; copper sulphate, 9 mg; ferric sulphate, 6 mg; potassium iodide, 0.5 mg; manganese oxide, 9.6 mg; sodium selenite, 0.01 mg; zinc sulphate,7.5 mg; sodium chloride, 400 mg; calcium carbonate, 1.86 g; excipient wheat middlings (PREMIX Lda, Portugal).

[12] (ROVIMIX E50, DSM Nutritional Products, Switzerland).

[13] Paramega PX (Kemin Europe NV, Belgium).

[14] Disproquímica (Portugal).

[15] ALIPHOS MONOCAL, 22.7 % P (ALIPHOS, Belgium).

[16] Carophyll Pink 10 % CWS (DSM Nutritional Products, Switzerland).

[17] Nucleoforce Salmonids (BioIbérica, Spain).

[18] Macrogard, 67.2 % beta-glucans (Biorigin, Brazil).

[19] L-Histidine 98 %, (Ajinomoto Eurolysine SAS, France).

[20] L-Tryptophan 98 %, (Ajinomoto Eurolysine SAS, France).

[21] DL-METHIONINE FOR AQUACULTURE 99 %, (EVONIK Nutrition & Care GmbH, Germany). [22] L-Taurine 98 %, (ORFFA, The Netherlands).

2.2. Experimental diets and growth trial

The feed ingredient composition, calculated and analyzed proximate composition of the experimental diets are presented in Tables 1 and 2, which were manufactured by SPAROS Lda. Olhao, Portugal. The diets were formulated to be isoproteic and isoenergetic on the basis of crude protein and gross energy content. A FM based diet was used as control (CTRL) and three experimental diets were formulated to replace

Table 2
Calculated and analyzed proximate nutrient composition of the experimental diets on a as fed basis (%).

	CTRL	PP25	PP50	PP75
Calculated				
Crude protein	53.9	53.9	53.9	53.9
Crude fat	13.4	13.4	13.4	13.4
Fiber	0.3	0.7	1.0	1.3
Starch	9.2	9.5	8.8	8.1
Ash	11.3	9.5	8.2	7.1
Gross Energy	20.0	20.2	20.3	20.4
Arginine	3.5	3.7	4.0	4.2
Histidine	1.4	1.4	1.4	1.4
Isoleucine	2.0	2.1	2.2	2.4
Leucine	3.8	3.9	4.0	4.1
Lysine	3.9	3.9	3.9	3.9
Tryptophan	0.5	0.5	0.5	0.5
Threonine	2.5	2.3	2.2	2.1
Valine	2.5	2.5	2.6	2.6
Methionine + Cysteine	2.3	2.0	2.0	2.0
Phenylalanine + Tyrosine	4.5	4.5	4.5	4.5
Taurine	1.2	1.2	1.2	1.2
Total Phosphorous	1.7	1.5	1.4	1.4
Vitamin C (mg/kg)	1000.0	1000.0	1000.0	1000.0
Vitamin E (mg/kg)	350.0	350.0	350.0	350.0
Eicosapentaenoic acid (EPA)	1.6	1.6	1.6	1.6
Docosahexaenoic acid (DHA)	2.0	1.9	1.8	1.6
EPA + DHA	3.5	3.4	3.4	3.4
Total phospholipids	2.6	2.6	2.6	2.6
Analyzed				
Dry matter	93.9	94.9	95.3	93.3
Crude protein	51.1	52.1	52.5	52.4
Crude fat	14.9	14.8	14.4	14.5
Ash	11.3	9.7	8.5	6.2
Gross Energy	20.5	20.8	20.8	20.8

25 % (PP25) 50 % (PP50) and 75 % (PP75) of the FM with a mixture of SPC and PPC (1:1 proportion). The remaining protein ingredients such as wheat gluten, krill meal, and CPSP 90 were kept constant. The diets were supplemented with L-tryptophan, DL-methionine, L-taurine and L-histidine to keep these ingredients similar among all diets. Wheat meal was used to balance the starch and carbohydrate content among the diets. Krill oil was used in increasing levels from CTRL to the PP75, to increase the content of EPA, DHA and phospholipids.

All dry ingredients were mixed in a double-helix mixer (model RM90, MAINCA Spain) passed through a 0.4 mm micro-pulverizer hammer mill (model SH1, Hosokawa-Alpine, Germany). Diets were extruded in a twin-screw extruder (model BC45, Clextral, France) with a 1.5 mm die and extruded pellets dried in a vibrating fluid bed dryer (model DR100, TGC Extrusion, France). Oils were added post-extrusion by vacuum coating (model PG-10VCLAB, Dinnissen, Netherlands). Experimental diets were stored at room temperature until they were used for feeding. The four diets were randomly allocated to triplicate tanks (n = 3 / feed group), and each tank was equipped with an automatic feeder (ArvoTec, Sterner, Norway). Fish were fed the experimental diets to apparent satiation, with the feeding rate of 2.5 % of their body mass. The feeding were closely monitored through visual inspections and the feed were provided eight time points every day, between 6:00 to 21:00 during the 54 day experimental period.

2.3. Sample collection

At the beginning and end of the growth trial, all fish were individually weighed to the nearest 0.5 g and their standard length and body height measured to the nearest 0.1 mm. In addition, at each sampling point liver and visceral weight were also recorded. A total of 20 fish per tank were sampled randomly for chemical composition analysis. Fish were pooled into 10 fish per pool and 2 pooled samples per tank (n = 6 fish / feed group), packed in plastic bags, and frozen at -40 °C until further analysis. Five fish were sampled per tank and used for the evaluation of muscle histology. All samples were taken at the start, 19, 35, and 54 days (19D, 35D, 54D) of the growth trial. Prior to sampling, fish were anaesthetized with MS-222 (Tricaine methane sulphonate; Argent Chemical Laboratories, USA; 30 g /L) and dispatched by a sharp blow to the head.

2.4. Biochemical analyses

The frozen whole fish samples were thawed for approximately 5 h at 4 °C and pooled samples were homogenized using a conventional food processor (Bosch GmbH, CNCM11, Slovenia). Part of this homogenate was used to determine the dry matter and ash content in whole fish. Remaining homogenate was freeze dried for 96 h at −70 °C (VirTis benchtop K Mod, Warminster, U.S.A) and dry matter was recorded. The freeze dried samples were frozen at −80 °C before being re-ground (3 × 15 s) into a fine powder for crude protein and crude fat (dry basis) analysis. The proximate composition of the feed pellets was also determined. In brief, moisture content was determined by drying whole fish (2.0 g) and feed (5.0 g) samples to a constant weight at 104 °C for 20 h (ISO 6496-1999). The whole fish samples were combusted in a muffle furnace to a constant weight at 540 °C for 16 h to determine the ash content at FBA, whereas, the feed was analysed by Eurofins (Moss, Norway) (ISO 5984-2002). Crude protein of fish and feed were determined from a 0.5 g samples using the Kjeldahl titration method (N x 6.25, KjeltecTM 2300, Foss Tecator AB, Höganäs, Sweeden ISO 5983-1987). Crude fat was determined gravimetrically using 2.0 g of freeze dried fish and 5.0 g of feed samples using the diethyl ester extraction method, according to the (Norwegian Standard Association., 1994) and feed energy analysed by bomb calorimeter (IKA C200, Staufen, Germany: ISO 9831: 1998). All biochemical analyses of the feed and whole fish were triplicated and duplicated respectively.

2.5. Fast muscle cellularity

To evaluate the muscle cellularity, a 5 mm thick cross sectional steak was cut just anterior to the second dorsal fin of juvenile lumpfish (Fig. 1) and photographed together with graph paper to measure the total fast muscle cross-sectional area (TCA) of the steak (SigmaScan pro. 5.0, Systat, Inc.). Depending on fish size, two to three muscle blocks (5 × 5 × 5 mm) from the dorsal left side of each fillet were taken for histological analysis. In brief, muscle blocks were mounted on cork sheets (1.5 × 1.5 cm) covered in cryomatrix (Shandon Cryomatrix, Thermo scientific) and frozen in 2-methyl butane (60 s) cooled to near its freezing point (−159 °C) in liquid nitrogen. Frozen blocks were stored at −80 °C until further analysis. Muscle blocks were sectioned (7 μm) at −18 °C in a cryostat (Cryostar NX50, Thermo Scientific, USA), air dried and stained with hematoxylin (Harris hematoxylin, Sigma Aldrich, Steinheim, Germany). The outlines of the muscle fibers (area) of 800 fibers per fish were examined using a light microscope (Axioscop 2 mot plus; Carl Zeiss INC., Germany) equipped with a camera, and area measured using the software Axio Vision (Rel.4.2, Carl Zeiss INC., Germany). All the parameters measured for muscle cellularity were normalized based on the size of fish, as described by Alami-Durante et al. (2010a).

2.6. Calculations

Condition factor (B^1) was calculated according to the formula proposed by Richter et al. (2000). B^1 (g cm^{-3}) = fish weight (g) / [fork length (cm) x body height ^{2} (cm)]. Somatic indices and Specific Growth Rate (SGR) were calculated employing the following formulae: Hepatosomatic index (HSI) = [liver weight (g) / fish weight (g)] × 100. Visero-somatic index (VSI) = [visceral weight (g) / fish weight (g)] × 100. SGR (% day $^{-1}$) = 100 × ln [final mean weight (g) - initial mean weight (g)] / number of feeding days.

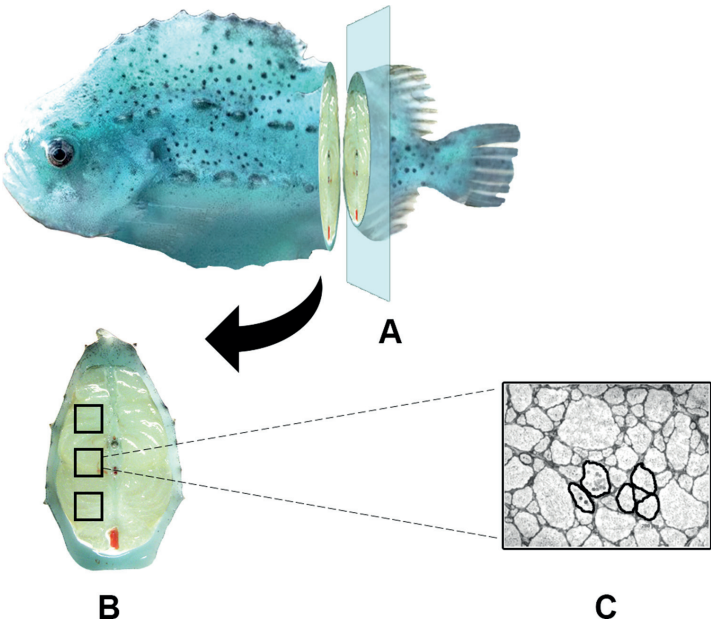

Fig. 1. A) Schematic view of sampling locations in lumpfish, B) sample sites of muscle blocks from the dorsal left side of the steak, C) fast muscle histological section (×10) highlight of the individual muscle fiber area measured.

2.7. Statistical analysis

The software Sigmaplot 14.0 (Systat software, San Jose, CA) was used for the statistical analyses. A Shapiro-Wilk test was used to assess the normality of distributions, and the Brown-Forsythe F-test to determine the equality of group variances. One way analysis of variance (ANOVA) was performed for the parametric data. Significant differences revealed in ANOVA were followed by Tukey's multiple comparison test. A Kruskal-Wallis one-way analysis of variance on ranks, followed by Tukey's multiple comparison test was used for the nonparametric data. Dunn's pairwise multiple comparison test was used only to assess the significance of the unequal size of growth-related data at the end of the experiment. Distribution of muscle fiber diameter was evaluated using smooth non parametric distributions where 800 measurements of fast fiber diameters were fitted using a kernel function (Bowman and Azzalini, 1997; Johnston et al., 1999). Experimental groups compared at the end of feeding period were of similar body length ($n = 12$ per group). Comparison of the distribution of muscle fiber diameters was done by applying the nonparametric Kolmogorov-Smirnov test, with the null hypothesis that one-dimensional probability density functions (PDF) of groups were equal over all the diameters. Bootstrap techniques were used to create the variability bands around the group PDFs using the mean smoothing parameter. This was used to identify which areas of the muscle fiber distribution of diameters contributed to significant differences. Significance was established when $p < 0.05$; data is presented as means ± SEM.

3. Results

3.1. Chemical composition of the experimental diets

Minor differences were observed between the calculated and analyzed proximate composition of the diets (Table 2). The chemical analysis showed that crude protein was slightly lower and crude lipid was slightly higher than the calculated values, while ash and energy were similar to the calculated values.

