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Effect of partial replacement of dietary fish meal and oil by pumpkin kernel cake and rapeseed oil on fatty acid composition and metabolism in Arctic charr (*Salvelinus alpinus*)

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ABSTRACT

The aim of this 15-month feeding study was to investigate the effects of more sustainable feeds on specific growth rate, fatty acid composition and metabolism of Arctic charr (*Salvelinus alpinus*). A control feed, formulated with fish meal and fish oil (F1), was compared with feeds where the marine ingredients were increasingly replaced by pumpkin kernel cake and rapeseed oil (feeds F2, F3, and F4). Arctic charr were randomly distributed into 12 tanks and fed one of the feeds in triplicate. The biomass of fish fed F1 and F2 diets was significantly higher compared to fish fed with diet F4 which was the highest replacement level. However, the dorsal and ventral muscle tissues had very similar total saturated, monounsaturated, and polyunsaturated fatty acid (PUFA) contents, irrespective of dietary supply. Although diets F3 and F4 contained 6-fold less fish oil than diets F1 and F2, fish fed diets F3 and F4 retained only 2-fold less highly desired omega-3 (n-3) long-chain (LC)-PUFA in their dorsal and ventral muscle tissues. Incubating isolated hepatocytes with ¹⁴C-labelled α-linolenic acid (18:3n-3) provided evidence that Arctic charr can bioconvert this essential dietary PUFA to n-3 LC-PUFA, including docosahexaenoic acid. The results suggested that tissue fatty acid compositions in Arctic charr are dependent, not only on dietary fatty acid supply, but also on their ability for endogenous synthesis of n-3 LC-PUFA. Finally, this long-term feeding study indicated that feeds containing pumpkinseed press cake and rapeseed oil produced fish with largely similar fatty acid composition to fish fed diets containing higher contents of fish meal and fish oil.

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1. Introduction

The availability, cost and environmental sustainability of feed fish are some of the main bottlenecks preventing the expansion of the aquaculture industry (Tocher et al., 1997; Worm et al., 2006). Farmed carnivorous fish are traditionally fed diets containing large amounts of marine fish meal (FM) and fish oil (FO) (Torstensen et al., 2008). Fish meal is the major protein source in feeds, while FO provides the major source of lipids, including omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA). Both proteins and lipids derived from FM and FO serve a variety of important biological functions in fish and are important in human nutrition (Drevon, 1992; Nyina-Wamwiza et al., 2010). On the basis of increasing global FM and FO costs, alternative protein and lipid sources are required to ensure the economic and environmental viability of the aquaculture industry (Bendiksen et al., 2011; Tacon and Metian, 2008; Turchini et al., 2009).

Fish oil contains high amounts of n-3 LC-PUFA, such as eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) (Kaushik et al., 1995; Turchini et al., 2009) that are highly retained in farmed fish (Bell et al., 2003; Torstensen et al., 2005). Despite lacking n-3 LC-PUFA, vegetable oils (VO) have been proposed as sustainable alternatives to dietary FO (Torstensen et al., 2005) with various studies finding no deleterious impact on the health or growth rate of farmed fish when FO was replaced with VO (Bell et al., 2001; Seierstad et al., 2005; Torstensen et al., 2000; Waagbø et al., 1995). However, it is widely accepted that complete or partial replacement of FO with VO reduces particularly the n-3 LC-PUFA content of fish tissues (Bell et al., 2003, 2004; Mourente and Bell, 2006; Torstensen et al., 2005), which is a concern for the general fish condition and nutritional value to the consumer.

Although tissue fatty acid compositions are closely correlated with those of dietary supply, many fish, including Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*) can convert α-linolenic acid (ALA; 18:3n-3) to EPA and DHA, albeit rather inefficiently (Tocher, 2003). Understanding and utilising this biosynthetic pathway through the provision of VO-derived precursors may enable farmed fish to meet their physiological n-3 LC-PUFA requirements, even if these

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n–3 LC-PUFA are not sufficiently supplied within the diet (Tocher, 2003). Rapeseed oil appears to be a particularly effective alternative due to its lower cost, but higher sustainability and relatively high amounts of the essential n–3 LC-PUFA precursor ALA (Bell et al., 1997, 2001; Tocher et al., 2001a,b; Turchini et al., 2009).

Sustainable alternatives to FM include vegetable meals containing 20–50% crude protein, which can approach the levels found in FM typically fed to intensively reared fish (Hertrampf and Piedad-Pascual, 2000; Van Weerd, 1995). Fish meal can be partially or totally replaced with alternative plant protein sources without affecting the survival or growth rate of farmed fish (Fagbenro, 1999; Gomes et al., 1995; Kaushik et al., 1995; Nyina-Wamwiza et al., 2010). However, the use of plant derived protein sources as feed ingredients is limited by the presence of anti-nutritional factors (ANFs) that inhibit specific metabolic pathways, decreasing digestibility and nutrient absorption (Francis et al., 2001).

