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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

Florence Perera Willora [a], Bjørn Grønevik [a], Cui Liu [b,c], Anjana Palihawadana [a], Mette Sørensen [a], Ørjan Hagen [a,*]

[a] Nord University, Faculty of Bioscience and Aquaculture, Norway
[b] University of Chinese Academy of Sciences, Beijing, 100049, China
[c] State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, 430072, China

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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, hepatosomatic index (HSI), visero-somatic index, condition factor (CF), and whole body chemical composition 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 CF, but increased the 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.

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.,

* Corresponding author at: Faculty of Bioscience and Aquaculture, Nord University, 8049, Bodø, Norway.
E-mail address: orjan.hagen@nord.no (Ø. Hagen).

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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, 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 European sea bass (*Dicentrarchus labrax*) (Montero et al., 2005), tilapia (*Oreochromis niloticus*) (Peng et al., 2016), carp (Ljubojević et al., 2015; Sun et al., 2011; Yang et al., 2020), senegalese sole (*Solea senegalensis*) (Pereira et al., 2019), sterlet sturgeon (*Acipenser ruthenus*) (Pourhosein Sarameh et al., 2019), yellow croaker (*Larimichthys crocea*) (Mu et al., 2020), and gilthead sea bream (*Sparus aurata*) (Sánchez-Moya et al., 2020). 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, to evaluate the growth performance, FA, and chemical composition of whole body and tissues in juvenile lumpfish.

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,

Table 1
Ingredient composition (g 100g-[1]) and analyzed proximate composition (%) of the experimental diets on an as fed basis. Values are expressed as mean of triplicate 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

[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, 20,000 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).

Table 2
Fatty acid composition of the experimental diets.

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

Values are expressed as mean value ± SEM of triplicate samples per diet.
Σ SFA is the sum of saturated fatty acids.
Σ MUFA is the sum of monounsaturated fatty acids.
Σ PUFA is the sum of polyunsaturated fatty acids.
n-3 is the sum of n-3 polyunsaturated fatty acids, includes C18:4.
Σ n-6 is the sum of n-6 polyunsaturated fatty acids.
n-3/n-6 is the ratio of Σ n-3 and Σ n-6.
Sum of EPA and DHA.

Portugal) and the extrusion process was earlier described by Willora et al. (2020). The oil mixtures were coated to the feeds using 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 over a 6 week period 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 in abundance at a level of 2.5 % of their body mass on a daily basis between 06:00 to 21:00 at eight time intervals to make sure the fish were fed to apparent satiation.

2.3. Lumpfish and experimental set-up

Juvenile lumpfish of 4 g 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 W, luminous flux1350 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 °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 h at −70 °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 h (ISO 6496−1999). 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 the FBA; the feed was analyzed 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 analyzed 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 0.8 mL of distilled water, 2 mL of methanol, and 1 mL of chloroform followed by addition of 1 mL of chloroform and 1 mL of distilled water. Samples were then centrifuged (2000 *g*) to separate the phases. 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 25 m 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 ^{2} (cm)]. K (g cm^{-3}) = [fish weight (g) / fork length^3 (cm)] × 100. 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.

2.9. Statistical analysis

All statistical analyses were performed and graphs generated using Sigmaplot 14.0 (Systat software, San Jose, CA). Data were tested for normality (Shapiro-Wilk test) and homogeneity of variances (Brown-Forsythe F-test). Individual means were compared by one-way analysis of variance (ANOVA), 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 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. All data were presented as means ± SE (standard error), and differences were considered significant only if their p-value was < 0.05. Correlation of selected main FAs present in the whole body, liver, and muscles of the dietary groups with the FAs in their respective feeds were determined using Pearson's correlation coefficient (r). The different strengths of (r) were defined as very high, high, moderate, low and negligible (Mukaka, 2012).