3.2. Growth performance

The experimental diets were well accepted and no mortalities were recorded. The final weight of fish increased 5–6 fold their initial weight (Table 3). Fish fed PP50 had significantly higher body weight, length and height compared to the other diets at the end of the experiment. The height of the fish increased from an average of 2.22 cm–4.22 cm during the course of the experiment. Length of the fish appeared to be proportional with weight gain and was significantly higher for fish fed PP50 compared to all other diet groups. The fish fed PP50 diet tended to have higher SGR ($p = 0.06$) compared to the other feding groups at the end of the feeding period. The B^1 ranged between 0.23 to 0.33 and was slightly, but significantly higher in fish fed plant diets than those on the CTRL diet at the end of the experiment. No significant differences were found for the VSI among the feeding groups. HSI varied from 2.1–2.5 with the lowest value for fish receiving PP25 and highest for fish on the PP75 diet ($p < 0.05$).

3.3. Chemical composition of fish

Crude fat, protein, ash and dry matter of whole body increased slightly during the experimental period (Table 4). The crude fat content of all groups showed a small numerical and temporal drop after being introduced to the experimental diets compared to the initial levels ($p > 0.05$). However, crude fat content increased for all four groups after 19 days on experimental diets. The CTRL group showed a higher crude fat content at the end of the experiment compared to PP25 and

Table 3
Growth parameters and condition indices of lumpfish fed diets with different levels of plant protein concentrates.

Parameter	Feeding trial period	Plant protein inclusion levels				p - Value
		CTRL	PP25	PP50	PP75	
Growth parameters						
Body weight (g)	Start (0 days)	6.88 ± 0.06	6.80 ± 0.06	6.83 ± 0.06	7.03 ± 0.06	0.246
	Continuous phase I (19D)	14.63 ± 0.31^{ab}	14.25 ± 0.24^{ab}	15.09 ± 0.26^{a}	13.72 ± 0.34^{b}	0.021
	Continuous phase II (35D)	26.34 ± 0.57	25.67 ± 0.59	26.92 ± 0.51	26.5 1 ± 0.63	0.286
	End (54 D)	40.75 ± 0.56^{b}	40.58 ± 0.59^{b}	46.26 ± 0.68^{a}	35.84 ± 0.94^{c}	< 0.001
Body length (cm)	Start (0 days)	4.59 ± 0.01	4.61 ± 0.02	4.64 ± 0.01	4.71 ± 0.07	0.432
	Continuous phase I (19D)	6.24 ± 0.05^{a}	6.20 ± 0.03^{a}	6.22 ± 0.04^{a}	6.03 ± 0.05^{b}	0.005
	Continuous phase II (35D)	7.65 ± 0.06^{ab}	7.59 ± 0.06^{ab}	7.72 ± 0.05^{a}	7.51 ± 0.06^{b}	0.043
	End (54 D)	8.69 ± 0.04^{b}	8.55 ± 0.05^{b}	8.91 ± 0.05^{a}	7.91 ± 0.08^{c}	< 0.001
Body height (cm)	Start (0 days)	2.23 ± 0.01	2.23 ± 0.01	2.22 ± 0.01	2.26 ± 0.01	0.283
	Continuous phase I (19D)	3.06 ± 0.03	3.07 ± 0.02	3.09 ± 0.02	3.05 ± 0.03	0.726
	Continuous phase II (35D)	3.78 ± 0.03^{c}	3.76 ± 0.03^{b}	3.82 ± 0.03^{abc}	3.91 ± 0.04^{a}	0.008
	End (54 D)	4.07 ± 0.03^{b}	4.27 ± 0.03^{bc}	4.48 ± 0.03^{a}	4.07 ± 0.05^{b}	< 0.001
SGR (% day $^{-1}$)	Start (0 days)	n.a	n.a	n.a	n.a	
	Continuous phase I (19D)	3.94 ± 0.14	3.89 ± 0.17	4.18 ± 0.002	3.49 ± 0.21	0.074
	Continuous phase II (35D)	3.84 ± 0.13	3.78 ± 0.07	3.92 ± 0.06	3.72 ± 0.11	0.579
	End (54 D)	3.30 ± 0.03	3.32 ± 0.05	3.55 ± 0.05	3.13 ± 0.16	0.062
Condition indices						
HSI	Start (0 days)	2.53 ± 0.08	2.58 ± 0.09	2.59 ± 0.08	2.44 ± 0.10	0.395
	Continuous phase I (19D)	2.47 ± 0.05	2.50 ± 0.11	2.27 ± 0.05	2.40 ± 0.07	0.066
	Continuous phase II (35D)	2.22 ± 0.05	2.20 ± 0.04	2.14 ± 0.04	2.26 ± 0.05	0.259
	End (54 D)	2.37 ± 0.06^{ab}	2.18 ± 0.04^{b}	2.22 ± 0.04^{b}	2.48 ± 0.04^{a}	< 0.001
VSI	Start (0 days)	12.84 ± 0.19	12.57 ± 0.20	12.83 ± 0.19	12.49 ± 0.31	0.210
	Continuous phase I (19D)	15.18 ± 0.22	15.32 ± 0.22	14.94 ± 0.33	14.56 ± 0.31	0.202
	Continuous phase II (35D)	13.86 ± 0.19	13.30 ± 0.26	13.04 ± 0.25	13.54 ± 0.21	0.086
	End (54 D)	13.72 ± 0.28	13.34 ± 0.17	13.51 ± 0.33	13.23 ± 0.19	0.144
Condition factor B^1 (g cm^{-3})	Start (0 days)	0.30 ± 0.001	0.30 ± 0.001	0.30 ± 0.001	0.30 ± 0.001	0.417
	Continuous phase I (19D)	0.25 ± 0.002^{b}	0.24 ± 0.002^{a}	0.25 ± 0.002^{b}	0.24 ± 0.002^{a}	0.001
	Continuous phase II (35D)	0.24 ± 0.001^{a}	0.24 ± 0.001^{a}	0.24 ± 0.00 ^{a}	0.23 ± 0.001^{b}	0.001
	End (54 D)	0.28 ± 0.002^{a}	0.32 ± 0.060^{b}	0.30 ± 0.050^{b}	0.33 ± 0.020^{b}	0.001

CTRL: Control, PP25: 25 % of SPC and PPC inclusion, PP50: 50 % of SPC and PPC inclusion, PP75: 75 % of SPC and PPC inclusion. Values represented as means ± SEM. Growth parameters and CF for week 0 are based on measurements of all fish. Similarly, growth parameters and CF at the end of the feeding trial based on both fish sampled and fish remaining after 54 days. Significant differences between treatment groups at the same time point indicated with different superscript letters ($p < 0.05$).

PP50 ($p < 0.05$). Additionally, the whole body crude protein content was higher in the PP50 group compared to the CTRL at the end of the experiment ($p < 0.05$).

3.4. Fast muscle cellularity

No differences were found for muscle cellularity among the diet groups, except for mean diameter and muscle fiber size category ranging from 50 µm to 70 µm (Table 5). At the start of the experiment, fish had an average fast muscle fiber number of 62659 ± 4645 and increased for all groups close to five fold during the experimental period. The daily recruited muscle fiber numbers were numerically higher in the PP50 group (4768) compared to the control (4274), PP25 (3882) and PP75 (4283). Size distribution of fast muscle fibers were categorized into 7 groups. Juvenile lumpfish showed a higher number of smaller fibers ranging from 10 to 70 µm and fewer of the larger fibers in the size range 90 − 120 µm. The fibers with diameters $10 < D \leq 30$ µm were the most common and abundant in all diet groups. The only significant difference among diets were for fiber diameters ranging from $50 < D \leq 70$ µm that were lower in fish fed PP75 compared to the other groups. The PDFs for fiber diameter distribution showed, however, no differences among the feeds (Fig. 2, $p > 0.05$).

4. Discussion

In the present study, the utilization of the plant protein concentrates of SPC and PPC in diets for juvenile lumpfish were evaluated based on growth performance, body chemical composition and muscle development.

4.1. Fish growth performance

In the wild, lumpfish feed on a variety of prey items including, plankton, jellyfish and polycheates (Daborn and Gregory, 1983; Davenport, 1985; Ingólfsson and Kristjánsson, 2002; Mitamura et al., 2012), as well as seaweeds and seagrass (Davenport, 1985). Lumpfish have the ability to switch their natural prey choice to whatever is available (Imsland et al., 2015a, b). This opportunistic feeding behavior combined with a gut length twice the body length (Davenport, 1985) indicate that lumpfish are omnivorous and may explain why the best growth performance (i.e. body weight, length, and height) was observed in fish fed the PP50 diet. The experiment was not designed to study feed intake, but all the groups were fed in excess to secure *ad libitum* feed intake, assumed to promote fast growth and maximize utilization of the feed. With regard to delousing, smaller juvenile stages (initial weight of 20 g) are more efficient compared to larger conspecifics (Imsland et al., 2016). Therefore, in order to achieve optimal

Table 4
Chemical composition [%] of whole body of lumpfish fed diets with different inclusion levels of plant protein concentrates.

Parameter	Feeding trial period	Plant protein inclusion levels				p - value
		CTRL	PP25	PP50	PP75	
Dry matter (%)	Start (0 days)	13.33 ± 0.13	13.05 ± 0.10	13.19 ± 0.17	12.89 ± 0.15	0.190
	Continuous phase I (19D)	13.40 ± 0.08	13.29 ± 0.09	13.32 ± 0.05	12.90 ± 0.18	0.138
	Continuous phase II (35D)	13.75 ± 0.12	13.37 ± 0.11	13.24 ± 0.14	13.29 ± 0.16	0.056
	End (54 D)	14.30 ± 0.12	13.79 ± 0.15	14.03 ± 0.08	13.94 ± 0.14	0.057
In dry matter, %						
Crude protein	Start (0 days)	60.47 ± 0.38	60.79 ± 0.34	59.76 ± 0.21	60.50 ± 0.33	0.255
	Continuous phase I (19D)	60.47 ± 0.38	60.89 ± 0.46	61.28 ± 0.12	61.54 ± 0.79	0.474
	Continuous phase II (35D)	61.16 ± 0.46	61.46 ± 0.67	61.75 ± 0.33	62.68 ± 0.77	0.373
	End (54 D)	60.67 ± 0.22^{b}	61.06 ± 0.39^{ab}	62.20 ± 0.24^{a}	61.16 ± 0.17^{ab}	0.012
Crude lipid	Start (0 days)	18.58 ± 0.29	18.17 ± 0.39	18.71 ± 0.46	18.27 ± 0.37	0.731
	Continuous phase I (19D)	17.59 ± 0.05	16.32 ± 0.52	17.29 ± 0.14	16.03 ± 0.66	0.108
	Continuous phase II (35D)	19.26 ± 0.77	18.04 ± 0.22	18.40 ± 0.37	17.14 ± 0.84	0.278
	End (54 D)	21.08 ± 0.44^{a}	19.27 ± 0.46^{b}	18.98 ± 0.38^{b}	20.13 ± 0.30^{ab}	0.006
Ash	Start (0 days)	1.41 ± 0.09	1.31 ± 0.04	1.45 ± 0.12	1.37 ± 0.08	0.670
	Continuous phase I (19D)	1.44 ± 0.14	1.47 ± 0.12	1.44 ± 0.12	1.42 ± 0.15	0.904
	Continuous phase II (35D)	1.62 ± 0.04	1.60 ± 0.01	1.58 ± 0.02	1.62 ± 0.03	0.624
	End (54 D)	1.62 ± 0.02	1.63 ± 0.03	1.66 ± 0.02	1.62 ± 0.02	0.716

CTRL: Control, PP25: 25 % of SPC and PPC inclusion, PP50: 50 % of SPC and PPC inclusion, PP75: 75 % of SPC and PPC inclusion. Values represented as means ± SEM (n = 6 / treatment). Significant differences between treatment groups at the same time point indicated with different superscript letters ($p < 0.05$).

delousing, it is essential to maintain their uniform and slow to moderate growth during the process of co-culture with hosts (Imsland et al., 2018). Fast growth rate is not desirable in lumpfish in the pens with the salmon, because fish larger than 350 g become less interested in louse (Imsland et al., 2014b). Growth performance is, however, established as a parameter to evaluate the efficiency of alternative feed ingredients (Shearer, 2000) and is considered to be an important welfare indicator (Huntingford and Kadri, 2014).