Methods such as cooking, dehulling, germination, roasting, soaking and extrusion cooking can reduce the presence of ANFs improving plant protein digestibility and utilisation by farmed fish (Nyina-Wamwiza et al., 2010). Many terrestrial meals, such as sunflower oil cake (Nyina-Wamwiza et al., 2010), palm kernel cake (Iluyemi et al., 2010), soybean seed meal (Robaina et al., 1995) and cottonseed meal (Robinson and Li, 1994), and recently pumpkin kernel cake are of particular interest as potential protein sources for farmed fish. Pumpkin seeds contain approximately 32% crude protein and, after oil extraction, up to 70% of dry matter in the kernel cake (Sharma et al., 1986). Furthermore, during a comparative nutritional study, Zdunczyk et al. (1999) reported that pumpkin kernel cake contained a higher crude protein content and fewer ANFs compared to soybean meal.

While many previous investigations identified how FM or FO replacements affected a variety of physical and biochemical variables, less is known about how dual replacement of both marine proteins and lipids with terrestrial alternatives affects the growth rate and fatty acid composition of farmed fish (Torstensen et al., 2008; Turchini et al., 2009). In addition, the use of pumpkin kernel cake as the main source of protein in feed has never been examined in farmed freshwater salmonids, such as Arctic charr (*Salvelinus alpinus*) that is increasingly farmed (FAO, 2012). Therefore, in the current study we address this question directly by examining the effect of partial replacement of dietary FM and FO with graded amounts of pumpkin kernel cake and rapeseed oil on the growth rate, tissue fatty acid profiles and metabolism in consumer-sized Arctic charr. Our null hypothesis was that there is no difference in the growth rate or tissue fatty acid profiles among the fish feeding on the different diets. Thus, our underlying assumption was that pumpkin kernel cake and rapeseed oil in fish feeds can fully replace commonly used FM and FO resulting in equal fish growth rates. In addition, fish provided with dietary rapeseed oil will endogenously convert dietary ALA to the n–3 LC-PUFA EPA and DHA and thus prevent any discernable differences in tissue fatty acid profiles compared to fish fed feeds containing typically high contents of FM and FO.

2. Materials and methods

2.1. Fish, husbandry and experimental diets

Arctic charr (15–20 g body weight) from the same strain (fish hatchery in Lunz am See, Austria) were held at the aquarium facilities at the WasserCluster Research Centre from August 2012 until October 2013. The experiment was conducted in a flow-through system containing twelve 1000-L rectangular tanks with a continuous supply of gravel filtered spring water (ca. 25 L min⁻¹). Waste water was drained using a sink hole covered by a 5 mm mesh screen. Fish were subjected to a natural photoperiod (latitude = 47.8604 °N), delivered by artificial fluorescent lighting and adjusted weekly. A total of 1200 juvenile Arctic charr were randomly distributed as 100 fish of mixed sexes per tank. Three replicate tanks per dietary treatment were used.

Table 1

Feed components (in %) of the gradual decrease in fish meal and increase in pumpkin kernel cake and rapeseed oil from feeds F1 to F4.

	F1	F2	F3	F4
Fish meal, anchovy, super prime, 67% CP	35.0	22.5	22.5	10.0
Pumpkin kernel cake, 59% CP, 11% C. lipids	–	12.5	12.5	25.0
Sunflower protein concentrate, 46% CP	16.8	13.8	13.8	11.0
Haemoglobin powder	7.5	7.5	7.5	7.5
Rapeseed cake, 32.5% CP, 9% CL	5.0	5.0	5.0	5.0
Wheat gluten 80% CP	–	3.34	3.34	6.27
Wheat, feed quality	10.5	9.7	9.7	8.5
Wheat feed flour	6.0	6.0	6.0	6.0
Fish oil (salmon oil)	18.1	17.8	3.0	3.0
Rapeseed oil	–	–	14.8	14.5
Monocalciumphosphate	–	0.6	0.6	1.45
Lysine–HCL	–	0.16	0.16	0.68
Premix	0.8	0.8	0.8	0.8
Diamol (marker)	0.3	0.3	0.3	0.3

Dissolved oxygen, pH and water temperature were recorded daily. Throughout this long-term feeding experiment, Arctic charr was exposed to natural variability of water temperature (3.7 °C to 12.3 °C; mean = 7.9 °C), dissolved oxygen (7.3 to 11.4 mg L⁻¹; mean = 9.2 mg L⁻¹) and circum-neutral pH values (6.7 to 7.7; mean = 7.4).

Four isocaloric fish feeds were formulated (Garant™, Austria) to provide sufficient lipid and protein to meet somatic requirements for salmonids (NRC, 2011). Fish in triplicate tanks were fed 1 of the 4 different diets that gradually contained less FM (35%–10%) and FO (i.e., salmon oil; 18%–3%; Table 1). Diets were dispensed daily into the tank by a clockwork belt feeder (Dryden Aqua Ltd) over a 12 h feeding period. The daily feed ration exceeded the recommended feeding rate for salmonids for the prevailing water temperature.

2.2. Sampling procedure

During the entire feeding experiment, every 2 weeks one third of the fish in each tank was randomly selected, weighed (g) and measured (cm) for the assessment of specific growth rates and biomass. The specific growth rate (SGR, % body weight day⁻¹) was calculated as $[(\ln W_1 - \ln W_0) / t] \times 100$, where W_0 and W_1 are weights in grams per fish at the start and at the end of the feeding period, respectively, and t is the time of feeding in days. Twelve fish were selected at random, 3 replicates per treatment, to determine lipid contents and fatty acid composition in liver as well as the dorsal and ventral muscle. A further 12 fish, 3 per treatment, were used for the preparation of isolated hepatocytes at the end of the trial. Fish were killed by a blow to the head, and a sub-sample of liver and muscle were dissected and stored in plastic vials (8 mL). Muscle samples were obtained by cutting a fillet from the fish and separating the two sections using the lateral line as a border between the dorsal and ventral tissue. Care was taken to prevent any skin or bone from being included in the sample. All tissue samples were stored at –40 °C overnight and freeze dried before analysis.