3. Results

3.1. Growth performance and somatic indices

Biometric parameters, condition factor, and somatic indices measured during the feeding trial are presented in Table 3. No mortalities occurred during the experiment and all fish appeared healthy. Fish grew from an average of 7 g to 34 − 39 g over the 6 weeks feeding trial. Body weight, length, and height of fish showed significant differences among fish fed the experimental diets. All these parameters in RO100 group were significantly lower than all other groups, while no differences were observed among the other three groups at the mid- and end-points of the experiment ($p < 0.05$). The lower weight gain of fish fed RO100 was characterized by a tendency towards a lower SGR ($p = 0.09$). HSI was slightly, but significantly, higher in fish fed RO100 diet at the end of the experiment. Viserosomatic index was not affected by the diet, but all groups showed a small numerical drop at week 3 and 6 compared to the initial levels ($p > 0.05$). Condition factor (B^1), was significantly lower in fish fed RO100 than CTRL and RO25 at the end of the experiment.

3.2. Chemical composition of whole body and liver

Chemical composition of crude protein, lipid, ash and energy in the whole body and liver are presented in Table 4. Whole body composition remained unaffected by dietary treatment up to 3 weeks of the feeding trial. At the end of the trial, whole body moisture was slightly higher in the fish fed RO100 ($p < 0.05$). The crude lipid was slightly, but significantly higher, while crude protein was significantly lower in the RO100 group 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

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	0.136
	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^{b}	0.25 ± 0.0010^{b}	0.24 ± 0.0011^{a}	0.24 ± 0.0001^{a}	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 (kJ/g)	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)	63.6 ± 1.1	64.6 ± 2.62	60..6 ± 0.67	62.2 ± 0.81	0.195
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 ($p < .05$).

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; however, the reduced growth for fish fed the RO100 suggest that nutrient requirement was not met. Fish fed the CTRL, RO25 and RO50 showed no differences in growth, suggesting that dietary EPA + DHA levels in the range 1.3–2.6%, corresponding to 9–18.5% of total FAs, satisfy the nutrient requirement. 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