The experimental diets were optimized to be isoenergetic and isoproteic. Assuming that fish were fed to satiation, reduced weight gain for fish fed the PP75 diet may be explained by the reduced utilization of energy or nutrients provided in this diet. Reduced utilization of plant based diets may be explained by ANF's palatability, as well as modified energy metabolism. ANF's such as lectins, saponins, glucosinolate and oligosaccharide are removed from the SPC (Colburn et al., 2012; Drew et al., 2007), while phytate and non-starch polysaccharides (NSP's) are still present in SPC as well as in PPC (Collins et al., 2013; Storebakken et al., 1998). Phytate is known to interfere with mineral absorption and growth (Baeverfjord et al., 2019). Atlantic salmon fed 50 % of untreated SPC diet showed reduced whole body element concentrations (Ca, Mg, and Zn) and a lower apparent digestibility coefficient of the same elements compared to the phytase treated SPC diet (Storebakken et al., 1998). Air classified PPC also contain saponins (Penn et al., 2011), and may result in growth arrest (González-Rodríguez et al., 2016; Tian et al., 2018), associated with histopathology of the distal intestine (Krogdahl et al., 2015). A study with Atlantic salmon fed 35 % air classified PPC in their feed resulted in reduced weight gain, with SGR and enteropathy present in the distal intestine (Penn et al., 2011). Research with the omnivore sharp-snout sea bream (*Diplodus puntazzo*) showed that inclusion of PPC at 160 and 320 g kg^{-1} gave poor growth, and alterations in the distal intestinal morphology associated with ANFs in the PPC (Nogales-Mérida et al., 2016). Hence, plant protein ingredients may be less palatable (Grey et al., 2009), and have a negative effect on feed intake (Kader and Koshio, 2012; Takakuwa et al., 2019). The diets in the present experiment were supplemented with feed attractants in order to enhance their acceptability and growth performance. Nucleotides (Burrells et al., 2001), krill meal (Hatlen et al., 2017; Kousoulaki et al., 2013; Zhang et al., 2012) and soluble fish

Table 5
Fast muscle cellularity of lumpfish; data normalized by total length.

	Start	End (54 D)				p - value
		CTRL	PP25	PP50	PP75	
Fiber number	62659 ± 4645	310233 ± 20243	284228 ± 21793	331334 ± 26872	298406 ± 20353	0.528
D mean	35.2 3 ± 1.18	33.47 ± 0.55^{a}	32.77 ± 0.83^{ab}	32.13 ± 1.11^{ab}	31.48 ± 0.73^{b}	0.047
D median	30.71 ± 1.63	25.97 ± 0.62	25.16 ± 0.79	24.59 ± 0.77	24.89 ± 0.75	0.376
D max	117.66 ± 14.64	171.38 ± 17.07	175.33 ± 21.23	163.72 ± 18.15	142.16 ± 4.98	0.113
D mean of upper 95th percentile	75.79 ± 2.31	81.77 ± 1.95	80.19 ± 2.73	80.12 ± 3.52	78.24 ± 1.18	0.185
Proportion (%) white muscle fibers with						
D ≤ 10 μm	3.80 ± 3.05	6.60 ± 0.97	5.35 ± 0.72	7.13 ± 1.25	6.33 ± 1.14	0.743
10 < D ≤ 30 μm	45.15 ± 7.47	52.54 ± 1.64	54.84 ± 1.57	55.34 ± 1.15	55.97 ± 1.97	0.210
30 < D ≤ 50 μm	30.21 ± 5.88	21.85 ± 1.60	22.67 ± 1.51	21.05 ± 1.36	22.21 ± 1.68	0.854
50 < D ≤ 70 μm	13.98 ± 3.77	11.08 ± 0.79^{a}	9.57 ± 0.71^{ab}	9.06 ± 0.81^{ab}	8.21 ± 0.71^{b}	0.046
70 < D ≤ 90 μm	5.37 ± 1.99	4.13 ± 0.40	4.19 ± 0.36	3.78 ± 0.42	4.03 ± 0.29	0.639
90 < D ≤ 120 μm	1.18 ± 0.99	2.68 ± 0.22	2.37 ± 0.26	2.46 ± 0.26	2.52 ± 0.13	0.607
D > 120 μm	0.29 ± 0.95	1.10 ± 0.22	0.99 ± 0.27	1.72 ± 0.35	0.71 ± 0.13	0.115

CTRL: Control, PP25: 25 % of SPC and PPC inclusion, PP50: 50 % of SPC and PPC inclusion, PP75: 75 % of SPC and PPC inclusion.
Values are represented as mean ± SEM. Raw means for the end of the feeding period with different superscript letters differ significantly ($p < 0.05$).

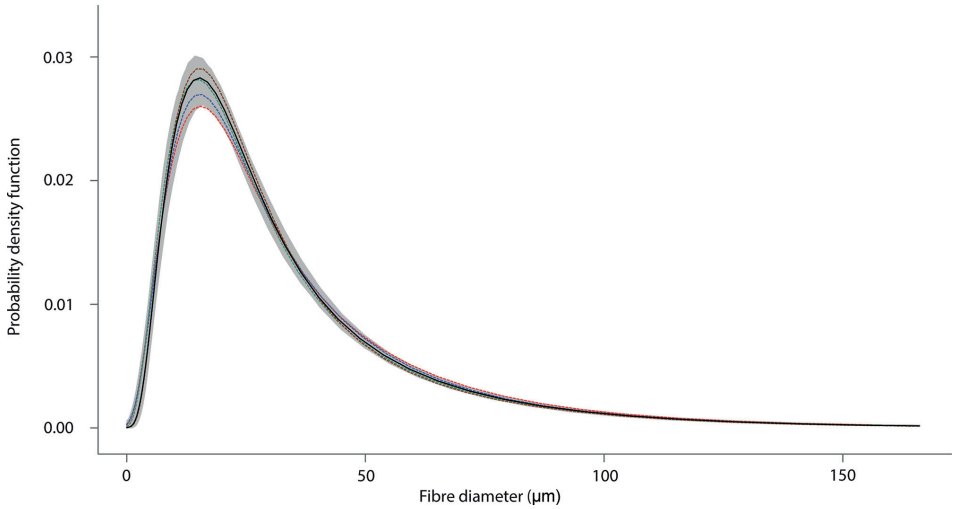

Fig. 2. Bootstrapping analysis comparing all four groups over all fast fiber diameters. Probability functions of mean fiber distribution in juvenile *C. lumpus* fed mix of SPC and PPC diets showing in; red (CTRL), blue (PP25), green (PP50) and brown (PP75) represent in dotted lines. Black solid line represent the overall mean of all four groups. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

protein concentrates (Kousoulaki et al., 2009, 2012) in the diets of Atlantic salmon and rainbow trout are known to have growth promoting effects.

Length-weight relation is used to monitor growth and to evaluate the nutritional status or condition of the fish (Jones et al., 1999). In the present study, length and height were doubled with the 5–6 fold increase in weight. The three dimensional growth pattern suggests that condition factor (CF) should not only be based on weight and length, but also height. The CF commonly reported in the scientific literature is mainly calculated based on Fulton's condition factor (1911), $K = 100\,wt\,(g) \times length\,(cm)^{-3}$. The K values at termination of the experiment were 6.15 ± 0.05, 6.37 ± 0.05, 6.44 ± 0.05 and 6.79 ± 0.06 for the CTRL, PP25, PP50 and PP75, respectively. These K-values were higher than the values of 2.6–4.2 reported earlier for lumpfish (50 % crude protein) (Imsland et al., 2018), suggesting that the fish were in a good nutritional condition. All K values were slightly reduced at termination compared to the start of the experiment, with the highest value noted for the PP75 diet and lowest for the CTRL. This is simillar to results reported by Imsland et al. (2018) were the highest K also was found for the group with lower weight gainIn conjugation with K values, the B^1 showed lower values for the CTRL diet compared to the other three experimental diets. Furthermore, B^1 did not show any decline in value between the start and end of the experiment, suggesting that B^1 may be a more robust measure than the traditional K value and should be considered in future studies with lumpfish. Fish liver is the major organ with respect to nutrient metabolism, producing bile-salts and storing lipid and glycogen (Brusle and Anadon, 1996). Liver size varies a lot among fish species and HSI can range from 1.2 to 1.6 in Atlantic salmon (Gong et al., 2019; Kiron et al., 2016; Sørensen et al., 2017) and up to 9–11 in Atlantic cod (Ingebrigtsen et al., 2014) depending on energy intake (Hatlen et al., 2007). The large liver in cod reflects its importance in storage of lipid; up to 80 % of the lipid content can be found in the liver (Albrektsen et al., 2006). The HSI values in lumpfish in the present experiment were higher than usually found in Atlantic salmon, but still in the lower range of Atlantic cod. The higher HSI in fish fed PP75 is in line with a study performed with juvenile gilthead sea bream, where HSI was higher (0.87 versus 0.80; $p < 0.05$) in fish on a diet where 75 % of FM was replaced with a mixture of corn gluten meal, wheat gluten, extruded peas, rapeseed meal and extruded whole wheat compared with those on FM diet (De Francesco et al., 2007). In contrast, studies with seabass (*Dicentrarchus labrax*) showed no effect on HSI when more than 50 % of FM was replaced with plant protein mixtures in their diets (Kaushik et al., 2004).

4.2. Chemical composition

The higher content of whole body protein in fish fed PP50 compared to those fed the CTRL diet confirm the higher growth of this group, as body protein is a key predictor for gain of body weight (Dumas et al., 2007). A correlation between protein deposition and body weight has also been reported for rainbow trout (Brinker and Reiter, 2011; Dumas et al., 2007). Crude lipid content of whole body decreased below the initial levels after introduction of the experimental diets. This observation indicates that lumpfish were in a negative energy balance and used body lipid during the acclimation period before they fully accepted the experimental diets. Fat seems to be the preferred energy source over protein in anorectic lumpfish.

The whole body lipid content showed minimal increase during the course of the experiment for all diet groups, indicating that lumpfish are not depositing much lipid in body tissues and organs. The low body lipid content should be reflected in the diets of this species. The significantly higher lipid content in lumpfish fed the CTRL compared to groups fed PP25 and PP50 indicated that lipid or energy utilization was affected by incorporation of plant protein concentrates in the diets. Altered lipid metabolism in fish fed plant proteins have been reported in a number of other studies such as Atlantic salmon fed air classified faba bean protein concentrate at 50 to 200 g kg^{-1} (De Santis et al., 2015), gilthead sea bream fed 100 % of rapeseed protein concentrate and 100 % SPC (Kissil et al., 2000), yellow croaker (*Larimichthys crocea*) fed 100 % SPC (Wang et al., 2017), and Senegalese sole (*Solea senegalensis*) fed plant protein mix ranged from 70 to 80% (Silva et al., 2009a). The changes in lipid metabolism may be explained by reduced re-absorption of bile acids (Romarheim et al., 2006; Sørensen et al., 2011) resulting in lower lipid digestibility, and reduced cholesterol in fish

plasma (Dias et al., 2005; Kortner et al., 2013). However, further studies are needed to establish the requirement for lipid in diets, and the relationship between lipid metabolism and plant protein ingredients in juvenile lumpfish.

4.3. Muscle cellularity

The muscle fiber distribution, analysed using both the PDFs and the muscle fiber size classes, illustrates that the growth of juvenile lumpfish mainly takes place through hyperplastic growth. The fast muscle fiber data in all groups showed a similar fiber distribution, being dominated by fast fibers of $< 30\,\mu m$, following a sharp decline in the presence of muscle fibers $> 30\,\mu m$. This is not unique to lumpfish but is ubiquitous, being described in several other juvenile fish species such as Atlantic salmon (Bjørnevik et al., 2003; Higgins and Thorpe, 1990), white seabass (*Atractoscion nobilis*) (Zimmerman and Lowery, 1999), Senegalese sole (Valente et al., 2016), and rainbow trout (Alami-Durante et al., 2010a). The significant differences observed for muscle fibers with diameters between $50 < D \leq 70\,\mu m$ of fish fed PP75 vs. CTRL diet were not supported by the PDFs. Fiber population analysis is a stronger statistical tool than individual measurements (Johnston et al., 1999), suggesting that diet had no effect on the fiber size distribution or fiber number. Similar results have been reported from feed experiments on Atlantic salmon with no or only minor influence on muscle cellularity (Bjørnevik et al., 2003; Johnston et al., 2002). In contrast, fiber analysis showed that the size distribution of fast muscle fibers of juvenile blackspot seabream (*Pagellus bogaraveo*) fed a protein rich diet favored muscle growth by hyperplasia (Silva et al., 2009b).

Relatively few studies have attempted to elucidate the effect of plant protein sources or FM replacement by plant ingredients on muscle cellularity (Alami-Durante et al., 2010a, b; Knutsen et al., 2019). Sensitivity to dietary protein source and amino acid profile was found in juvenile rainbow trout on a diet of high soybean meal inclusion. This resulted in a lower median fiber diameter of white muscle than fish with high wheat and pea inclusions (Alami-Durante et al., 2010b). Clearly then, plant protein ingredients lead to significant changes in reported muscle fiber cellularity (Alami-Durante et al., 2010a). When replacement of FM with a mix of plant protein ingredients at 75–100 % showed a significantly lower median diameter of white muscle fibers in juvenile rainbow trout (Alami-Durante et al., 2010a), this was suggested to be a consequence of increased cathepsin D expression, an enzyme involved in proteolysis. Furthermore, total replacement of FM with rice protein concentrate (RPC) resulted in a decrease in the large diameter ($> 50\,\mu m$) of white muscle fiber of blunt snout bream (*Megalobrama amblycephala*) (Cai et al., 2018). Changes in muscle fiber growth was used to explain the poor growth performance of fish fed RPC in that experiment. However, diets used in the present study were isoproteic, and balanced with essential amino acids; thus poor growth in fish receiving PP75 is not explained by changes in muscle cellularity.