2.3. Proximate analysis

The gross nutrient composition of the four experimental diets was determined as below (Table 2). Moisture was determined by drying to constant weight in an oven at 110 °C for 24 h (Bell et al., 2003). Sample

Table 2

Proximate composition of experimental diets (g/100 g of diet).

	F1	F2	F3	F4
Protein	43.2 ± 1.0	43.7 ± 2.4	44.6 ± 2.1	44.0 ± 4.0
Lipid	25.1 ± 2.3	24.5 ± 1.4	24.4 ± 1.1	23.8 ± 3.4
Ash	10.2 ± 1.3	8.4 ± 0.0	8.0 ± 0.1	8.5 ± 0.9
Moisture	7.2 ± 0.3	5.8 ± 0.3	8.1 ± 0.3	8.8 ± 1.3

Table 3
Selected fatty acid contents (mg FA/g dry weight) of diets F1 to F4.

	F1	F2	F3	F4
<i>Fatty acids</i>				
14:0	7.0 ± 1.3	6.4 ± 1.6	2.4 ± 0.4	2.3 ± 0.4
16:0	23.3 ± 5.4	22.7 ± 3.6	17.2 ± 0.6	17.9 ± 3.1
18:0	4.7 ± 1.2	4.8 ± 1.6	4.2 ± 0.1	4.6 ± 0.1
<i>Total SFAFA contents^a</i>				
36.6 ± 8.2	35.3 ± 7.1	25.1 ± 1.3	26.2 ± 3.8	
16:1(n-7)	7.8 ± 1.3	7.2 ± 2.7	2.7 ± 0.1	2.5 ± 0.1
18:1(n-7)	54.5 ± 11.4	54.4 ± 1.1	84.6 ± 1.9	82.3 ± 3.3
18:1(n-7)	5.1 ± 1.2	4.9 ± 1.6	5.0 ± 0.8	4.3 ± 0.9
20:1(n-9)	4.3 ± 1.2	4.3 ± 1.6	2.1 ± 0.7	2.1 ± 0.7
22:1(n-9)	0.5 ± 0.0	0.5 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
24:1(n-9)	0.4 ± 0.0	0.4 ± 0.1	0.2 ± 0.0	0.2 ± 0.0
<i>Total MUFA contents^b</i>				
69.3 ± 14.4	68.5 ± 18.6	90.4 ± 2.8	88.0 ± 4.4	
18:2(n-6)	23.8 ± 11.0	25.1 ± 2.9	36.4 ± 1.0	38.9 ± 3.2
20:2(n-6)	3.1 ± 1.2	2.4 ± 0.7	1.0 ± 0.0	1.1 ± 0.4
20:4(n-6)	0.7 ± 0.1	0.5 ± 0.2	0.2 ± 0.0	0.2 ± 0.0
<i>Total (n-6)PUFA contents^c</i>				
30.0 ± 12.9	30.4 ± 4.2	38.5 ± 1.1	41.0 ± 4.1	
18:3(n-3)	7.4 ± 1.4	7.3 ± 2.7	12.0 ± 1.9	12.0 ± 2.1
20:5(n-3)	8.5 ± 1.8	7.0 ± 1.9	2.8 ± 0.2	2.1 ± 0.7
22:5(n-3)	1.7 ± 0.4	1.6 ± 0.4	0.5 ± 0.0	0.5 ± 0.1
22:6(n-3)	8.4 ± 1.5	7.0 ± 2.7	2.5 ± 0.8	2.0 ± 0.7
Total (n-3) PUFA contents ^d	26.5 ± 5.1	23.4 ± 7.7	17.8 ± 2.9	16.7 ± 3.7

Values are means of two replicate measurements.

^a Includes 12:0, 15:0, 20:0, 22:0 and 24:0.

^b Includes 16:1(n-9), 20:1(n-11) and 20:1(n-7).

^c Includes 18:3(n-6), 20:3(n-6) and 22:4(n-6).

^d Includes 20:3(n-3), 18:4(n-3) and 20:4(n-3).

weight was recorded before drying and after removal from the oven. Process was repeated at 1 h intervals until weight change was <5 mg. Total protein content in experimental diets was determined by a modified Bradford assay (Murray et al., 2013) and total lipids by solvent extraction and gravimetric determination (Heissenberger et al., 2010). Ash content was determined by placing pre-weighed diets in a muffle furnace at 550 °C for 8 h or until white ash was obtained (Bell et al., 2003) that was subsequently weighed.