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	RO25	RO50	RO100	
Saturates (SFAs)						
C14:00	2.77 ± 0.03	3.48 ± 0.02 ^{d}	2.79 ± 0.02 ^{c}	2.27 ± 0.02 ^{b}	1.18 ± 0.01 ^{a}	< 0.001
C16:00	14.52 ± 0.40	14.68 ± 0.08 ^{d}	12.84 ± 0.05 ^{c}	11.61 ± 0.04 ^{b}	8.66 ± 0.06 ^{a}	< 0.001
C18:0	4.71 ± 0.05	4.45 ± 0.05^{b}	4.09 ± 0.03 ^{bc}	3.88 ± 0.02 ^{ac}	3.53 ± 0.02 ^{a}	< 0.001
∑SFA [1]	21.00 ± 0.16	22.61 ± 0.86 ^{b}	19.72 ± 0.75^{ab}	17.76 ± 0.67 ^{a}	13.37 ± 0.53 ^{a}	< 0.001
Monounsturates (MUFAs)						
C16:1	4.07 ± 0.03	4.66 ± 0.04 ^{b}	3.66 ± 0.02 ^{bc}	2.85 ± 0.02 ^{ac}	1.48 ± 0.01 ^{a}	< 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 ^{d}	4.69 ± 0.02 ^{c}	4.39 ± 0.01 ^{b}	4.00 ± 0.02 ^{a}	< 0.001
C20:1 n-11	1.43 ± 0.01	2.67 ± 0.02 ^{d}	2.41 ± 0.01 ^{c}	2.21 ± 0.01 ^{b}	1.86 ± 0.01 ^{a}	< 0.001
C22:1 n-11	0.69 ± 0.01	1.75 ± 0.02 ^{b}	1.45 ± 0.01 ^{ab}	1.20 ± 0.01 ^{a}	0.68 ± 0.01 ^{a}	< 0.001
∑MUFA[2]	30.19 ± 1.15	33.35 ± 1.08 ^{b}	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 ^{b}	0.25 ± 0.00 ^{a}	0.25 ± 0.01 ^{a}	0.27 ± 0.01 ^{b}	< 0.001
C18:3 n-3 (ALA)	1.96 ± 0.01	1.82 ± 0.01^{a}	2.80 ± 0.01 ^{ab}	3.39 ± 0.30 ^{b}	5.30 ± 0.03 ^{b}	< 0.001
C18:4 n-3	1.34 ± 0.01	2.05 ± 0.01^{d}	1.55 ± 0.01 ^{c}	1.77 ± 0.01 ^{b}	0.50 ± 0.01 ^{a}	< 0.001
C20:3 n-3	1.31 ± 0.02	0.98 ± 0.01 ^{d}	0.78 ± 0.01 ^{c}	0.60 ± 0.01 ^{b}	0.30 ± 0.01 ^{a}	< 0.001
C20:4 n-3	0.59 ± 0.01	0.72 ± 0.01 ^{d}	0.57 ± 0.01 ^{c}	0.43 ± 0.01 ^{b}	0.20 ± 0.01 ^{a}	< 0.001
C20:5 n-3 (EPA)	9.95 ± 0.07	10.24 ± 0.07 ^{d}	8.06 ± 0.05 ^{c}	6.32 ± 0.05 ^{b}	3.15 ± 0.04 ^{a}	< 0.001
C22:5 n-3	1.37 ± 0.01	1.24 ± 0.02^{b}	1.00 ± 0.01^{ab}	0.76 ± 0.00 ^{a}	0.38 ± 0.00^{a}	< 0.001
C22:6 n-3 (DHA)	13.94 ± 0.08	12.00 ± 0.13^{b}	9.88 ± 0.07 ^{ab}	7.79 ± 0.05 ^{a}	4.11 ± 0.03 ^{a}	< 0.001
∑PUFA[3]	45.32 ± 0.63	39.78 ± 0.42 ^{b}	38.1 ± 0.41 ^{ab}	36.76 ± 0.43 ^{ab}	34.55 ± 0.58 ^{a}	< 0.001
∑n-3 [4]	30.46 ± 0.62	29.05 ± 0.55 ^{b}	24.64 ± 0.43^{b}	21.06 ± 0.34^{b}	13.94 ± 0.23^{a}	< 0.001
∑n-6 [5]	14.86 ± 1.09	10.73 ± 0.80 ^{a}	13.46 ± 1.01 ^{a}	15.70 ± 1.20 ^{a}	20.61 ± 1.73 ^{b}	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 ^{d}	17.94 ± 0.43 ^{c}	14.11 ± 0.41 ^{b}	7.26 ± 0.44 ^{a}	< 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^{d}	1.88 ± 0.02^{c}	1.62 ± 0.03^{b}	0.89 ± 0.03^{a}	< 0.001
C16:00	12.03 ± 0.04	11.74 ± 0.11^{d}	10.70 ± 0.12^{c}	9.82 ± 0.11^{b}	7.19 ± 0.06^{a}	< 0.001
C18:0	5.23 ± 0.03	5.60 ± 0.10^{b}	5.33 ± 0.08^{b}	4.60 ± 0.06^{ab}	3.92 ± 0.03^{a}	< 0.001
∑SFA [1]	19.20 ± 0.35	19.59 ± 0.67^{b}	17.91 ± 0.62^{ab}	16.04 ± 0.58^{ab}	12.00 ± 0.44^{a}	0.003
Monounsturates (MUFAs)						
C16:1	4.33 ± 0.03	11.74 ± 0.12^{b}	10.70 ± 0.12^{ab}	9.82 ± 0.11^{a}	7.19 ± 0.06^{a}	< 0.001
C18:1 n-9 (OA)	27.12 ± 0.20	25.98 ± 0.40^{a}	32.94 ± 0.60^{a}	37.52 ± 0.63^{ab}	46.38 ± 0.83^{b}	< 0.001
C18:1 n-7	7.04 ± 0.33	7.55 ± 0.06^{d}	6.82 ± 0.04^{c}	5.96 ± 0.06^{b}	4.83 ± 0.04^{a}	< 0.001
C20:1 n-11	1.41 ± 0.01	2.40 ± 0.02^{b}	2.09 ± 0.02^{ab}	1.89 ± 0.01^{a}	1.57 ± 0.02^{a}	< 0.001
C22:1 n-11	0.42 ± 0.01	1.07 ± 0.02 ^{b}	0.79 ± 0.01 ^{ab}	0.68 ± 0.02 ^{a}	0.42 ± 0.01 ^{a}	< 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^{a}	16.71 ± 0.11^{a}	19.80 ± 0.18^{ab}	23.72 ± 0.08^{b}	< 0.001
C18:3 n-3 (ALA)	2.48 ± 0.03	2.11 ± 0.01^{a}	3.23 ± 0.02^{a}	4.26 ± 0.05^{ab}	5.81 ± 0.04^{a}	< 0.001
C18:4 n-3	1.50 ± 0.02	2.28 ± 0.03^{b}	1.68 ± 0.02^{b}	1.29 ± 0.03^{ab}	0.55 ± 0.03^{a}	< 0.001
C20:4 n-3	0.91 ± 0.01	1.13 ± 0.01^{b}	0.92 ± 0.01^{ab}	0.66 ± 0.01^{a}	0.35 ± 0.00^{a}	< 0.001
C20:5 n-3 (EPA)	8.03 ± 0.11	8.93 ± 0.09^{d}	6.36 ± 0.06^{c}	4.67 ± 0.07^{b}	1.91 ± 0.13^{a}	< 0.001
C22:6 n-3 (DHA)	6.45 ± 0.11	5.86 ± 0.10^{b}	3.72 ± 0.06^{ab}	2.54 ± 0.03^{a}	1.07 ± 0.07^{a}	< 0.001
∑PUFA[3]	39.74 ± 0.41	35.35 ± 0.52^{b}	33.92 ± 0.64^{ab}	34.24 ± 0.78^{ab}	33.73 ± 0.99^{a}	< 0.001
∑n-3 [4]	21.37 ± 0.03	22.13 ± 0.38^{b}	17.21 ± 0.25^{b}	14.44 ± 0.21^{b}	10.01 ± 0.27^{a}	< 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^{b}	10.08 ± 0.28^{bc}	7.21 ± 0.23^{b}	2.98 ± 0.11^{a}	< 0.001