5. Conclusion

Based on the results of the present study, it can be concluded that lumpfish are capable of utilizing mixtures of plant protein concentrates in their diet. The FM in diets for juvenile lumpfish can be replaced with up to 50 % of SPC and PPC without adversely affecting growth performance, body chemical composition, or fast muscle fiber cellularity.

CRediT authorship contribution statement

Florence Perera Willora: Conceptualization, Formal analysis, Investigation, Writing - original draft, Visualization. **Nimalan Nadanasabesan:** Formal analysis, Investigation. **Helene Rønquist Knutsen:** Formal analysis, Validation, Visualization. **Cui Liu:** Investigation. **Mette Sørensen:** Conceptualization, Methodology, Investigation, Resources, Writing - review & editing, Supervision, Project administration. **Ørjan Hagen:** Conceptualization, Methodology, Investigation, Resources, Writing - review & editing, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.aqrep.2020.100352.

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Paper II

Total replacement of marine oil by rapeseed oil in plant protein rich diets of juvenile lumpfish (*Cyclopterus lumpus*): effects on growth performance, chemical and fatty acid composition

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Abstract

Lumpfish is used to control sea lice in open net-pen farming of Atlantic salmon, but little is known about their nutritional requirements. The aim of this study was to investigate the effects of replacing marine oil (MO) with rapeseed oil (RO), in diets incorporating 50% plant protein concentrates, on the growth, chemical and fatty acid (FA) composition of juvenile lumpfish. Four extruded diets, nearly iso-lipidic (14 – 15% DM) and iso-nitrogenous (53 – 54% DM) were produced with either 10% MO (fish oil : krill oil constant proportion 2.3 : 1; Control), or the MO replaced with either 25%, 50% or 100% replacement with RO to give the diets identified as RO25, RO50 and RO100, respectively. Triplicate groups of fish (7 ± 0.18 g) were fed the experimental diets ad libitum during 6 weeks. No significant effects were found on growth parameters, Specific Growth Rate, condition indices, whole body chemical composition or FA profile in liver and whole body when 50% of MO was replaced by RO. Monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) in whole body, liver and muscles were also not affected by the 50% replacement of MO. Total substitution of MO with RO significantly reduced the growth performance, and condition factor, but increased the hepatosomatic index (HSI), and crude lipid in whole body and liver, accompanied by lipid deposition. At the end of the experiment, saturated fatty acids (SFA), PUFA, n-3 FA and eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) in whole body, muscles, and liver decreased ($p<0.05$), while MUFA, and total n-6 FA increased ($p<0.05$) in fish fed RO100. In conclusion, the results of the present study suggest that dietary inclusion of 50% RO in diets where the protein content was derived from marine/plant origin (50/50), did not impair the growth of juvenile lumpfish.

Key words: Robustness, feed ingredients, rapeseed oil, growth, chemical composition, fatty acids

1. Introduction

Lumpfish (*Cyclopterus lumpus*), also known as lumpsucker, are used as a biological means of preventing or reducing sea lice infestations in open net-pen farming of Atlantic salmon (Imsland et al., 2014a; 2014b; 2014c; Powell et al., 2018). This has resulted in a rapid increase in their production, reaching 42.4 million fish in 2019 (Norwegian Directorate of Fisheries, 2019), making lumpfish the second most important aquaculture species in Norway. Increasingly, attention has been paid to the welfare of lumpfish, warranting studies to improve knowledge of fish nutrition and tolerance to plant ingredients to improve fish health. Recent experiments showed that 50% of fishmeal (FM) could be replaced with soy and pea protein concentrate without a negative effect on growth and development (Willora et al., 2020). To our knowledge, no studies have been performed to investigate the replacement of fish oil (FO) with plant oil (PO) in feeds for lumpfish.

Aquaculture is the major user of FO with approximately 73% used for aquafeeds, but the current direct human consumption (17%) is increasing (IFFO, 2018). Fish oil is a unique source of long-chain polyunsaturated fatty acids (LC-PUFA), particularly EPA (C20:5 n-3) and DHA (C22:6 n-3), essential to marine fish and incorporated in feeds to maintain fish growth, health, and physiological functions (Peng et al., 2016; Tocher, 2015; Tocher et al., 2010). The aquafeed industry cannot rely solely on dwindling fisheries resources to supply FO (Chen et al., 2020; Delgado et al., 2003; Gatlin et al., 2007). Other marine derived oils that may become more available in the future are from underutilized species in lower trophic levels, such as mesopelagic fish, copepods (Melle et al., 2004; Olsen et al., 2010; Olsen et al., 2004), and krill (Hewitt et al., 2002; Olsen et al., 2010; Sprague et al., 2017). Antarctic krill (*Euphasia superba*) oil has a higher content of phospholipid-bound n-3 LC-PUFA (Kolakowska et al., 1994; Le Grandois et al., 2009); with a high bio-efficacy and bioavailability than FO, which is dominated by triacylglycerol-bound EPA and DHA (Salem and Kuratko, 2014).

The largest and most widely used oil alternatives for aquafeeds comes from terrestrial plants. Over the past 20 years, a variety of plant oils have been considered as dietary substitutes for MO in feeds for commercially important aquaculture species, representing a more reliable source of production of the bulk ingredient (Naylor et al., 2009; Turchini et al., 2009; USDA, 2020). Rapeseed (*Brassica napus*) is the third most produced PO, after palm oil and soybean oil, being used for both food and nonfood purposes (USDA, 2020; Wu et al., 2019). Global production of RO has reached 26.98 million metric tons (USDA, 2020), and is characterized by substantial levels of MUFA, PUFA and low levels of SFA (7%) (Lewinska et

al., 2015). In RO, oleic acid (OA: C18:1 n-9) is the most abundant FA, accounting for 59%, followed by linoleic acid (LA: C18:2 n-6) (19%) and alpha-linolenic acid (ALA: C18:3 n-3) (9%), but it lacks LC-PUFAs such as EPA and DHA (Turchini et al., 2010). In Norwegian salmon feeds RO together with camelina oil accounts for 19.8% of the bulk content compared to FO derived from forage fish and trimmings from both capture and culture fisheries which makes up 10.4% (Aas et al., 2019). In addition to its incorporation in salmon diets, studies have also investigated the possibilities of replacing FO with RO, either alone or in combination with other POs in diets of several species such as tilapia (*Oreochromis niloticus*) (Peng et al., 2016), carp (Ljubojević et al., 2015; Sun et al., 2011; Yang et al., 2020), European sea bass (*Dicentrarchus labrax*) (Montero et al., 2005), gilthead sea bream (*Sparus aurata*) (Sánchez-Moya et al., 2020), yellow croaker (*Larimichthys crocea*) (Mu et al., 2020), and Senegalese sole (*Solea senegalensis*) (Pereira et al., 2019). The total replacement of FO by POs which are devoid of DHA and EPA poses a major challenge in assuring the recommended levels of such FAs for fish growth (EFSA, 2010). An unfavorable n-6 : n-3 ratio with increasing incorporation of PO may lead to adverse health effects, such as excessive lipid deposition in the liver, resulting in an alteration of liver morphology and functions (Boonanuntanasarn et al., 2019; Peng et al., 2014; Torrecillas et al., 2017), as well as arresting growth (Bou et al., 2017a) and promoting inflammation in the distal intestine (Bou et al., 2017b; Moldal et al., 2014).

The aim of the present study was to investigate the effect of replacing MO (fish oil : krill oil constant proportion 2.3 : 1) with 25, 50 or 100% RO in feeds where 50% of the protein was derived from plant protein concentrates. Growth performance, FA, and chemical composition of whole body and tissues were evaluated during a 6 week feeding trial.

2. Materials and Methods

2.1 Ethics statement

The feeding trial was approved by the ethics and animal welfare committee at Nord University, Norway. All fish handling protocols comply with guidelines under the Norwegian animal welfare act (LOV-2009-06-19-97) and European Union act (EU/2010/63). MS-222 (Tricaine methane sulphonate; Argent Chemical Laboratories, USA; 30 g /L) was used to anesthetize the animals before handling or euthanasia; the latter administered by a sharp blow to the head.

2.2 Experimental diets and feeding trial

Four experimental diets were formulated to be nearly iso-lipidic (14 – 15% DM) and iso-nitrogenous (53 – 54% DM). Feed ingredient composition, the analyzed proximate composition, and the FA profiles of experimental diets are presented in Tables 1 and 2 respectively. The protein and carbohydrate ingredients were constant and the feed differed in the inclusion of RO from 0 (control, CTRL) to the three experimental diets consisting of 25% (RO25), 50% (RO50) and 100% (RO100) replacement of the MO used in the CTRL diet. The key protein ingredients were FM, soy protein concentrate, pea protein concentrate, and wheat gluten in diets supplemented with L-tryptophan, DL-methionine, L-taurine and L-histidine to balance essential amino acids. Experimental diets were manufactured by SPAROS Lda. (Olhao, Portugal). All dry ingredients were mixed in a double-helix mixer (model RM90, MAINCA Spain), passed through a 0.4 mm micro-pulverizer hammer mill (model SH1, Hosokawa-Alpine, Germany). Diets were extruded using a twin-screw extruder (model BC45, Clextral, France) with a 1.5 mm die; extruded pellets were dried in a vibrating fluid bed dryer (model DR100, TGC Extrusion, France). Oil fraction was subsequently added under vacuum conditions in a Pegasus vacuum coater (model PG-10VCLAB, Dinnissen, Netherlands) at room temperature. Experimental diets were stored under chilled conditions until used.

The feeding trial was conducted at Nord University research station, Faculty of Biosciences and Aquaculture (FBA). The four feeds were randomly assigned to triplicate tanks (n = 3 / feed group), each equipped with an automatic feeder (ArvoTec, Sterner, Norway). Fish were fed the experimental diets over a period of 6 weeks to apparent satiation with a feeding rate of 2.5% of their body mass, eight times a day between 06:00 to 21:00.

2.3 Lumpfish and experimental set-up

Juvenile lumpfish of 4g initial mean body weight were provided by Mørkvedbukta AS, Bodø, Norway. The fish were randomly allocated into 12 indoor rearing tanks (500 L) with 200 fish per tank and acclimated to laboratory conditions for 16 days before the start of the feeding trial, during which time they were fed a commercial diet (Gemma Silk, Skretting, Stavanger, Norway). During acclimation fish grew to approximately 7 ± 0.18 g for all groups. Tanks were supplied with constant seawater flow (500 l / h) with water drawn from a depth of 250 m from Saltenfjorden. The average salinity was 34‰ and the oxygen level remained above 86.7 ± 0.11%, with an average temperature of 7.6 ± 0.9°C. Light intensity was controlled by four florescent lamps (24 h) (Grunda Viktor work lamps, 38 watt, luminous flux 1350 lm) facing upwards to provide similar light conditions to those in commercial lumpfish farms. Critical physical and chemical parameters; temperature, salinity and dissolved oxygen were monitored daily.

2.4 Sample collection

At start and termination of the experiment, all fish were anesthetized before individual body weight (g), length, and height (cm) were measured. Additionally, liver and visceral weights were recorded during the course of the experiment; at the start and after 3 and 6 weeks. A total of 28 fish per tank were sampled and stored at −40^{o}C for subsequent whole body chemical composition and FA analysis. Samples of muscle (dorsal loin from left fillet) and liver from 10 fish per tank were also collected for determination of chemical composition and FA profile.

2.5 Sample preparation for chemical and fatty acid analyses

Whole body, liver, and muscle samples were thawed and divided into two groups of pooled samples, containing 14 whole fish, 5 livers and 5 muscle samples per pool (n= 6 pooled samples / feed group). Fish were homogenized and part of this homogenate used to determine the moisture and ash content. Liver, muscle, and feed samples were also homogenized and freeze dried for 72 hours at −70^{o}C using a VirTis benchtop K Mod (SP industries, Warminster, U.S.A) and dry matter recorded.

2.6 Chemical analyses

All chemical analyses followed standard methods. Experimental diets and tissue samples were performed in triplicate and duplicate respectively. In brief, moisture content was determined by drying whole fish (2.0 g) and feed (5.0 g) samples to a constant weight at 104°C for 20 hours (ISO 6496-1999). Whole fish samples were combusted in a muffle furnace to a constant weight at 540°C for 16 hours to determine the ash content at the FBA; the feed was analysed by Eurofins (Moss, Norway) (ISO 5984-2002). Crude protein of whole body (0.5 g), feed (0.5 g), and liver were determined by the Kjeldahl titration method (N x 6.25, KjeltecTM 2300, Foss Tecator AB, Höganäs, Sweeden ISO 5983-1987). Crude fat in whole body (2.0 g), feed (5.0 g), and liver (0.2 g) were determined gravimetrically using the diethyl ester extraction method, according to the Norwegian Standard Association (1994). Also energy in feed and whole body were analysed using a bomb calorimeter (IKA C200, Staufen, Germany: ISO 9831-1998).