2.4. Lipid extraction and fatty acid analysis

Total lipids from homogenised, freeze-dried liver samples (15–20 mg) and dorsal and ventral muscle samples (25–35 mg) were analysed as in Heissenberger et al. (2010). In brief, samples were sonicated and vortexed (4 ×) in a chloroform–methanol (2:1) mixture. Organic layers were removed and transferred into solvent-rinsed vials. For gravimetric determination of total lipid contents (i.e., mg lipids g dry weight⁻¹), subsamples (100 µL) of the extracts (duplicates) were evaporated and weighed. Fatty acids were derivatised to obtain fatty acid methyl esters (FAME) using toluene and sulphuric acid–methanol solution (1% v/v, 16 h at 50 °C). In contrast to Heissenberger et al. (2010), hexane without butylated hydroxytoluene (BHT) was used for each washing step after methylation to avoid BHT-related peak interference in chromatograms (data not shown). FAME were identified by comparison with known standards (Supelco37 FAME Mix) using a gas chromatograph (Thermo Scientific TRACE GC Ultra™) equipped with a flame ionisation detector (FID) and a Supelco™ SP-2560 column (100 m, 25 mm i.d., 0.2 µm film thickness). Quantification of FA was performed by comparison with a known concentration of the internal standard using Excalibur 1.4™ (Thermo Electron Corporation).

2.5. Preparation of isolated hepatocytes

Preparation of liver cells and fatty acid bioassay was carried out as described by Tocher et al. (2001a,b) with some modifications. In brief, fish were killed with a blow to the head and the liver was quickly dissected. The gall bladder was removed carefully and the liver was perfused using solution A (Hanks balanced salt solution (HBSS) + 10 mM HEPES), using a syringe fitted with a 2-gauge needle, to clear blood from the tissue. The liver was chopped finely with scissors and incubated with 20 mL of solution B (solution A + 1 mg mL⁻¹ collagenase) on an orbital shaker at ambient water temperature for 60 min. The digested liver was filtered through 100 µm nylon gauze and washed with solution C (solution A + 1% fatty acid free bovine serum albumin (FAF-BSA)). Hepatocyte cells were collected by centrifugation at 500 ×g for 2 min. The cell pellet was washed with 20 mL of solution A and re-centrifuged. The hepatocytes were re-suspended in 10 mL medium 199 containing 10 mM HEPES. A 100 µL aliquot of cell suspension was retained for protein determination using the modified Bradford assay (Bradford, 1976) described by Murray et al. (2013).

2.6. Assay of hepatocyte fatty acyl desaturation/elongation activities

Samples of 5 mL of each hepatocyte suspension were dispensed into a 25 cm² tissue culture flask. Hepatocytes were incubated with 0.25 µCi of [1-¹⁴C]18:3n-3 (ARC®, USA), added as a complex with FAF-BSA. After the addition of the isotope, the solution was mixed carefully and incubated at 10 °C for 1 h. After incubation, the cell layer was dislodged by gentle rocking and transferred to glass conical test tubes and the flasks washed with 1 mL ice-cold HBSS containing 1% FAF-BSA. The cell suspensions were centrifuged at 400 ×g for 4 min, the supernatant was decanted and the pellet washed in 5 mL ice-cold HBSS/FAF-BSA. The supernatant was discarded and tubes were placed upside down and carefully blotted dry for 15–20 s before lipid extraction as described above using the modified Heissenberger et al. (2010) method.

Total lipids were methylated and FAME prepared as described above. The methyl esters were re-dissolved in hexane (100 µL) and applied as 2.5 cm origins to a TLC plate impregnated with silver nitrate (2 g) in acetonitrile (20 mL) and pre-activated at 110 °C for 30 min. Plates were fully developed in toluene/acetonitrile (95:5, v/v). Autoradiography was performed with Kodak MR2 film for 6 days at room temperature. Silica corresponding to ALA, EPA and DHA was scraped into scintillation vials containing 2.5 mL of scintillation fluid (Ultima Gold™ AB, PerkinElmer®) and radioactivity was determined in a scintillation counter (model 1002A, PerkinElmer®). Results were corrected for counting efficiency, quenching of ¹⁴C and number of live hepatocyte cells.

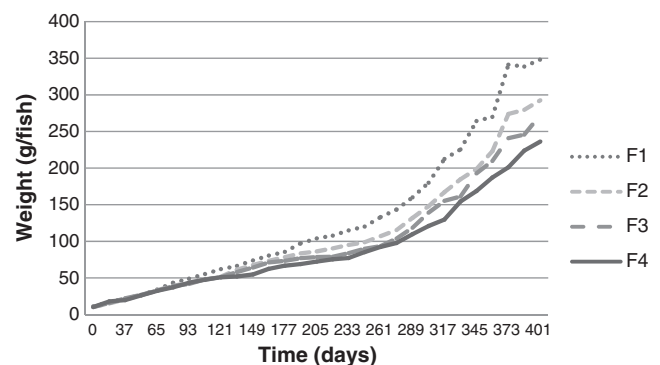


Fig. 1. Average biomass of Arctic charr fed diets containing decreasing concentrations of FM and FO and increasing concentrations of pumpkin kernel cake and rapeseed oil (F1 → F4).

Table 4
Total lipid and fatty acid contents (mg FAME/g dw) of dorsal and ventral muscle tissue from fish fed the different diets (F1–F4).