Muscles

Fatty acid	Week 6				p - Value
% of total fatty acids	CTRL	RO25	RO50	RO100	
Saturates (SFAs)					
C14:00	4.19 ± 0.03^{d}	3.32 ± 0.04 ^{c}	2.66 ± 0.04^{b}	1.27 ± 0.03^{a}	< 0.001
C16:00	15.82 ± 0.15^{d}	14.15 ± 0.11^{c}	12.51 ± 0.11^{b}	8.99 ± 0.05^{a}	< 0.001
C18:0	3.71 ± 0.04^{b}	3.69 ± 0.06^{b}	3.42 ± 0.05^{c}	3.08 ± 0.03^{a}	< 0.001
∑SFA [1]	23.72 ± 0.96^{c}	21.43 ± 0.86^{bc}	18.59 ± 0.77^{ab}	13.26 ± 0.56^{a}	< 0.001
Monounsturates *(MUFAs)*					
C16:1	5.20 ± 0.05^{d}	4.01 ± 0.05^{c}	3.06 ± 0.04^{b}	1.45 ± 0.03^{a}	< 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^{c}	4.33 ± 0.03^{bc}	4.10 ± 0.02^{ab}	3.83 ± 0.01^{a}	< 0.001

(*continued on next page*)

Table 5 (*continued*)