2.7 Fatty acid analysis

An optimum total lipid extraction of freeze dried feeds, whole body, liver, and muscle (n= 6 pooled samples / feed group) samples was carried out according to the chloroform and methanol gravimetric determination described by Bligh and Dyer (1959). All analyses were performed in triplicate (feed) and duplicate (tissues). Briefly, homogenization of freeze dried samples was carried out by mixing 1.8 ml of distilled water, 2 ml of methanol, and 1 ml of chloroform followed by addition of 1ml of chloroform and 1ml of distilled water. Samples were then centrifuged (4000 rpm). The lower chloroform phase containing lipids was transferred into a Kimax tube and dried under a gentle nitrogen flow to prevent FA oxidation. Fatty acid methyl esters (FAMEs) of samples were obtained by transesterification and methylation according to the AOCS Official Method Ce 1b-89. FAMEs analyses were performed in a gas chromatograph, (SCION 436-GC) fitted with a flame ionization detector, at 250 °C in duplicate. Separation was achieved using a wax embedded column of 25m length, 0.25 mm internal diameter, and 0.2 µm film thickness (Agilent Technologies). Standard mixtures of FAMEs were used for identification and quantification of common FAs in samples (FAME MIX 2/GLC-473, Nu-Chek Prep, Elysian, MN, USA) and quantified using the relative percentage area of the total FA using Compass CDS, Bruker Co-operation software.

2.8 Calculations

Condition factor was calculated according to the formulae B^1 and K proposed by Richter et al. (2000) and Fulton, (1911). B^1 (g cm^{-3}) = fish weight (g) / [fork length (cm) x body height

233 ^{2} cm. K (g cm^{-3}) = [fish weight (g) / fork $length^{3}$ (cm)] × 100. Somatic indices and Specific
234 Growth Rate (SGR) were calculated employing the following formulae: Hepatosomatic index
235 (HSI) = [liver weight (g) / fish weight (g)] × 100. Visero-somatic index (VSI) = [visceral weight
236 (g) / fish weight (g)] × 100. SGR (% day^{-1}) = 100 × ln [final mean weight (g) - initial mean
237 weight (g)] / number of feeding days.

238

239 ## 2.9 Statistical analysis

240 All statistical analyses were performed and graphs generated using Sigmaplot 14.0 (Systat
241 software, San Jose, CA). Data were tested for normality (Shapiro-Wilk test) and
242 homogeneity of variances (Brown-Forsythe F-test). Individual means were compared by
243 one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test. A
244 Kruskal-Wallis one-way analysis of variance on ranks, followed by Tukey's multiple
245 comparison test, was used for nonparametric data. Dunn's pairwise multiple comparison
246 test was used only to assess the significance of the unequal size of growth-related data at
247 the end of the experiment. All data were presented as means ± SE (standard error), and
248 differences were considered significant only if their p-value was < 0.05. Correlation of
249 selected main FAs present in the whole body, liver, and muscles of the dietary groups with
250 the FAs in their respective feeds were determined using Pearson's correlation coefficient
251 (r). The different strengths of (r) were defined as very high, high, moderate, low and
252 negligible (Mukaka, 2012).

253

254 # 3. Results

255 ## 3.1 Growth performance and somatic indices

256 Biometric parameters, condition factor, and somatic indices measured during the feeding
257 trial are presented in Table 3. No mortalities occurred during the experiment and all fish
258 appeared healthy. Fish grew from an average of 7 g to 34 − 39 g over the 6 week feeding
259 trial. Body weight, length, and height of fish showed significant differences among fish fed
260 the experimental diets. The indices of the RO100 group were significantly lower than all
261 other groups, while no differences were observed among the other three groups at the mid-
262 and end-points of the experiment ($p<0.05$). The lower weight gain of fish fed RO100 was
263 characterized by a tendency towards a lower SGR ($p=0.09$). HSI was slightly, but significantly,
264 higher in fish fed RO100 diet at the end of the experiment but the VSI remained unaffected.
265 Condition factor (B^{1}), was significantly lower in fish fed RO100 than CTRL and RO25 at the
266 end of the experiment.

3.2 Chemical composition of whole body and liver

Chemical composition of the whole body and liver are presented in Table 4. Whole body moisture, crude protein, crude lipid, and ash remained unaffected by dietary treatment up to week 3 of the feeding trial. At the end of the trial, whole body moisture and lipid were slightly, but significantly higher in the RO100 group, with a significantly lower protein level compared to the control diet. Ash content was lower in RO50 fed fish compared to the other three diets ($p < 0.05$). Whole body energy content was similar among all groups ($p>0.05$). Changes in liver protein and lipid followed a similar trend as whole body protein and lipid at the end of the experimental period. The liver lipid content increased in the RO100 group compared to other dietary groups, and protein content was reduced compared to the CTRL diet ($p<0.05$).

3.3 Fatty acid composition

Fatty acid composition of the whole body, liver, and muscle are given in Table 5. Total SFA, PUFA, n-3, and amount of EPA and DHA of whole body, liver, and muscle were significantly different in all treatments, with the highest value in fish fed CTRL and the lowest in those fed 100% RO ($p<0.05$), reflecting the FA profile of each feed. Whole body FAs in fish fed RO25, RO50 and RO100 diets were dominated by MUFA (37 − 51%), followed by PUFA (34 − 38%), and SFA (13 − 19%). Total MUFA and n-6 was higher in fish fed RO100 compared to the CTRL group ($p<0.05$). Muscle FAs showed a similar trend as whole body FA, while FA of the liver in all experimental feeds was dominated by MUFA (47 − 60%), followed by PUFA (32 − 33%), and SFA (12 − 19%). SFA in whole body, muscles and liver comprised mostly of myristic acid (C:14), palmitic acid (C16:0) and stearic acid (C:18), and were reduced with increasing levels of RO in the diets ($p<0.05$). Palmitic acid represented the majority of SFA and was lower in fish fed RO100 diet compared to the CTRL ($p<0.05$). MUFA was the dominant lipid class in whole body and muscle for all experimental groups fed RO, and the dominating fatty acid OA (C18:1 n-9) was higher in fish fed RO100 compared to the CTRL group ($p<0.05$). PUFAs were the second most prevalent FAs and were dominated by LA, ALA, EPA, and DHA. Rapeseed oil in the feed increased LA and ALA and reduced the content of EPA and DHA ($p<0.05$), in the whole body, liver and muscles ($p<0.05$). The n-3 : n-6 ratios were higher in fish fed CTRL diet compared to RO100 ($p<0.05$). The different FAs (PA, OA, LA, ALA, EPA, and DHA) and total amounts of SFA, MUFA and PUFA measured in whole body, liver and muscles, correlated ($r = 0.69 − 0.99$) with the contents of experimental diets (Fig. 1). A moderate positive correlation was noted between dietary and liver PUFA ($r=0.69$, $p=0.03$), while the other FA classes showed very high positive correlations ($r>0.99$, $p<0.01$).

4. Discussion

4.1 Growth performance

Lipid is essential in fish diets to provide energy and essential FAs. The diets were formulated to be iso-proteinic and iso-lipidic and the differences noted in weight gain and SGR is therefore explained by changes in FA composition. The long-chain PUFAs, EPA and DHA, were remarkably reduced with increasing levels of RO in the feed (Table 2). The dietary requirement of EPA and DHA for juvenile lumpfish is not known, but the present study indicated that RO100 had too low a level. In the present study, fish fed the CTRL, RO25 and RO50 showed no differences in growth, suggesting that dietary ∑PUFA levels in the range 2.1% – 3.2%, correspond to 29.5% and 31.8% of total FAs, satisfy the nutrient requirement of juvenile fish growth. Full replacement of MO with RO resulted in lower final body weight and SGR, suggesting too low a level of essential FAs to support growth. Growth arrest is reported in fish fed diets deficient in EPA and DHA (Bou et al., 2017b; Tocher et al., 2010) and has been reported for a number of species such as silver perch (*Bidyanus bidyanus*) (Smith et al., 2004), yellow tail king fish (*Seriola lalandi*) (Bowyer et al., 2012), Atlantic salmon (Bell et al., 2001) and fingerling black carp (*Mylopharyngodon piceus*) (Sun et al., 2011), sea bream (Benedito-Palos et al., 2008) and yellow croaker (Mu et al., 2020). The optimal replacement of MO with RO was not determined in this experiment, but studies with other species have shown that growth was unaffected by substituting FO with RO up to 60% in sea bass (Mourente et al., 2005), 75% in gilthead sea bream (Izquierdo et al., 2005; Sánchez-Moya et al., 2020), 50% in Atlantic salmon (Rosenlund et al., 2001), and 70% in red sea bream (*Pagrus major*) (Huang et al., 2007).

4.2 Condition factor and somatic indices

The condition factor was calculated with both Fulton's condition factor (K) and by the alternative B^1, taking into consideration the three dimensional growth pattern of lumpfish. The K-values were higher than the values of 4.3 to 4.8 reported earlier for lumpfish fed with commercial feed containing 50% crude protein and 10% lipid (Imsland et al., 2020), suggesting that the fish were in a good nutritional condition. However, the K values presented in the present study showed the highest value for fish fed the RO100 diet, while the lowest value was found for the CTRL. The B^1 showed significantly lower values for the RO100, coinciding with lower growth found for the RO100 group compared to CTRL, suggesting that B^1 may be a more robust measure than the traditional K value and should be considered in future studies of lumpfish.

The present study showed a significantly higher HSI for fish fed RO100 diet, which agrees with former studies reporting a trend of increasing HSI when FO was totally replaced by RO in diets in aquaculture species (Bowyer et al., 2012; Fountoulaki et al., 2009; Mu et al., 2020; Sun et al., 2011). HSI value correlates with fat deposition (Gao et al., 2012). Increasing fat deposition is associated with decreasing n-3 : n-6 ratios reported in other studies (Kjær et al., 2008a; Reis et al., 2014) and may have adverse effects on both liver morphology and function (Boonanuntanasarn et al., 2019; Peng et al., 2014; Torrecillas et al., 2017). Lipid deposition in the liver is a complex process, including hepatic secretion, oxidation, transport and uptake of lipid (Kjær et al., 2008a; Vegusdal et al., 2005); many key enzymes and transcription factors, such as peroxisome proliferator-activated receptors (*PPAR-α*, *PPAR-β*, *PPAR-γ;* Burri et al., 2010; Kjær et al., 2014; Li et al., 2016) are involved in this process. The PPARγ is a key transcription factor for differentiation, lipogenesis, and is involved in lipid deposition in hepatocytes (Poulsen et al., 2012). A previous study in European seabass (*Dicentrarchus labrax*) reported that n-3 LC-PUFA deficiency increased the expression of *PPAR* (Vagner et al., 2009), promoting lipid synthesis and deposition (Burri et al., 2010). The same mechanism is likely to explain the increased lipid accumulation in the liver of juvenile lumpfish fed RO100 diet, which had a 2.5 times lower n-3 LC-PUFA content than the CTRL.

4.3 Fatty acid and chemical composition of whole body, liver and muscles

Tissue FA composition is known to be affected by diet in fish at all stages of their life cycle (Olsen and Skjervold, 1995). The SFA and PUFA in all analyzed tissues showed a linear decrease with RO incorporation (CTRL > RO25 > RO50 > RO100). The relatively low deposition of SFAs C16:00 and C14:00 is because these FAs are the preferred substrate for β-oxidation over MUFA and PUFA, respectively, depending on FA availability (Tocher et al., 2003; Turchini et al., 2009).

It is well known that some organs have the ability to retain EPA or DHA to a greater extent (Thomassen et al., 2017). In this study, muscle and whole body seemed to have a selectively higher deposition of DHA than EPA. High retention of DHA in lumpfish muscles corroborates with other studies on salmonids (Bell et al., 2001; Bell et al., 2003a; Caballero et al., 2002; Torstensen et al., 2004), Senegalese sole (Pereira et al., 2019), sea bream and sea bass (Fountoulaki et al., 2009; Montero et al., 2005). The effect of different dietary levels of EPA and DHA on salmon tissue composition was explained by Bou et al. (2017a); fish fed with EPA as the main source of n-3 led to retention values of DHA above 100%, indicating net synthesis of this FA in the body. However, DHA as the main source of dietary n-3, regardless of level, increased the cellular DHA level only about 70%. This suggests that EPA is less

conserved than DHA due its different biological functions; such as conversion to DHA, and metabolization into eicosanoid compounds and/or energy production through β-oxidation, whereas dietary DHA is more resistant to β-oxidation (Bou et al., 2017a; 2017b; 2017c; Rosenlund et al., 2016; Thomassen et al., 2012).

