Murray DS, Hager H, Tocher DR & Kainz MJ (2014) 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), *Aquaculture*, 431, pp. 85-91.

This is the peer reviewed version of this article

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*)

D.S. Murray[a], H. Hager[a], D.R. Tocher[b], M.J. Kainz[a]

[a] *WasserCluster – Biologische Station Lunz, 3929 Lunz am See, Austria.*

[b]*Institute of Aquaculture, University of Stirling, Stirling, FK9 4LA, Scotland, UK.*

Email: david.murray@wcl.ac.at; hannes.hager@wcl.ac.at; d.r.tocher@stir.ac.uk; martin.kainz@donau-uni.ac.at

Corresponding author: David S. Murray

Office phone number: (+43) 7486-20060

Mobile number: (+43) 6802-202422

Fax number: (+43) 7486-2006020

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 diet F4 with 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 ^{14}C-labeled 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.

Keywords: fatty acids; physiology; tissue; retention.

1. Introduction

The availability, cost and environmental sustainability of feed fish are some of the main bottlenecks preventing the expansion of aquaculture industry (Tocher 2009; 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 (Tacon *et al.*, 2006; 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.*, 2004). 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; Seirestad *et al.*, 2005; Torstensen *et al.*, 2000, Waagbo *et al.*, 1991). 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, 2004; 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 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*., 2001; 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 (Hertrampt and Pascual, 2003; 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 (Iluymi *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 (Sharama *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 2010). 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 natural photoperiod (latitude = 47.8604 ^{o}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.

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 ^{o}C to 12.3 ^{o}C; mean = 7.9 ^{o}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^{TM}, 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 hr 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 wks 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 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 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 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.

169

170 *2.4. Lipid extraction and fatty acid analysis*

171 Total lipids from homogenised, freeze-dried liver samples (15-20 mg) and dorsal
172 and ventral muscle samples (25-35 mg) were analysed as in Heissenberger *et al*.
173 (2010). In brief, samples were sonicated and vortexed (4X) in a chloroform-
174 methanol (2:1) mixture. Organic layers were removed and transferred into
175 solvent-rinsed vials. For gravimetrical determination of total lipid contents (i.e.,
176 mg lipids g dry weight^{-1}), subsamples (100 µL) of the extracts (duplicates) were
177 evaporated and weighed. Fatty acids were derivatised to obtain fatty acid
178 methyl esters (FAME) using toluene and sulphuric acid-methanol solution (1%
179 v/v, 16 h at 50°C). In contrast to Heissenberger *et al*. (2010), hexane without
180 butylated hydroxytoluene (BHT) was used for each washing step after
181 methylation to avoid BHT-related peak interference in chromatograms (data not
182 shown). FAME were identified by comparison with known standards (Supelco37
183 FAME Mix) using a gas chromatograph (Thermo Scientific TRACE GC Ultra™)
184 equipped with a flame ionisation detector (FID) and a Supelco™ SP-2560 column
185 (100 m, 25 mm i.d., 0.2 µm film thickness). Quantification of FA was performed
186 by comparison with a known concentration of the internal standard using
187 Excalibur 1.4™ (Thermo Electron Corporation).
188
189 *2.5. Preparation of isolated hepatocytes*

190 Preparation of liver cells and fatty acid bioassay was carried out as described by
191 Tocher *et al*. (2001) with some modifications. In brief, fish were killed with a
192 blow to the head and the liver was quickly dissected. The gall bladder was
193 removed carefully and the liver was perfused using solution A (Hanks balanced
194 salt solution (HBSS) +10 mM HEPES), using a syringe fitted with a 2-gauge needle,
195 to clear blood from the tissue. The liver was chopped finely with scissors and
196 incubated with 20 ml of solution B (solution A + 1 mg mL^{-1} collagenase) on an
197 orbital shaker at ambient water temperature for 60 min. The digested liver was
198 filtered through 100 µm nylon gauze and washed with solution C (solution A + 1 %
199 fatty acid free bovine serum albumin (FAF-BSA)). Hepatocyte cells were
200 collected by centrifugation at 500 x g for 2 min. The cell pellet was washed with
201 20 mL of solution A and re-centrifuged. The hepatocytes were re-suspended in

10 ml medium 199 containing 10mM 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 cm^2 tissue culture flask. Hepatocytes were incubated with 0.25 µCi of [1-^{14}C]18:3n-3 (ARC®, USA), added as a complex with FAF-BSA. After 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 x 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 ^{14}C and number of live hepatocyte cells.