Muscles					
Fatty acid	Week 6				p - Value
% of total fatty acids	CTRL	RO25	RO50	RO100	
Saturates (SFAs)					
C20:1 n-11	3.13 ± 0.03^{c}	2.9 ± 0.02^{bc}	2.54 ± 0.02^{ab}	2.04 ± 0.01^{a}	< 0.001
C22:1 n-9	0.43 ± 0.01^{c}	0.40 ± 0.00^{bc}	0.37 ± 0.00^{ab}	0.28 ± 0.00^{a}	< 0.001
C22:1 n-11	2.32 ± 0.03^{d}	1.89 ± 0.02^{c}	1.48 ± 0.02^{b}	0.77 ± 0.02^{a}	< 0.001
C24:1 n-9	0.40 ± 0.00^{d}	0.37 ± 0.00^{c}	0.31 ± 0.01^{b}	0.25 ± 0.00^{a}	< 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^{c}	1.58 ± 0.03 ^{bc}	1.16 ± 0.02 ^{b}	0.49 ± 0.02 ^{a}	< 0.001
C20:5 n-3 (EPA)	9.66 ± 0.05^{c}	7.57 ± 0.08 ^{bc}	5.73 ± 0.15 ^{ab}	3.06 ± 0.07 ^{a}	< 0.001
C22:5 n-3	1.27 ± 0.01^{d}	1.02 ± 0.01^{c}	0.74 ± 0.01 ^{b}	0.37 ± 0.01 ^{a}	< 0.001
C22:6 n-3 (DHA)	13.53 ± 0.12^{c}	11.00 ± 0.24 ^{bc}	8.24 ± 0.28 ^{b}	4.58 ± 0.12 ^{a}	< 0.001
∑PUFA[3]	39.24 ± 0.46 ^{b}	37.46 ± 0.44 ^{ab}	35.48 ± 0.46 ^{ab}	33.44 ± 0.65 ^{a}	< 0.001
∑n-3 [4]	29.75 ± 0.49 ^{b}	25.07 ± 0.39 ^{ab}	20.53 ± 0.30 ^{ab}	14.04 ± 0.21 ^{a}	0.028
∑n-6 [5]	9.49 ± 0.92 ^{a}	12.39 ± 1.21 ^{ac}	14.95 ± 0.82 ^{c}	19.40 ± 0.12 ^{b}	< 0.001
n-3/n-6 [6]	2.94	1.79	1.11	0.44	
EPA + DHA [7]	23.19 ± 0.41^{c}	18.57 ± 0.38 ^{c}	13.97 ± 0.31 ^{b}	7.64 ± 0.17 ^{a}	< 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).
Σ SFA is the sum of saturated fatty acids.
Σ MUFA is the sum of monounsaturated fatty acids.
Σ PUFA is the sum of polyunsaturated fatty acids.
Σ n-3 is the sum of n-3 polyunsaturated fatty acids, includes C20:3 and, C20:4 only for the muscles.
Σ n-6 is the sum of n-6 polyunsaturated fatty acids, also includes C22:4 for the whole body and muscles.
n-3/n-6 is the ratio of Σ n-3 and Σ n-6.
Sum of EPA and DHA.

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 4.3–4.8 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). Viserosomatic index value is an important indicator directly affecting the fish yield (Wang et al., 2005). Increased VSI is associated with lipid content in the diet (Bendiksen et al., 2003; Han et al., 2014; Jobling et al., 1998) or energy intake (Hatlen et al., 2007). Lumpfish in the present study were fed nearly iso-lipidic diets (15 %) and no differences were noted for VSI among the diets. The VSI was slightly reduced at the end of the experiment, while the body weight increased from an average of 6.8 g–37.9 g. Willora et al. (2020) observed a similar reduction in VSI over time when feeding juvenile lumpfish plant protein incorporated diets.

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, 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, b, c; 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 and Frayn, 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