	Dorsal				Ventral			
	F1	F2	F3	F4	F1	F2	F3	F4
Total lipids	72.8 ± 16.5	83.1 ± 38.9	77.8 ± 13.1	72.8 ± 17.9	86.5 ± 21.7	84.2 ± 26.6	223.1 ± 118.9	103.1 ± 66.8
SAFA	9.4 ± 3.0	10.9 ± 5.1	8.8 ± 1.1	8.5 ± 1.4	10.5 ± 2.0	11.2 ± 4.4	25.2 ± 11.5	11.3 ± 7.1
MUFA	5.4 ± 2.4	6.9 ± 3.7	4.6 ± 0.6	4.3 ± 0.9	6.1 ± 2.7	6.9 ± 2.7	14.7 ± 6.6	5.9 ± 4.2
PUFA	22.8 ± 8.0	28.4 ± 17.7	25.9 ± 10.1	27.0 ± 6.6	29.1 ± 10.7	28.7 ± 11.6	109.6 ± 73.1	41.9 ± 37.8
n–3 PUFA	13.8 ± 4.2	14.7 ± 5.3	11.7 ± 0.5	10.1 ± 1.2	14.9 ± 2.0	14.8 ± 5.3	27.3 ± 9.2	12.0 ± 5.9
n–6 PUFA	25.5 ± 8.4	32.8 ± 20.3	30.5 ± 12.9	33.4 ± 8.4	32.7 ± 13.2	32.9 ± 13.2	129.6 ± 89.0	51.5 ± 46.7
ALA	1.2 ± 0.4	1.5 ± 0.9	1.5 ± 0.7	1.5 ± 0.4	1.6 ± 0.7	1.5 ± 0.5	6.2 ± 4.1	2.1 ± 1.9
EPA	2.6 ± 0.9	3.0 ± 0.9	2.1 ± 0.3	1.8 ± 0.2	0.4 ± 0.0 ^{ab}	0.5 ± 0.1 ^{ab}	0.6 ± 0.1 ^a	0.2 ± 0.1 ^b
DHA	9.9 ± 2.3	9.3 ± 1.8	7.8 ± 2.0	6.3 ± 0.3	9.0 ± 0.6	9.4 ± 3.1	10.6 ± 2.1	6.3 ± 0.8

Values are mean ± S.D. Values in the same row with different superscript letters are significantly different ($P < 0.05$).

2.7. Data analysis

Principle components analysis (PCA) was used to reduce the number of individual FA into a single FA composition score (Adams et al., 2007; Turnbull et al., 2005) and used to analyse the difference between dietary and tissue FA compositions. Significant differences between dietary treatments were determined by one-way ANOVA. Differences between means were determined by Tukey's HSD test. Data identified as nonhomogeneous, using a variance test, were subjected to log transformation before applying the statistical tests. The Minitab®16 statistical software package was used for data analysis. Fatty acid retention ratios were determined as the quotient of fatty acids in fish muscle tissues (mg FA per unit biomass) and fatty acids in the respective diet. We define retention as the ability of fish to regulate and control ingested fatty acids.

3. Results

3.1. Diet composition

All feeds contained similar contents of total proteins (~43–45%), total lipids (~23–25%), total ash (~8–10%), and moisture (~6–9%; Table 2). The contents (mg FA per unit biomass) for total saturated fatty acids (SAFA) decreased 1.4-fold from diets F1 to F4 (Table 3). There was a 1.6-fold decrease in total n–3 PUFA contents between diets F1 and F4, specifically a 4.0 and 4.2-fold decrease in DHA and EPA, respectively (Table 3). Alternatively, total monounsaturated fatty acid (MUFA) contents increased 1.3-fold, n–6 PUFA by 1.4-fold and ALA by 1.6-fold between diets F1–F4 (Table 3).

3.2. Biomass and specific growth rate

After 191 days of feeding, fish biomass started differing significantly among the 4 dietary treatments ($F_{[3-11]} = 11.03$; $R^2 = 0.805$; $P = 0.003$) (Fig. 1). Fish fed diets F4 (69.2 ± 8.9) and F3 (77.8 ± 8.2) diets had a significantly lower biomass (mean g fish⁻¹ ± SD) than fish fed diet F1 (97.6 ± 2.8). This trend continued to the end of the experiment

and fish biomass was significantly lower ($F_{[3-11]} = 26.09$; $R^2 = 0.873$; $P < 0.001$) for fish feeding on F4 (236.3 ± 17.0 g fish⁻¹), higher for F2 (291.9 ± 12.5 g fish⁻¹) and highest for F1 (350.0 ± 22.8 g fish⁻¹) (Fig. 1). Biomass of fish fed F3 (270.3 ± 8.0 g fish⁻¹) was also significantly smaller than fish from F1 tanks, but not from F2 or F4 tanks (Fig. 1).

Specific growth rates for the entire feeding period (with water temperatures ranging from 3.7 °C to a maximum of 12.3 °C) were highest in F1 fish ($0.86 \pm 0.01\%$) and decreased gradually in fish fed F2 ($0.83 \pm 0.01\%$), F3 ($0.81 \pm 0.02\%$), and F4 ($0.78 \pm 0.02\%$). Fish fed F4 had significantly lower SGR than F1 and F2 fish ($F_{[3-11]} = 8.19$; $R^2 = 0.66$; $P = 0.008$), but not significantly different than F3 fish.

Regression analysis showed no linear relationship between fish weight and dietary or tissue lipid contents or any individual fatty acids or fatty acid groups (including MUFA, SAFA, PUFA, n–3 PUFA, n–6 PUFA, ALA, EPA and DHA) (data not shown).