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 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 acids (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 F1 tanks, but not 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 ± 0.01

%) and decreased gradually in fish fed F2 (0.83 ± 0.01 %), F3 (0.81 ± 0.02 %), and F4 (0.78 ± 0.02 %). Fish fed F4 had significantly lower SGR than F1 and F2 fish ($F_{[3\text{-}11]} = 8.19$; $R^2 = 0.66$; $p = 0.008$), 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 ($F_{[3\text{-}11]} = 4.45$; $R^2 = 0.630$; $P < 0.05$)(Table 4). There was no significant difference in 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 ($F_{[3\text{-}11]} = 23.73$; $R^2 = 0.861$; $P < 0.001$; Table 5). Retention of EPA in dorsal muscle was also higher in F3 and F4 fish compared to F1 fish ($F_{[3\text{-}11]} = 11.79$; $R^2 = 0.746$; $P = 0.003$) (Table 5). Retention of DHA in ventral muscle of F3 and F4 fish was higher than that of F1 and F2 fish ($F_{[3\text{-}11]} = 33.96$; $R^2 = 0.900$; $P < 0.001$), F3 and F4 fish also retained more EPA in ventral muscle than F1 and F2 fish ($F_{[3\text{-}11]} = 22.19$; $R^2 = 0.853$; $P < 0.001$) (Table 5). SAFA ($F_{[3\text{-}11]} = 4.95$; $R^2 = 0.650$; $P = 0.031$) ventral muscle retention ratios were 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 ($F_{[3\text{-}11]} = 5.42$; $R^2 = 0.693$; $P = 0.025$) (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

292 component (PC) scores from fish fed the F1 ($F_{[1-3]}$ = 154.54; R^2 = 0.981; P =
293 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 <
294 0.001) and F4 ($F_{[1-3]}$ = 1033.48; R^2 = 0.997; P < 0.001) treatments contained
295 significantly different fatty acid compositions to those present within dietary PC
296 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
298 to corresponding dietary fatty acid scores, but there was no significant
299 differences between F3 and F4 ventral muscle and dietary scores (Fig. 3).

300 *3.5. Hepatocyte fatty acid desaturation/elongation activities*

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

307

308 **4. Discussion**

309 This study demonstrated that partial replacement of FM and FO with pumpkin
310 kernel cake and rapeseed oil resulted in reduced specific growth rates and a
311 decrease in Arctic charr biomass, particularly with the highest inclusion levels in
312 diet F4, compared to fish fed the F1 diet. These results are in contrast to
313 previous studies that showed no significant impact of individual replacement of
314 either vegetable meals (Gomes *et al.*, 1995; Guillou *et al.*, 1995; Kaushik *et al.*,
315 1995) or rapeseed oil (Pettersson *et al.*, 2009) on growth rate or final fish
316 weights in farmed fish. It is suggested that preferential retention of DHA and
317 EPA in muscle tissues indicates that Arctic charr are either sufficiently supplied
318 with dietary DHA and EPA by all test diets and/or able to endogenously convert
319 dietary ALA to n-3 LC-PUFA.

320 The F4 diet yielded lower fish biomass than diets containing >2-fold more FM and
321 6-fold more marine FO (i.e., F1 and F2), which suggests that such a decrease of

dietary biochemical quality had a negative effect 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, 2001; Torstensen *et al*., 2000). Results of the current study 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 wks 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 (Suara-Calixto *et al*. 1983) that affect digestive functions by increasing intestinal flow rates (Lienner 1980, Huisman *et al*. 1989, Krogdahl 1989; Nyina-Wamwiza *et al*. 2010), which may reduce the retention of dietary nutrients (Krogdahl 1989; Meyer *et al*., 1988). 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 the higher 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 3x 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 muscles 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 ß-oxidation as a result of the complex metabolic pathway of this fatty acid (Tocher *et al.*, 2001).