The higher lipid level in whole body and liver in the present study are in line with fish fed RO either as a single source or in combination with other PO in Seneglase sole (Pereira et al., 2019), large yellow croaker (Mu et al., 2020), black carp (Sun et al., 2011) and Atlantic salmon (Bell et al., 2003b; Kjær et al., 2008a; Todorčević et al., 2008). Liver is the key organ in FA metabolism, facilitating the FA entrance, synthesis and disposal (Hodson et al., 2011). Deposition of SFA in liver followed a similar pattern as muscles and whole body. The OA, LA and ALA in feeds and deposition in liver showed a linear increase with incorporation of RO (CTRL < RO25 < RO50 < RO100). At the end of the experimental period, these FAs in liver of fish fed RO100 diet was higher compared to those in whole body and muscles. The relatively higher retention of OA, LA and ALA in liver is in agreement with previous reports of fingerling black carp (Sun et al., 2011) and Senegalese sole (Pereira et al., 2019) fed diets containing RO. Both whole body and muscle seemed to have a selective retention of DHA in the present study, while EPA seemed to be retained in the liver. This suggests selective retention of the essential n-3 PUFA differs in various tissues.

Excess dietary FAs are exported from the liver in the form of lipoproteins, accumulated and stored in the form of TAG in target lipid storage sites (Tocher et al., 2003). Studies with Atlantic salmon has shown an increase in neutral lipids such as TAG (Bou et al., 2017b; Ruyter et al., 2006; Todorčević et al., 2008) and glycerolipids (Kjær et al., 2008a; Vegusdal et al., 2005) in the liver with decreasing levels of EPA and DHA. In contrast, increasing levels of n-3 FAs may reduce TAG synthesis, and three possible mechanisms involved in the lowering effect were discussed by Kjær et al. (2008b). Moreover, diets short of EPA and DHA stimulate the n-6 pathway by increasing the levels of 20:3n-6 and 20:4n-6 in the polar lipid (phospholipid) fraction of hepatocytes (Bou et al., 2017c). Increased lipid deposition in fish fed the RO100 diet in the present study is most likely explained by too low EPA and DHA levels in the RO100 diet and stimulation of lipogenesis via the activation of PPAR (Burri et al., 2010; Valenzuela et al., 2011).

Increased lipid deposition may also be explained by increasing levels of OA and LA in the experimental diets when MO was replaced with RO. Fish fed 100% RO diet received 3 and

2 fold higher OA and LA respectively, compared to those fed the CTRL. A study with large yellow croaker showed that increased dietary LA induced hepatic lipid accumulation (Mu et al., 2018). Increasing ratio of OA : n-3 HUFA may also give increased lipid deposition in salmon hepatocytes and more OA were deposited in TAGs than EPA and DHA in all differentiated stages of adipocytes (Todorčević et al., 2008). These findings indicate reduced levels of n-3 HUFA in fish diets, when the traditional FO is replaced by n-6 and n-9 FA rich PO.

Following termination of the experiment, crude protein in whole body and liver for fish fed the RO100 group was significantly lower, compared to CTRL; as these fish also had a significantly higher crude lipid, the lower protein content can just as well be a result of the composition changes and not the dietary oil effect.

Conclusion

Total substitution of MO with RO significantly reduced growth performance and condition factor concurrent with an increase in whole body and liver fat. The FA composition of the whole body, muscle and liver also reflected changes in the feed as MO was replaced with RO. In conclusion, the results of the present study suggest that dietary inclusion of 50% RO in diets where the protein content was derived from marine / plant origin (50/50), did not have adverse effect on growth. A significant increased deposition of fat in the liver may suggest that the optimal RO level is lower.

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716 ## Figure legends

717 **Figure 1**: Relationship between dietary FA level (black, dashed) and their respective FA
718 levels in the liver (blue) whole body (red) and muscle (green) of palmitic (C16:0), oleic (C18:1
719 n-9), linoleic (18:2n-6), alpha-linolenic (18:3n-3), EPA (20:5n-3) and DHA (22:6n-3), as well
720 as total amounts of SFA, MUFA and PUFA in juvenile lumpfish fed with CTRL, OR25, OR 50
721 and OR100. TFA = Total Fatty Acids, r= Pearson's correlation coefficient, p = significant
722 relationship between tissue FA and their respective dietary FA in the correlation ($P > 0.05$).
723 Data are represented as mean ± SEM. Standard error bars are plotted but some are within
724 the boundaries of the data points. r* and p* values for PUFA are only valid for whole body
725 and muscle while values for liver are presented in the text body.

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729 **Table 1**: Ingredient composition (g 100g-[1]) and analyzed proximate composition (%) of
730 the experimental diets on an as fed basis. Values are expressed as mean of triplicate
731 samples per diet.

Ingredients	CTRL	RO25	RO50	RO100
Fish meal [1]	29.00	29.00	29.00	29.00
Soy protein concentrate [2]	14.45	14.45	14.45	14.45
Pea protein concentrate [3]	14.45	14.45	14.45	14.45
CPSP 90 [4]	2.50	2.50	2.50	2.50
Krill meal [5]	5.00	5.00	5.00	5.00
Wheat gluten [6]	7.00	7.00	7.00	7.00
Wheat meal [7]	6.95	6.95	6.95	6.95
Pea starch [8]	5.35	5.35	5.35	5.35
Fish oil [9]	7.00	5.28	3.52	0.00
Krill oil [10]	3.05	2.26	1.51	0.00
Rapeseed oil [11]	0.00	2.51	5.03	10.05
Vit & Mineral Premix [12]	1.00	1.00	1.00	1.00
Lutavit E50 [13]	0.05	0.05	0.05	0.05
Antioxidant powder [14]	0.20	0.20	0.20	0.20
Sodium propionate [15]	0.10	0.10	0.10	0.10
MCP [16]	0.98	0.98	0.98	0.98
Carophyll Pink [17]	0.05	0.05	0.05	0.05
Nucleotides [18]	0.50	0.50	0.50	0.50
Garlic extract [19]	0.50	0.50	0.50	0.50
L-Histidine [20]	0.25	0.25	0.25	0.25
L-Tryptophan [21]	0.17	0.17	0.17	0.17
DL-Methionine [22]	0.35	0.35	0.35	0.35
L-Taurine [23]	1.10	1.10	1.10	1.10
Proximate composition				
Dry matter	95.4	96.5	97.2	97.8
As fed %				
Crude Protein	52.9	53.7	54.0	53.9
Crude lipid	14.3	14.8	14.8	15.1
Ash	8.5	8.5	8.6	8.7
Energy (kJ / g)	20.8	21.0	21.3	21.7

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[1] NORVIK LT 70 : 70.3% crude protein (CP) 5.8% crude fat (CF) (Sopropêche, France).

[2] Soycomil : 63% CP, 0.8% CF (ADM, The Netherlands). [3] Lysamine GPS: 78% CP, 0.9% CF (Roquette Frères, France). [4] Soluble fish protein hydrolysate: 82.6% CP, 9.6% CF (Sopropêche, France). [5] 61.1% CP, 17.4% CF (Aker Biomarine, Norway). [6] VITAL: 83.7% CP, 1.6% CF, (Roquette, Frères, France). [7] 10.2% CP; 1.2% CF (Casa Lanchinha, Portugal). [8] NASTAR 90% starch, (Cosucra, Belgium). [9] (SAVINOR UTS, Portugal). [10] (Aker Biomarine, Norway). [11] Henry Lamotte Oils (GmbH, Germany). [12] Vitamins (IU or mg kg^{-1} diet): DL-alpha tocopherol acetate, 100 mg; sodium menadione bisulphate, 25 mg; retinyl acetate, 20000 IU; DL-cholecalciferol, 2000 IU; thiamin, 30 mg; riboflavin, 30 mg; pyridoxine, 20 mg; cyanocobalamin, 0.1 mg; nicotinic acid, 200 mg; folic acid, 15 mg; ascorbic acid, 1000 mg; inositol, 500 mg; biotin, 3 mg; calcium panthotenate, 100 mg; choline chloride, 1000 mg, betaine, 500 mg. Minerals (g or mg kg-1 diet): cobalt carbonate, 0.65 mg; copper sulphate, 9 mg; ferric sulphate, 6 mg; potassium iodide, 0.5 mg; manganese oxide, 9.6 mg; sodium selenite, 0.01 mg; zinc sulphate,7.5 mg; sodium chloride, 400 mg; calcium carbonate, 1.86 g; excipient wheat middlings (PREMIX Lda, Portugal).

[13] (ROVIMIX E50, DSM Nutritional Products, Switzerland). [14] Paramega PX (Kemin Europe NV, Belgium) [15] Disproquímica (Portugal). [16] ALIPHOS MONOCAL, 22.7% P (ALIPHOS, Belgium) [17] Carophyll Pink 10% CWS (DSM Nutritional Products, Switzerland). [18] Nucleoforce Salmonids (BioIbérica, Spain). [19] Macrogard, 67.2% beta-glucans (Biorigin, Brazil). [20] L-Histidine 98%, (Ajinomoto Eurolysine SAS, France). [21] L-Tryptophan 98%, (Ajinomoto Eurolysine SAS, France). [22] DL-Methionine for aquaculture 99%, (EVONIK Nutrition & Care GmbH, Germany). [23] L-Taurine 98%, (ORFFA, The Netherlands).

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770 **Table 2**: Fatty acid composition of the experimental diets

Fatty acid (%)	CTRL	RO25	RO50	RO100
Saturates (SFAs)				
C14:00	6.81 ± 0.11	5.41 ± 0.07	4.02 ± 0.02	1.63 ± 0.02
C16:00	22.03 ± 0.38	18.25 ± 0.15	15.03 ± 0.13	9.23 ± 0.07
C18:0	3.98 ± 0.02	3.42 ± 0.01	3.01 ± 0.03	2.20 ± 0.02
∑SFAs [1]	32.82 ± 2.97	27.08 ± 2.46	22.06 ± 2.04	13.06 ± 1.29
Monounsturates (MUFAs)				
C16:1: n-9	5.92 ± 0.03	4.71 ± 0.43	3.49 ± 0.01	1.39 ± 0.01
C18:1 n-9 (OA)	16.10 ± 0.08	25.70 ± 0.04	34.67 ± 0.17	49.30 ± 0.13
C18:1 n-7	4.40 ± 0.02	4.17 ± 0.02	4.05 ± 0.44	3.66 ± 0.01
C20:1 n-11	3.97 ± 0.04	3.27 ± 0.29	3.12 ± 0.23	2.37 ± 0.12
C22:1 n-11	4.61 ± 0.03	3.75 ± 0.03	3.02 ± 0.02	1.77 ± 0.01
∑MUFAs[2]	35.00 ± 1.27	41.6 ± 2.41	48.35 ± 3.45	58.49 ± 5.18
Polyunsturates (PUFAs)				
C18:2 n-6 (LA)	9.32 ± 0.05	12.61 ± 0.04	15.11 ± 0.01	19.65 ± 0.13
C18:3 n-3 (ALA)	1.84 ± 0.01	3.09 ± 0.02	4.04 ± 0.07	5.80 ± 0.07
C20:5 n-3 (EPA)	9.33 ± 0.09	7.08 ± 0.02	4.79 ± 0.11	1.70 ± 0.03
C22:6 n-3 (DHA)	9.15 ± 0.12	6.68 ± 0.09	4.30 ± 0.17	1.41 ± 0.04
∑PUFA[3]	31.84 ± 0.98	31.27 ± 1.04	29.53 ± 1.31	28.56 ± 2.07
∑n-3 [4]	22.52 ± 1.13	18.66 ± 0.71	14.42 ± 0.43	8.91 ± 0.64
∑n-6 [5]	9.32 ± 0.05	12.61 ± 0.04	15.11 ± 0.01	19.65 ± 0.13
n-3/n-6 [6]	2.42	1.48	0.95	0.45
EPA + DHA [7]	18.48 ± 0.07	13.76 ± 0.11	9.09 ± 0.15	3.11 ± 0.75

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775 Values are expressed as mean value ± SEM of triplicate samples per diet.
776 1. Σ SFA is the sum of saturated fatty acids.
777 2. Σ MUFA is the sum of monounsaturated fatty acids.
778 3. Σ PUFA is the sum of polyunsaturated fatty acids.
779 4. n-3 is the sum of n-3 polyunsaturated fatty acids, includes C18:4
780 5. Σ n-6 is the sum of n-6 polyunsaturated fatty acids,
781 6. n-3/n-6 is the ratio of Σ n-3 and Σ n-6.
782 7. Sum of EPA and DHA
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Table 3: Growth parameters and condition indices of lumpfish fed diets with different levels of rapeseed oil.