3.3. Total lipid content and fatty acid composition

There were no significant differences in total lipid contents in dorsal or ventral muscle tissue between dietary treatment groups (Table 4). In dorsal muscle tissue there was no significant difference in the content of SAFA, MUFA, PUFA, n–3 PUFA, n–6 PUFA or individual FA (ALA, EPA and DHA) among dietary treatments (Table 4). Fish fed diet F3 had higher EPA in their ventral muscle compared to fish fed diet F4 ($F_{[3-11]} = 4.45$; $R^2 = 0.630$; $P < 0.05$) (Table 4). There was no significant difference in the content of fatty acid groups, ALA or DHA in the ventral muscle of fish fed F1–F4 diets.

In dorsal muscle, F3 and F4 fish retained more DHA compared to F1 and F2 fish ($F_{[3-11]} = 23.73$; $R^2 = 0.861$; $P < 0.001$; Table 5). Retention of EPA in dorsal muscle was also higher in F3 and F4 fish compared to F1 fish ($F_{[3-11]} = 11.79$; $R^2 = 0.746$; $P = 0.003$) (Table 5). Retention of DHA in ventral muscle of F3 and F4 fish was higher than that of F1 and F2 fish ($F_{[3-11]} = 33.96$; $R^2 = 0.900$; $P < 0.001$), and F3 and F4 fish also retained more EPA in ventral muscle than F1 and F2 fish ($F_{[3-11]} = 22.19$; $R^2 = 0.853$; $P < 0.001$) (Table 5). SAFA ($F_{[3-11]} = 4.95$; $R^2 = 0.650$; $P = 0.031$) ventral muscle retention ratios were

Table 5
Fatty acid retention ratios of dorsal and ventral muscle tissue from fish fed 4 different diets (F1–F4).

	Dorsal				Ventral			
	F1	F2	F3	F4	F1	F2	F3	F4
SAFA	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.0	0.3 ± 0.1	0.3 ± 0.1 ^a	0.3 ± 0.1 ^a	1.0 ± 0.5 ^b	0.4 ± 0.3 ^{ab}
MUFA	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0
PUFA	0.4 ± 0.2	0.5 ± 0.3	0.5 ± 0.2	0.5 ± 0.1	0.5 ± 0.2	0.5 ± 0.2	2.0 ± 1.3	0.7 ± 0.7
n–3 PUFA	0.5 ± 0.2	0.6 ± 0.2	0.7 ± 0.0	0.6 ± 0.1	0.6 ± 0.1 ^a	0.6 ± 0.2 ^a	1.5 ± 0.5 ^b	0.7 ± 0.4 ^a
n–6 PUFA	0.8 ± 0.3	1.1 ± 0.7	0.8 ± 0.3	0.8 ± 0.3	1.1 ± 0.4	1.1 ± 0.4	3.4 ± 2.3	1.3 ± 1.1
ALA	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	0.5 ± 0.3	0.2 ± 0.2
EPA	0.3 ± 0.1 ^a	0.4 ± 0.1 ^{ab}	0.8 ± 0.1 ^{bc}	0.8 ± 0.1 ^c	0.3 ± 0.0 ^a	0.4 ± 0.1 ^a	1.3 ± 0.2 ^b	0.9 ± 0.3 ^b
DHA	1.2 ± 0.3 ^a	1.3 ± 0.3 ^a	3.2 ± 0.8 ^b	3.2 ± 0.2 ^b	1.1 ± 0.1 ^a	1.3 ± 0.4 ^a	4.3 ± 0.8 ^b	3.2 ± 0.4 ^b

Values are mean ± S.D. Values in the same row with different superscript letters are significantly different ($P < 0.05$).

higher in F3 fish compared to F1 and F2 fish. F3 fish also retained more $n-3$ PUFA in their ventral muscle compared to F1 and F2 fish ($F_{[3-11]} = 5.42$; $R^2 = 0.693$; $P = 0.025$) (Table 5).

3.4. Dietary versus muscle tissue FA compositions

The fatty acid compositions of muscle tissue did not fully reflect dietary fatty acid compositions. There was no significant linear relationship between muscle fatty acid scores and dietary fatty acid scores. Dorsal muscle principle component (PC) scores from fish fed the F1 ($F_{[1-3]} = 154.54$; $R^2 = 0.981$; $P = 0.001$), F2 ($F_{[1-3]} = 96.59$; $R^2 = 0.970$; $P = 0.002$), F3 ($F_{[1-3]} = 1171.0$; $R^2 = 0.997$; $P < 0.001$) and F4 ($F_{[1-3]} = 1033.48$; $R^2 = 0.997$; $P < 0.001$) treatments contained significantly different fatty acid compositions to those present within dietary PC scores (Fig. 2). PC scores for F1 ($F_{[1-3]} = 164.98$; $R^2 = 0.982$; $P = 0.001$) and F2 ($F_{[1-3]} = 123.88$; $R^2 = 0.976$; $P = 0.002$) ventral muscle FA were significantly different to corresponding dietary fatty acid scores, but there was no significant differences between F3 and F4 ventral muscle and dietary scores (Fig. 3).

3.5. Hepatocyte fatty acid desaturation/elongation activities

The LC-PUFA biosynthesis activity in hepatocytes, determined at the end of the trial, was highest in liver cells of fish fed diet F4 albeit not significantly (Fig. 4). Production of EPA was higher than that of DHA in all treatments. There was no significant linear relationship between desaturation/elongation activity and individual dietary FA concentrations (ALA, EPA and DHA) or physical variables (weight and length) (data not shown).