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; Alexander and Adams, 2000; Skulason and Smith, 1995). In addition, Morais *et al.* (2011) found that expression of genes associated with LC-PUFA metabolism were 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.

Acknowledgements

This work received financial support by the Austrian Ministry of Life (project Nr. 100837; BMLFUW-LE.1.3.2/0051-II/1/2012) and GARANT Tiernahrung Austria. We are grateful for technical assistance and support from Eduard Schneeberger, and veterinary supervision by Heinz Heistinger. We also thank Katharina Drucker, Katharina Hader, Katharina Winter, and Zahra Changizi for their laboratory assistance.

References

Adams, C.E., Woltering, C., Alexander, G., 2003. Epigenetic regulation of trophic morphology through feeding behaviour in Arctic charr, *Salvelinus alpinus*. Biological Journal of the Linnean Society 78, 43-49.

Adams, C.E., Turnbull, J.F., Bell, A., Bron, J.E., Huntingford, F.A., 2007. Multiple determinants of welfare in farmed fish: stocking density, disturbance, and aggression in Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquatic Sci. 64, 336-344.

Bell, J.G., Tocher, D.R., Farndale, B., Cox, D., McKinney, R., Sargent, J., 1997. The effect of dietary lipid on polyunsaturated fatty acid metabolism in Atlantic salmon (*Salmo salar*) undergoing parr-smolt transformation. Lipids 32, 515-525.

Bell, J.G., McEvoy, J., Tocher, D.R., McGhee, F., Campbell, P.J., Sargent, J.R., 2001. Replacement of Fish Oil with Rapeseed Oil in Diets of Atlantic Salmon (*Salmo salar*) Affects Tissue Lipid Compositions and Hepatocyte Fatty Acid Metabolism. J. Nutr. 131, 1535-1543.

Bell, J.G., Henderson, R.J., Tocher, D.R., McGhee, F., Dick, J.R., Porter, A., Smullen, R.P., Sargent, J.R., 2002. Substituting Fish Oil with Crude Palm Oil in the Diet of Atlantic Salmon (*Salmo salar*) Affects Muscle Fatty Acid Composition and Hepatic Fatty Acid Metabolism. J. Nutr. 132, 222-230.

Bell, J.G., Tocher, D.R., Henderson, R.J., Dick, J.R., Crampton, V.O., 2003. Altered Fatty Acid Compositions in Atlantic Salmon (*Salmo salar*) Fed Diets Containing Linseed and Rapeseed Oils Can Be Partially Restored by a Subsequent Fish Oil Finishing Diet. J. Nutr. 133, 2793-2801.

Bell, J.G., Henderson, R.J., Tocher, D.R., Sargent, J.R., 2004. Replacement of dietary fish oil with increasing levels of linseed oil: Modification of flesh fatty acid compositions in Atlantic salmon (*Salmo salar*) using a fish oil finishing diet. Lipids 39, 223-232.

Bendiksen, E.Ã., Johnsen, C.A., Olsen, H.J., Jobling, M., 2011. Sustainable aquafeeds: Progress towards reduced reliance upon marine ingredients in

453 diets for farmed Atlantic salmon (*Salmo salar* L.). Aquaculture 314, 132-
454 139.

455 Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of
456 microgram quantities of protein utilizing the principle of protein-dye
457 binding. Analyt. Biochem. 72, 248-254.

458 Brenner, R.R., 1981. Nutritional and hormonal factors influencing desaturation
459 of essiential fatty acids. Prog. Lipid Res. 20, 41-47.

460 Burel, C., Boujard, T., Tulli, F., Kaushik, S.J., 2000. Digestibility of extruded
461 peas, extruded lupin, and rapeseed meal in rainbow trout (*Oncorhynchus*
462 *mykiss*) and turbot (*Psetta maxima*). Aquaculture 188, 285-298.

463 Drevon, C.A., 1992. Marine Oils and Their Effects. Nutrition Rev. 50, 38-45.

464 Fagbenro, O.A., 1999. Comparative evaluation of heat-processed Winged bean
465 (*Psophocarpus tetragonolobus*) meals as partial replacement for fish meal
466 in diets for the African catfish (*Clarias gariepinus*). Aquaculture 170, 297-
467 305.