Parameter	Feeding trial period	CTRL	RO25	RO50	RO100	p - Value
Growth parameters						
Body weight (g)	Start	6.68 ± 0.17	6.93 ± 0.18	6.89 ± 0.18	6.78 ± 0.18	0.177
	Mid (3 W)	19.34 ± 0.41^{b}	19.52 ± 0.42^{b}	19.13 ± 0.34^{b}	17.18 ± 0.41^{a}	< 0.001
	End (6 W)	38.86 ± 0.48^{b}	39.05 ± 0.45^{b}	39.76 ± 0.49^{b}	34.25 ± 0.45^{a}	< 0.001
Body length (cm)	Start	4.41 ± 0.04	4.44 ± 0.04	4.45 ± 0.04	4.43 ± 0.04	0.711
	Mid (3 W)	6.17 ± 0.05^{b}	6.10 ± 0.04^{b}	6.13 ± 0.04^{b}	5.90 ± 0.05^{a}	< 0.001
	End (6 W)	8.49 ± 0.04^{b}	8.54 ± 0.03^{b}	8.53 ± 0.04^{b}	8.08 ± 0.04^{a}	< 0.001
Body height (cm)	Start	2.23 ± 0.02	2.25 ± 0.03	2.25 ± 0.03	2.22 ± 0.03	0.185
	Mid (3 W)	3.50 ± 0.03^{b}	3.53 ± 0.03^{b}	3.53 ± 0.03^{b}	3.38 ± 0.03^{a}	0.050
	End (6 W)	4.25 ± 0.02^{b}	4.27 ± 0.02^{b}	4.34 ± 0.02^{b}	4.16 ± 0.02^{a}	0.001
SGR (% day $^{-1}$)	Start	n.a	n.a	n.a	n.a	-
	Mid (3 W)	4.29 ± 0.22	4.33 ± 0.10	4.29 ± 0.10	3.80 ± 0.44	0.298
	End (6 W)	3.81 ± 0.07	3.86 ± 0.02	3.83 ± 0.09	3.55 ± 0.16	0.090
Condition indices						
HSI	Start	2.26 ± 0.04	2.43 ± 0.04	2.63 ± 0.06	2.35 ± 0.04	0.046
	Mid (3 W)	2.11 ± 0.05	2.10 ± 0.04	2.24 ± 0.89	2.01 ± 0.05	0.249
	End (6 W)	2. 23 ± 0.07^{b}	2.25 ± 0.05^{b}	2.27 ± 0.05^{b}	2.54 ± 0.05^{a}	0.001
VSI	Start	15.08 ± 0.16	15.58 ± 0.09	15.91 ± 0.11	15.21 ± 0.12	0.163
	Mid (3 W)	14.65 ± 0.25	15.36 ± 0.27	14.84 ± 0.33	15.17 ± 0.25	0.050
	End (6 W)	14.19 ± 0.17	14.05 ± 0.26	14.18 ± 0.28	14.52 ± 0.24	0.545
CF, K (g cm^{-3})	Start	8.33 ± 0.97	8.04 ± 0.38	7.69 ± 0.15	7.72 ± 0.22	0.119
	Mid (3 W)	8.15 ± 0.09^{a}	8.47 ± 0.08^{b}	8.20 ± 0.07^{ab}	8.23 ± 0.07^{ab}	0.023
	End (6 W)	6.31 ± 0.05^{b}	6.22 ± 0.04^{b}	6.35 ± 0.04^{ab}	6.47 ± 0.07^{a}	0.002

CF, B^1 (g cm^{-3})	Start	0.30 ± 0.0022	0.31 ± 0.0025	0.30 ± 0.0015	0.30 ± 0.0017	0.061
	Mid (3 W)	0.25 ± 0.0022^{ab}	0.25 ± 0.0022^{a}	0.24 ± 0.0014^{b}	0.24 ± 0.0016^{ab}	0.034
	End (6 W)	0.25 ± 0.0016^{a}	0.25 ± 0.0010^{a}	0.24 ± 0.0011^{b}	0.24 ± 0.0001^{b}	0.001

Values represented as means ± SEM. Growth parameters and CF for week 0 are based on measurements of all fish. Similarly, growth parameters and CF at the end of the feeding trial are based on both fish sampled and fish remaining after 42 days. Significant differences between treatment groups at the same time point are indicated by different superscript letters ($p < .05$).

Table 4: Chemical composition of the whole body and liver of lumpfish fed diets with different inclusion levels of rapeseed oil

Parameter	Feeding trial period	CTRL	RO25	RO50	RO100	p - value
Whole fish						
Moisture	Start	87.1 ± 0.08	87.2 ± 0.13	87.1 ± 0.17	86.8 ± 0.17	0.457
	Mid (3 W)	87.1 ± 0.07	87.0 ± 0.09	87.1 ± 0.05	87.3 ± 0.09	0.226
	End (6 W)	86.5 ± 0.05^{a}	86.3 ± 0.06^{a}	86.3 ± 0.09^{a}	86.7 ± 0.11^{b}	< 0.001
In dry matter, %						
Crude protein	Start	64.6 ± 0.26	64.7 ± 0.27	64.5 ± 0.83	64.9 ± 0.33	0.896
	Mid (3 W)	63.4 ± 0.47	63.2 ± 0.48	63.2 ± 0.41	62.6 ± 0.20	0.483
	End (6 W)	62.8 ± 0.35^{a}	62.2 ± 0.26^{ab}	61.8 ± 0.22^{ab}	61.5 ± 0.39^{b}	0.031
Crude lipid	Start	14.8 ± 0.27	14.8 ± 0.38	14.5 ± 0.19	15.2 ± 0.24	0.306
	Mid (3 W)	16.2 ± 0.19	16.8 ± 0.40	16.4 ± 0.13	15.8 ± 0.28	0.095
	End (6 W)	18.6 ± 0.32^{a}	18.6 ± 0.43^{a}	19.7 ± 0.38^{ab}	20.5 ± 0.78^{b}	0.021
Ash	Start	1.60 ± 0.03	1.50 ± 0.09	1.49 ± 0.05	1.45 ± 0.05	0.350
	Mid (3 W)	1.63 ± 0.01	1.60 ± 0.02	1.64 ± 0.01	1.64 ± 0.02	0.558
	End (6 W)	1.52 ± 0.01^{ab}	1.54 ± 0.02^{ab}	1.44 ± 0.03^{b}	1.54 ± 0.01^{ab}	0.031
Energy	End (6 W)	22.2 ± 0.24	22.5 ± 0.12	22.4 ± 0.13	22.5 ± 0.15	0.489
Liver						
Moisture	End (6 W)	36.4 ± 1.11	34.8 ± 2.28	39.3 ± 0.67	37.8 ± 0.81	0.102
In dry matter, %						
Crude protein	End (6 W)	24.8 ± 0.18^{a}	23.5 ±0.31^{ab}	22.4 ± 0.19^{b}	21.7 ± 0.46^{b}	0.003
Crude lipid	Start	50.9 ± 2.54	56.3 ± 0.23	58.5 ± 0.80	56.5 ± 1.10	0.168
	End (6 W)	69.2 ± 0.61^{a}	69.7 ± 0.47^{a}	73.8 ± 0.28^{b}	77.4 ± 0.41^{c}	< 0.001

Values represented as means ± SEM (n = 6 / treatment). Significant differences between treatment groups at the same time point are indicated by different superscript letters ($p < .05$).

Table 5: Fatty acid composition of the whole body and liver at the start (week 0) and at the end of the feeding trial (week 6). Muscle fatty acid composition shown only for the end of the feeding trial.

Whole body

Fatty acid	Start	Week 6				p - Value
% of total fatty acids		CTRL	OR25	OR50	OR100	
Saturates (SFAs)						
C14:00	2.77 ± 0.03	3.48 ± 0.02 [a]	2.79 ± 0.02 [b]	2.27 ± 0.02 [c]	1.18 ± 0.01 [d]	< 0.001
C16:00	14.52 ± 0.40	14.68 ± 0.08 [a]	12.84 ± 0.05 [b]	11.61 ± 0.04 [d]	8.66 ± 0.06 [d]	< 0.001
C18:0	4.71 ± 0.05	4.45 ± 0.05[a]	4.09 ± 0.03 [ac]	3.88 ± 0.02 [bc]	3.53 ± 0.02 [b]	< 0.001
∑SFA [1]	21.00 ± 0.16	22.61 ± 0.86 [a]	19.72 ± 0.75[ab]	17.76 ± 0.67 [b]	13.37 ± 0.53 [b]	< 0.001
Monounsturates (MUFAs)						
C16:1	4.07 ± 0.03	4.66 ± 0.04 [a]	3.66 ± 0.02 [ac]	2.85 ± 0.02 [bc]	1.48 ± 0.01 [b]	< 0.001
C18:1 n-9 (OA)	19.13 ± 0.14	19.14 ± 0.15[a]	25.58 ± 0.15[b]	31.62 ± 0.15 [c]	43.32 ± 0.15 [d]	< 0.001
C18:1 n-7	4.87 ± 0.03	5.13 ± 0.03 [a]	4.69 ± 0.02 [b]	4.39 ± 0.01 [c]	4.00 ± 0.02 [d]	< 0.001
C20:1 n-11	1.43 ± 0.01	2.67 ± 0.02 [a]	2.41 ± 0.01 [b]	2.21 ± 0.01 [c]	1.86 ± 0.01 [d]	< 0.001
C22:1 n-11	0.69 ± 0.01	1.75 ± 0.02 [a]	1.45 ± 0.01 [ab]	1.20 ± 0.01 [b]	0.68 ± 0.01 [b]	< 0.001
∑MUFA[2]	30.19 ± 1.15	33.35 ± 1.08 [a]	37.79 ± 1.54[ab]	42.27 ± 1.98[ab]	51.34 ± 2.82 [b]	0.004
Polyunsturates (PUFAs)						
C18:2 n-6 (LA)	13.70 ± 0.10	10.05 ± 0.07 [a]	12.87 ± 0.05 [b]	15.18 ± 0.09 [c]	20.34 ± 0.07 [d]	< 0.001
C20:2 n-6	0.29 ± 0.10	0.26 ± 0.00 [a]	0.25 ± 0.00 [b]	0.25 ± 0.01 [b]	0.27 ± 0.01 [a]	< 0.001
C18:3 n-3 (ALA)	1.96 ± 0.01	1.82 ± 0.01[b]	2.80 ± 0.01 [ba]	3.39 ± 0.30 [a]	5.30 ± 0.03 [a]	< 0.001
C18:4 n-3	1.34 ± 0.01	2.05 ± 0.01[a]	1.55 ± 0.01 [b]	1.77 ± 0.01 [c]	0.50 ± 0.01 [d]	< 0.001
C20:3 n-3	1.31 ± 0.02	0.98 ± 0.01 [a]	0.78 ± 0.01 [b]	0.60 ± 0.01 [c]	0.30 ± 0.01 [d]	< 0.001
C20:4 n-3	0.59 ± 0.01	0.72 ± 0.01 [a]	0.57 ± 0.01 [b]	0.43 ± 0.01 [c]	0.20 ± 0.01 [d]	< 0.001
C20:5 n-3 (EPA)	9.95 ± 0.07	10.24 ± 0.07 [a]	8.06 ± 0.05 [b]	6.32 ± 0.05 [c]	3.15 ± 0.04 [d]	< 0.001
C22:5 n-3	1.37 ± 0.01	1.24 ± 0.02[a]	1.00 ± 0.01[ab]	0.76 ± 0.00 [b]	0.38 ± 0.00[b]	< 0.001
C22:6 n-3 (DHA)	13.94 ± 0.08	12.00 ± 0.13[a]	9.88 ± 0.07 [ab]	7.79 ± 0.05 [b]	4.11 ± 0.03 [b]	< 0.001
∑PUFA[3]	45.32 ±0.63	39.78 ± 0.42 [a]	38.1 ± 0.41 [ab]	36.76 ± 0.43 [ab]	34.55 ± 0.58 [b]	< 0.001
∑n-3 [4]	30.46 ± 0.62	29.05 ± 0.55 [a]	24.64 ± 0.43[a]	21.06 ± 0.34[a]	13.94 ± 0.23[b]	< 0.001

∑n-6 [5]	14.86 ± 1.09	10.73 ± 0.80 [b]	13.46 ± 1.01 [b]	15.70 ± 1.20 [b]	20.61 ± 1.73 [a]	0.009
n-3/n-6 [6]	2.05	2.70	1.83	1.34	0.68	
EPA + DHA [7]	23.44 ± 0.20	22.24 ± 0.42 [a]	17.94 ± 0.43 [c]	14.11 ± 0.41 [d]	7.26 ± 0.44 [b]	< 0.001