4. Discussion

This study demonstrated that partial replacement of FM and FO with pumpkin kernel cake and rapeseed oil resulted in reduced specific growth rates and a decrease in Arctic charr biomass, particularly with the highest inclusion levels in diet F4, compared to fish fed the F1 diet. These results are in contrast to previous studies that showed no significant impact of individual replacement of either vegetable meals (Gomes et al., 1995; Guillou et al., 1995; Kaushik et al., 1995) or rapeseed oil (Pettersson et al., 2009) on growth rate or final fish weights in farmed fish. It is suggested that preferential retention of DHA and EPA in muscle tissues indicates that Arctic charr are either sufficiently supplied with dietary DHA and EPA by all test diets and/or able to endogenously convert dietary ALA to $n-3$ LC-PUFA.

The F4 diet yielded lower fish biomass than diets containing >2 -fold more FM and 6-fold more marine FO (i.e., F1 and F2), which suggests that such a decrease of dietary biochemical quality had a negative effect

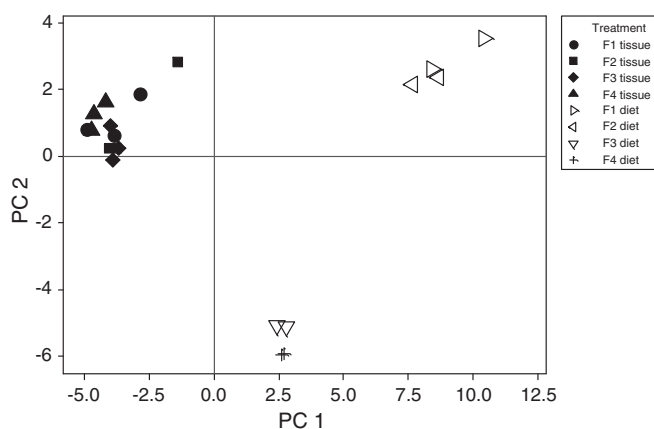


Fig. 2. Principle components analysis of dorsal muscle tissue and dietary fatty acid compositions.

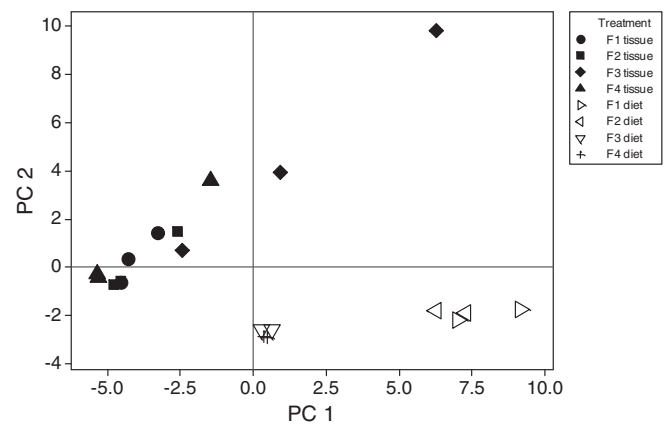


Fig. 3. Principle components analysis of ventral muscle tissue and dietary fatty acid compositions.

on fish biomass accrual. Differences in specific growth rates were also observed during individual time points within the study. Fish fed F1, F2 and F3 diets had significantly higher specific growth rates after 37 days compared to fish fed F4 diets. However, these differences were not consistently observed and only identified again after 373 days, whereby only F1 fish had higher specific growth rates than F4 fish. Nevertheless, these results suggest that the reduction of FM and/or inclusion of rapeseed oil in F3 and F4 resulted in a variable reduction of specific growth rate in Arctic charr compared to those fed F1 diet.

The majority of studies examining the effect of dietary VO on specific growth rates of farmed fish were performed over relatively short periods of time (Turchini et al., 2009). For example, studies reporting no significant differences in specific growth rates between fish consuming commercial feeds and feeds containing a wide span of rapeseed oil (14–100% of added oil) were performed for between 12 and 21 weeks (Bell et al., 2001; Tocher et al., 2000, 2001a,b; Torstensen et al., 2000). Results of the current study which are based on Arctic charr grown to their harvest weight for 400 days, indicated that the impact of reduced dietary FM and increased rapeseed oil on specific growth rates may be time dependent. This argument is supported by a study by Bell et al. (2003), which found that after 50 weeks of feeding Atlantic salmon fed diets containing 100% rapeseed oil or 100% VO blend (linseed oil/rapeseed oil, 2:1) had significantly higher final weights compared to fish fed 100% FO. The higher final weights of fish reported by Bell et al. (2003) may have been caused by the relatively high FM contained in the feeds used in the earlier trial compared to the current study. Therefore, the lower growth rate in the present study was probably more a consequence of the replacement of FM than the replacement of FO. Pumpkins contain high contents of neutral detergent fibre and acid detergent fibre (Saura-Calixto et al., 1983) that affect digestive functions