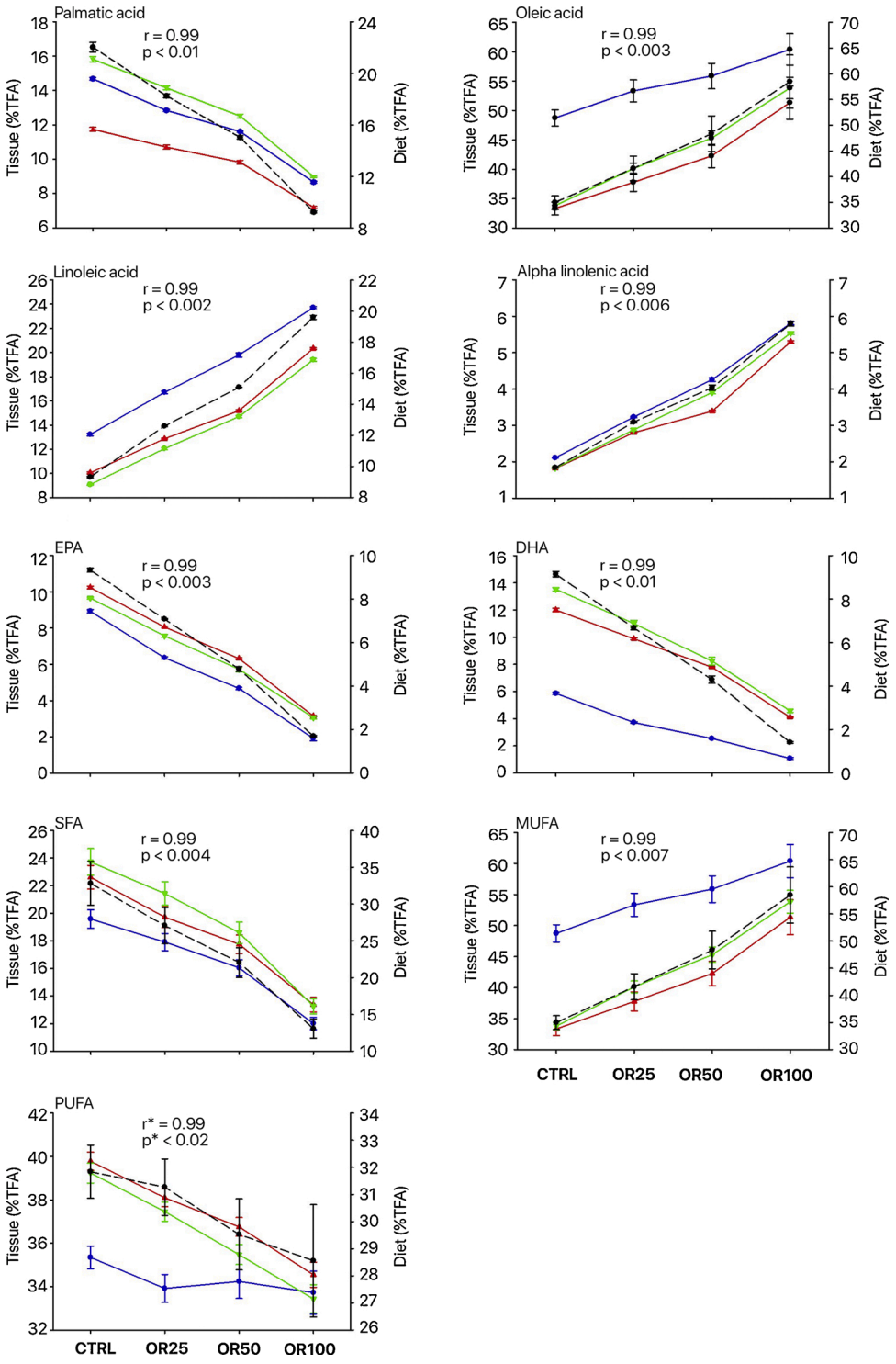

Fig. 1. Relationship between dietary FA level (black, dashed) and their respective FA levels in the liver (blue) whole body (red) and muscle (green) of palmitic (C16:0), oleic (C18:1 n-9), linoleic (18:2n-6), alpha-linolenic (18:3n-3), EPA (20:5n-3) and DHA (22:6n-3), as well as total amounts of SFA,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

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 triacylglycerol (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 salmon diets. 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 deficient in 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. 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. In general, variations in chemical composition of body and tissues in aquatic species depend on internal factors such as age, gender and size (Shearer, 1994). At the end of the experiment, fish fed RO100 was significantly smaller compared to the other dietary groups. Small fish tend to have a higher moisture content than bigger fish as water is replaced with lipid in growing salmon (Bjerkeng et al., 1997; Shearer, 1994). More noteworthy was the higher lipid content in fish fed RO100 concurrent with the higher water content. Increased lipid content is also previously reported in fish with high inclusion level of plant oils (Bell et al., 2003b; Kjær et al., 2008a; Sun et al., 2011; Todorčević et al., 2008).

5. 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.

CRediT authorship contribution statement

Florence Perera Willora: Conceptualization, Formal analysis, Investigation, Writing - original draft, Visualization. **Bjørn Grønevik:** Investigation. **Cui Liu:** Investigation. **Anjana Palihawadana:** Investigation. **Mette Sørensen:** Conceptualization, Methodology, Investigation, Resources, Writing - review & editing, Supervision, Project administration. **Ørjan Hagen:** Conceptualization, Investigation, Project administration, Resources, Supervision, Validation, Writing - original draft, Writing - review & editing.

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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