468 FAO, 2012. State of world aquaculture, FAO fisheries technical paper, Rome, pp.
469 209.

470 Francis, G., Makkar, H.P.S., Becker, K., 2001. Antinutritional factors present in
471 plant-derived alternate fish feed ingredients and their effects in fish.
472 Aquaculture 199, 197-227.

473 Folch, J., Lees, M., Stanley, G.H.S., 1957. A simple method for the isolation and
474 purification of total lipdes from animal tissue. J. Biol. Chem. 226, 497-
475 509.

476 Gomes, E.d.F., Rema, P., Kaushik, S.J., 1995. Replacement of fish meal by plant
477 proteins in the diet of rainbow trout (*Oncorhynchus mykiss*): digestibility
478 and growth performance. Aquaculture 130, 177-186.

479 Guillou, A., Soucy, P., Khalil, M., Adambounou, L., 1995. Effects of dietary
480 vegetable and marine lipid on growth, muscle fatty acid composition and
481 organoleptic quality of flesh of brook charr (*Salvelinus fontinalis*).

Aquaculture 136, 351-362.

Izquierdo, M.S., Obach, A., Arantzamendi, L., Montero, D., Robaina, L., Rosenlund, G., 2003. Dietary lipid sources for seabream and seabass: growth performance, tissue composition and flesh quality. Aquacult. Nutr. 9, 397-407.

Jonsson, B., Jonsson, N., 2001. Polymorphism and speciation in Arctic charr. J. Fish Biol. 58, 605-638.

Kaushik, S.J., Cravedi, J.P., Lalles, J.P., Sumpter, J., Fauconneau, B., Laroche, M., 1995. Partial or total replacement of fish meal by soybean protein on growth, protein utilization, potential estrogenic or antigenic effects, cholesterolemia and flesh quality in rainbow trout, *Oncorhynchus mykiss*. Aquaculture 133, 257-274.

Kaushik, S.J., Coves, D., Dutto, G., Blanc, D., 2004. Almost total replacement of fish meal by plant protein sources in the diet of a marine teleost, the European seabass, *Dicentrarchus labrax*. Aquaculture 230, 391-404.

Kiessling, A., Pickova, J., Johansson, L., Ãsgard, T., Storebakken, T., Kiessling, K.H., 2001. Changes in fatty acid composition in muscle and adipose tissue of farmed rainbow trout (*Oncorhynchus mykiss*) in relation to ration and age. Fd. Chem. 73, 271-284.

Krogdahl, Ã., 1989. Alternative protein sources from plants contain antinutrients affecting digestion in salmonids, The current status of fish nutrition in aqua-culture. Proceedings of the third international symposium on feeding and nutrition in fish. August, pp. 253-261.

Leaver, M.J., Taggart, J.B., Villeneuve, L., Bron, J.E., Guy, D.R., Bishop, S.C., Houston, R.D., Matika, O., Tocher, D.R., 2011. Heritability and mechanisms of n-3 long chain polyunsaturated fatty acid deposition in the flesh of Atlantic salmon. Comp. Biochem. Physiol. 6D, 62-69.

Leger, C., Fremont, L., Marion, D., Nassour, I., Desfarges, M.F., 1981. Essential fatty acids in trout serum lipoproteins, vitellogenin and egg lipids. Lipids 16, 593-600.

512 Liener, I., 1969. Protease inhibitors. In: Liener, I. (ed.): Toxic constituents of
513 plant foodstuffs. Elsevier, pp 7-68.

514 Morais, S., Pratoomyot, J., Taggart, J.B., Bron, J.E., Guy, D.R., Bell, J.G.,
515 Tocher, D.R., 2011. Genotype-specific responses in Atlantic salmon
516 (*Salmo salar*) subject to dietary fish oil replacement by vegetable oil: a
517 liver transcriptomic analysis. BMC Genomics 12, 255-277.

518 Murray, D.S., Bain, M.M., Adams, C.E., 2013. Adhesion mechanisms in European
519 whitefish *Coregonus lavaretus* eggs: is this a survival mechanism for high-
520 energy spawning grounds? J. Fish Biol. 83, 1221-1233.