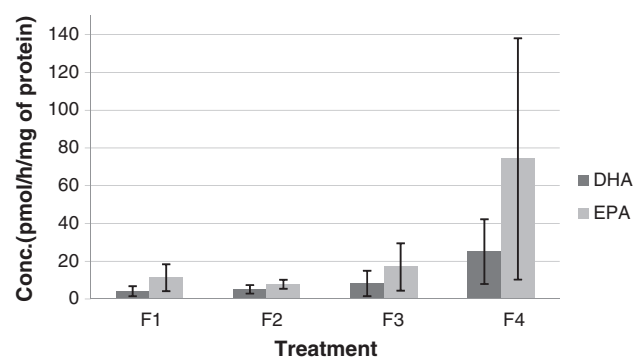


Fig. 4. Production (mean \pm SD) of EPA and DHA from ^{14}C -labelled ALA by isolated hepatocytes from Arctic charr fed diets containing decreasing amounts of fish meal and fish oil.

by increasing intestinal flow rates (Liener 1969, Pusztai et al., 2004; Krogdahl, 1989; Nyina-Wamwiza et al., 2010), which may reduce the retention of dietary nutrients (Krogdahl, 1989; Francis et al., 2001). This suggests that a 2-fold increase in pumpkin kernel cake in the present study may have affected nutrient absorption and general metabolism resulting in the lower growth rates of Arctic charr fed F4 diets in comparison to fish fed with a higher content of FM.

Dietary fatty acid compositions did not fully predict the fatty acid compositions in dorsal muscle tissues of the Arctic charr. Furthermore, there were no significant differences in dorsal muscle fatty acid contents among treatments, but the retention of DHA and EPA was between 3- and 4-fold higher in fish fed diet F4 compared to fish fed F1. In ventral muscle tissue, F1 and F2 ventral fatty acid compositions were significantly different from the associated diets. Also, fish fed diets F1 and F2 retained 3 × less DHA and EPA in their ventral muscle tissue compared to fish fed diets F3 and F4. Differences between dietary and tissue fatty acid compositions and retention ratios are possibly due to differences in lipid classes within different muscle tissues. Leaner dorsal tissues contain more polar lipids which act as building blocks of cell membranes, while more fatty ventral muscle tissues are predominantly neutral lipids which are used for energy storage (Kiessling et al., 2001; Testi et al., 2006). It is likely that particular fatty acids are regulated to meet species-specific cell requirements and thus not a 'simple' function of dietary fatty acid supply.

Although there was a clear trend, there was no statistically significant difference in tissue contents of DHA between fish charr fed diets containing 15% rapeseed oil, which does not contain DHA, (F3 and F4) and fish fed F1 and F2 containing only fish oil (18%), which has large amounts of DHA. Fish fed diets F3 and F4 retained between 3- and 4-fold more DHA in their dorsal and ventral muscle tissues compared to fish fed diets without rapeseed oil (F1 and F2). Fatty acid composition in muscle tissues can vary due to species, size, age-specific differences and selective retention and/or metabolism of individual fatty acids in fish (Bell et al., 2001, 2002), thus suggesting that fish with lower dietary DHA supply have higher activity of fatty acyl transferases for DHA or, more likely, relative resistance of DHA to β-oxidation as a result of the complex metabolic pathway of this fatty acid (Tocher et al., 2001a,b).

Diet is known to directly affect desaturase enzyme activity in mammals (Brenner, 1981). Previous studies have shown that increasing dietary content of VO and VO blends, increased desaturation and elongation activity in salmonid hepatocytes (Bell et al., 1997; Leaver et al., 2011; Tocher et al., 1997, 2000). In the present study, there was also a trend for increased hepatic conversion of ALA to DHA by partially replacing FO with rapeseed oil. However, there was also a large amount of individual variation within treatments that prevented the results from being significantly different, suggesting that the ability to convert ALA to DHA is not entirely driven by dietary VO concentrations. Previous studies have reported that Arctic charr populations are highly variable with many intra-population life-history strategies, phenotypic plastic traits and an increased potential for sympatric morphological divergence (Adams et al., 2003; Jonsson and Jonsson, 2001; Skulason and Smith, 1995). In addition, Morais et al. (2011) found that expression of genes associated with LC-PUFA metabolism was differentially affected by diet but that genetic background of the fish was also a strong influencing factor. In the current study, genotypic factors, such as gene regulation of desaturases (Morais et al., 2011; Zheng et al., 2005), may have influenced the ability of individual Arctic charr to convert ALA to DHA, irrespective of dietary rapeseed oil concentrations.

In summary, the present study suggests that inclusion of 25% pumpkin kernel cake and 15% rapeseed oil with 10% FM and 3% FO in the diets of Arctic charr, over an entire life-cycle, reduces their growth rate and biomass. However, dietary inclusion of 12.5% pumpkin kernel cake produced fish with similar specific growth rates and biomass compared to fish fed with standard commercial diets containing mainly FM and FO. Although there was a downward trend, the inclusion of 15% rapeseed oil with a 6-fold reduction in FO in diets for Arctic charr did not

significantly reduce EPA and DHA contents in muscle tissues, which clearly points to selective retention of DHA and, to a certain extent, EPA. Combined with an observed trend in generally increased hepatic conversion of ALA to EPA and DHA in fish fed diets containing rapeseed oil, the results indicated that the nutritional benefits of n – 3 LC-PUFA in Arctic charr supplied with pumpkinseed kernel cake and rapeseed oil will not be considerably reduced and thus the fish will retain health benefits for human consumers.

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