Liver

Fatty acid	Start	Week 6				p - Value
% of total fatty acids		CTRL	RO25	RO50	RO100	
Saturates (SFAs)						
C14:00	2.03 ± 0.02	2.25 ± 0.02^{a}	1.88 ± 0.02^{b}	1.62 ± 0.03^{c}	0.89 ± 0.03^{d}	< 0.001
C16:00	12.03 ± 0.04	11.74 ± 0.11^{a}	10.70 ± 0.12^{b}	9.82 ± 0.11^{c}	7.19 ± 0.06^{d}	< 0.001
C18:0	5.23 ± 0.03	5.60 ± 0.10^{a}	5.33 ± 0.08^{a}	4.60 ± 0.06^{ab}	3.92 ± 0.03^{b}	< 0.001
∑SFA [1]	19.20 ± 0.35	19.59 ± 0.67^{a}	17.91 ± 0.62^{ab}	16.04 ± 0.58^{ab}	12.00 ± 0.44^{b}	0.003
Monounsturates (MUFAs)						
C16:1	4.33 ± 0.03	11.74 ± 0.12^{a}	10.70 ± 0.12^{ab}	9.82 ± 0.11^{b}	7.19 ± 0.06^{b}	< 0.001
C18:1 n-9 (OA)	27.12 ± 0.20	25.98 ± 0.40^{b}	32.94 ± 0.60^{b}	37.52 ± 0.63^{ab}	46.38 ± 0.83^{a}	< 0.001
C18:1 n-7	7.04 ± 0.33	7.55 ± 0.06^{a}	6.82 ± 0.04^{b}	5.96 ± 0.06^{c}	4.83 ± 0.04^{d}	< 0.001
C20:1 n-11	1.41 ± 0.01	2.40 ± 0.02^{a}	2.09 ± 0.02^{ab}	1.89 ± 0.01^{b}	1.57 ± 0.02^{b}	< 0.001
C22:1 n-11	0.42 ± 0.01	1.07 ± 0.02 [a]	0.79 ± 0.01 [ab]	0.68 ± 0.02 [b]	0.42 ± 0.01 [b]	< 0.001
∑MUFA [2]	40.30 ± 0.72	48.74 ± 1.40^{a}	53.34 ±1.86^{ab}	55.87 ± 2.15^{ab}	60.39 ± 2.67^{b}	0.025
Polyunsaturates (PUFAs)						
C18:2 n-6 (LA)	18.37 ± 0.18	13.22 ± 0.11^{b}	16.71 ± 0.11^{b}	19.80 ± 0.18^{ab}	23.72 ± 0.08^{a}	< 0.001
C18:3 n-3 (ALA)	2.48 ± 0.03	2.11± 0.01^{b}	3.23 ± 0.02^{b}	4.26 ± 0.05^{ab}	5.81± 0.04^{b}	< 0.001
C18:4 n-3	1.50 ± 0.02	2.28 ± 0.03^{a}	1.68 ± 0.02^{a}	1.29 ± 0.03^{ab}	0.55 ± 0.03^{b}	< 0.001
C20:4 n-3	0.91 ± 0.01	1.13 ± 0.01^{a}	0.92 ± 0.01^{ab}	0.66 ± 0.01^{b}	0.35 ± 0.00^{b}	< 0.001
C20:5 n-3 (EPA)	8.03 ± 0.11	8.93 ± 0.09^{a}	6.36 ± 0.06^{b}	4.67 ± 0.07^{c}	1.91 ± 0.13^{d}	< 0.001
C22:6 n-3 (DHA)	6.45 ± 0.11	5.86 ± 0.10^{a}	3.72 ± 0.06^{ab}	2.54 ± 0.03^{b}	1.07 ± 0.07^{b}	< 0.001
∑PUFA[3]	39.74 ± 0.41	35.35 ± 0.52^{a}	33.92 ± 0.64^{ab}	34.24 ± 0.78^{ab}	33.73 ± 0.99^{b}	< 0.001
∑n-3 [4]	21.37 ± 0.03	22.13 ± 0.38^{a}	17.21 ± 0.25^{a}	14.44 ± 0.21^{a}	10.01 ± 0.27^{b}	< 0.001
∑n-6 [5]	18.37 ± 0.11	13.22 ± 0.11^{a}	16.71 ± 0.11^{b}	19.80 ± 0.18^{ab}	23.72 ± 0.08^{b}	< 0.001
n-3/n-6 [6]	1.05	1.53	1.95	0.67	0.41	
EPA + DHA [7]	14.48 ± 0.09	14.79 ± 0.33^{a}	10.08 ± 0.28^{ac}	7.21 ± 0.23^{c}	2.98 ± 0.11^{b}	< 0.001

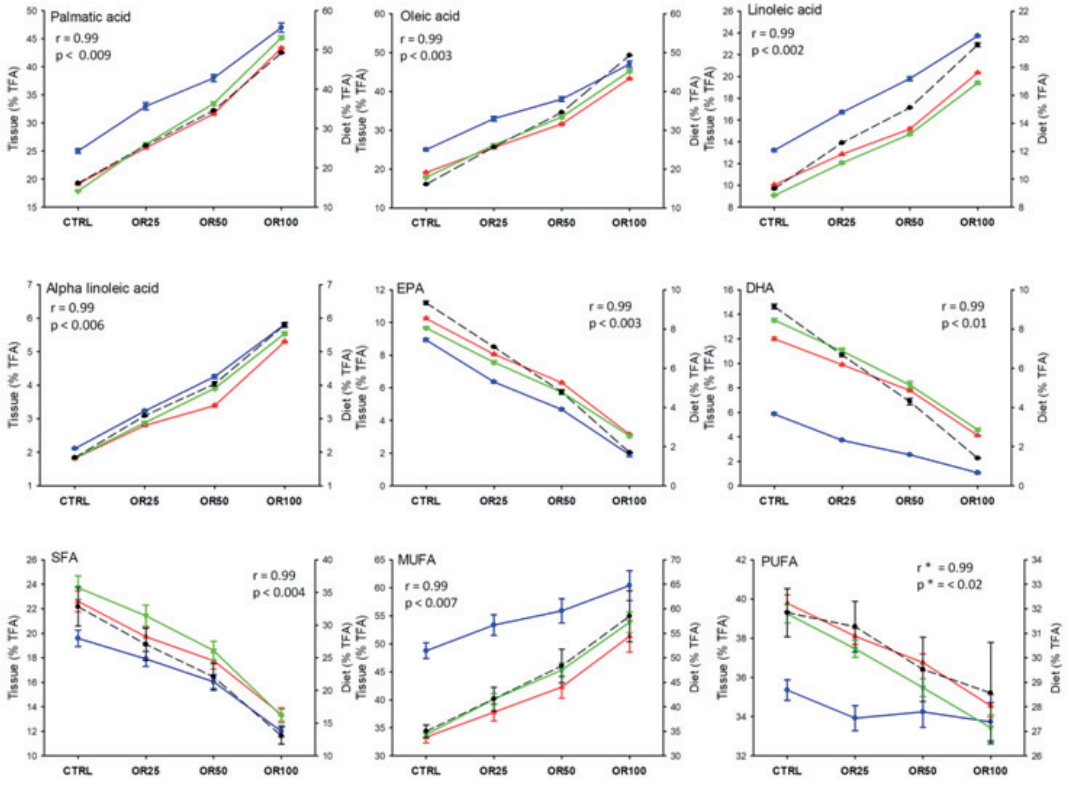
Muscles

Fatty acid	Week 6				p - Value
% of total fatty acids	CTRL	OR25	OR50	OR100	
Saturates (SFAs)					
C14:00	4.19 ± 0.03[a]	3.32 ± 0.04 [b]	2.66 ± 0.04[c]	1.27 ± 0.03[d]	< 0.001
C16:00	15.82 ± 0.15[a]	14.15 ± 0.11[b]	12.51± 0.11[c]	8.99 ± 0.05[d]	< 0.001
C18:0	3.71 ± 0.04[a]	3.69 ± 0.06[a]	3.42 ± 0.05[c]	3.08 ± 0.03[b]	< 0.001
∑SFA [1]	23.72 ± 0.96[a]	21.43 ± 0.86[ab]	18.59 ± 0.77[bc]	13.26 ± 0.56[c]	< 0.001
Monounsturates (MUFAs)					
C16:1	5.20 ± 0.05[a]	4.01 ± 0.05[b]	3.06 ± 0.04[c]	1.45 ± 0.03[d]	< 0.001
C18:1 n-9	17.87 ± 0.08[a]	26.22 ± 0.16[ab]	33.45 ± 0.30[bc]	45.21 ± 0.27[c]	< 0.001
C18:1 n-7	4.46 ± 0.04[a]	4.33 ± 0.03[ab]	4.10 ± 0.02[bc]	3.83 ± 0.01[c]	< 0.001
C20:1 n-11	3.13 ± 0.03[a]	2.9 ± 0.02[ab]	2.54 ± 0.02[bc]	2.04 ± 0.01[c]	< 0.001
C22:1 n-9	0.43 ±0.01[a]	0.40 ±0.00[ab]	0.37 ±0.00[bc]	0.28 ± 0.00[c]	< 0.001
C22:1 n-11	2.32 ± 0.03[a]	1.89 ± 0.02[b]	1.48 ± 0.02[c]	0.77 ± 0.02[d]	< 0.001
C24:1 n-9	0.40 ± 0.00[a]	0.37 ± 0.00[b]	0.31± 0.01[c]	0.25 ± 0.00[d]	< 0.001
∑MUFA [2]	33.81 ± 0.62[a]	40.12 ± 0.96[ab]	45.31 ± 1.24[ab]	53.83 ± 1.83[b]	0.025
Polyunsaturates (PUFAs)					
C18:2 n-6 (LA)	9.07 ± 0.04 [a]	12.05 ± 0.10 [d]	14.70 ± 0.09 [c]	19.40 ± 0.12 [b]	< 0.001
C18:3 n-3 (ALA)	1.83 ± 0.01[a]	2.88 ± 0.03 [ab]	3.91± 0.04 [b]	5.54 ± 0.04 [c]	< 0.001
C18:4 n-3	2.15± 0.01[a]	1.58 ± 0.03 [ab]	1.16 ± 0.02 [b]	0.49 ± 0.02 [c]	< 0.001
C20:5 n-3 (EPA)	9.66 ± 0.05[a]	7.57 ± 0.08 [ab]	5.73 ± 0.15 [cb]	3.06 ± 0.07 [c]	< 0.001
C22:5 n-3	1.27 ± 0.01[a]	1.02 ± 0.01[b]	0.74 ± 0.01 [c]	0.37 ± 0.01 [d]	< 0.001
C22:6 n-3 (DHA)	13.53 ± 0.12[a]	11.00 ± 0.24 [ab]	8.24 ± 0.28 [b]	4.58 ± 0.12 [c]	< 0.001
∑PUFA[3]	39.24 ± 0.46 [a]	37.46 ± 0.44 [ab]	35.48 ± 0.46 [ab]	33.44 ± 0.65 [b]	< 0.001
∑n-3 [4]	29.75 ± 0.49 [a]	25.07 ± 0.39 [ab]	20.53 ± 0.30 [ab]	14.04 ± 0.21 [b]	0.028
∑n-6 [5]	9.49 ± 0.92 [b]	12.39 ± 1.21 [cb]	14.95 ± 0.82 [c]	19.40 ± 0.12 [a]	< 0.001
n-3/n-6 [6]	2.94	1.79	1.11	0.44	
EPA + DHA [7]	23.19 ± 0.41[a]	18.57 ± 0.38 [a]	13.97 ± 0.31 [b]	7.64 ± 0.17 [c]	< 0.001

Values are presented as mean ± standard error. (n = 6 / diet group). Values with different superscript letters in the same row indicate significant differences between dietary treatments (P < .05).

1. Σ SFA is the sum of saturated fatty acids.
2. Σ MUFA is the sum of monounsaturated fatty acids.
3. Σ PUFA is the sum of polyunsaturated fatty acids.
4. Σ n-3 is the sum of n-3 polyunsaturated fatty acids, includes C20:3 and, C20:4 only for the muscles.
5. Σ n-6 is the sum of n-6 polyunsaturated fatty acids, also includes C22:4 for the whole body and muscles.
6. n-3/n-6 is the ratio of Σ n-3 and Σ n-6.
7. Sum of EPA and DHA

Figure 1


Palmatic acid
r = 0.99
p < 0.009
Oleic acid
r = 0.99
p < 0.003
Linoleic acid
r = 0.99
p < 0.002
Alpha linoleic acid
r = 0.99
p < 0.006
EPA
r = 0.99
p < 0.003
DHA
r = 0.99
p < 0.01
SFA
r = 0.99
p < 0.004
MUFA
r = 0.99
p < 0.007
PUFA
r * = 0.99
p * = < 0.02
Tissue (% TFA)
Diet (% TFA)
CTRL
OR25
OR50
OR100

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Lumpfish (*Cyclopterus lumpus*) is currently used as a biological control against the sea-lice in farming of Atlantic salmon. In Norway, the use of farmed lumpfish has increased 40 folds during last seven years. However, there are substantial knowledge gaps about nutrient requirements and how well this species utilize commercially available plant feed ingredients. The aim of this PhD thesis was therefore to investigate the potential of lumpfish to utilize plant protein concentrates and rapeseed oil as replacement for fishmeal and marine oil. Protein ingredients evaluated were mixtures of soy and pea protein concentrates replacing fishmeal in a dose response design. Replacement of marine oil in the diet with rapeseed oil was also designed as a dose response replacing up to 100% of the marine oil. Changes in fish growth, muscle cellularity, chemical and fatty acid compositions, digestive enzymes and gut histology were studied. Replacing up to 50% of fishmeal and/or marine oil did not compromise the growth, while a reduction in growth was observed at high inclusion levels of plant ingredients i.e. 75% of plant protein concentrates and 100% rapeseed oi. Overall, the results of present thesis suggests that plant protein concentrates and rapeseed oil are successful replacers for marine-based diets of juvenile lumpfish at modest incorporation levels.

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