521 National Research Council (NRC), 2011. Nutrient requirements of fish and
522 shrimp. The National Academies Press,Washington D.C.

523 Nyina-Wamwiza, L., Wathelet, B., Richir, J., Rollin, X., Kestemont, P., 2010.
524 Partial or total replacement of fish meal by local agricultural by-products
525 in diets of juvenile African catfish (*Clarias gariepinus*): growth
526 performance, feed efficiency and digestibility. Aquacult. Nutr. 16, 237-
527 247.

528 Parrish, C.C., 1999. Determination of total lipid, lipid classes, and fatty acids in
529 aquatic samples, Lipids in freshwater ecosystems. Springer, pp. 4-20.

530 Pettersson, A., Pickova, J., Brännäs, E., 2009. Effects of crude rapeseed oil on
531 lipid composition in Arctic charr *Salvelinus alpinus*. J. Fish Biol. 75, 1446-
532 1458.

533 Robaina, L., Izquierdo, M.S., Moyano, F.J., Socorro, J., Vergara, J.M., Montero,
534 D., Fernandez-Palacios, H., 1995. Soybean and lupin seed meals as
535 protein sources in diets for gilthead seabream (*Sparus aurata*): nutritional
536 and histological implications. Aquaculture 130, 219-233.

537 Robinson, E.H., Li, M.H., 1994. Use of Plant Proteins in Catfish Feeds:
538 Replacement of Soybean Meal with Cottonseed Meal and Replacement of
539 Fish Meal with Soybean Meal and Cottonseed Meal. J. World Aquaculture
540 Soc. 25, 271-276.

541 Saura-Calixto, F., Canellas, J., Garcia-Raso, J., 1983. Determination of

542 hemicellulose, cellulose and lignin contents of dietary fibre and crude
543 fibre of several seed hulls. Data comparison. Zeitschrift fur Lebensmittel-
544 Untersuchung und Forschung 177, 200-202.

545 Sargent, J., Bell, G., McEvoy, L., Tocher, D.R., Estevez, A., 1999. Recent
546 developments in the essential fatty acid nutrition of fish. Aquaculture
547 177, 191-199.

548 Sharma, P.B., Lal, B.M., Madaan, T.R., Chatterjee, S.R., 1986. Studies on the
549 nutritional quality of some cucurbit kernel proteins. J. Sci. Fd. Agriculture
550 37, 418.

551 Skulason, S.l., Smith, T.B., 1995. Resource polymorphisms in vertebrates. Trends
552 in Ecology & Evolution 10, 366-370.

553 Tacon, A.G.J., Metian, M., 2008. Global overview on the use of fish meal and
554 fish oil in industrially compounded aquafeeds: Trends and future
555 prospects. Aquaculture 285, 146-158.

556 Testi, S., Bonaldo, A., Gatta, P.P., Badiani, A., 2006. Nutritional traits of dorsal
557 and ventral fillets from three farmed fish species. Fd. Chem. 98, 104-111.

558 Tocher, D.R., Bell, J.G., Dick, J., Sargent, J.R., 1997. Fatty acyl desaturation in
559 isolated hepatocytes from Atlantic salmon (*Salmo salar*): Stimulation by
560 dietary borage oil containing γ-linolenic acid. Lipids 32, 1237-1247.

561 Tocher, D.R., Bell, J.G., Dick, J.R., Henderson, R.J., McGhee, F., Michell, D.,
562 Morris, P.C., 2000. Polyunsaturated fatty acid metabolism in Atlantic
563 salmon (*Salmo salar*) undergoing parr-smolt transformation and the
564 effects of dietary linseed and rapeseed oils. Fish Physiol. Biochem. 23, 59-
565 73.

566 Tocher, D.R., Agaba, M., Hastings, N., Bell, J.G., Dick, J.R., Teale, A.J., 2001.
567 Nutritional regulation of hepatocyte fatty acid desaturation and
568 polyunsaturated fatty acid composition in zebrafish (*Danio rerio*) and
569 tilapia (*Oreochromis niloticus*). Fish Physiol. Biochem. 24, 309-320.

570 Tocher, D.R., Bell, J.G., MacGlaughlin, P., McGhee, F., Dick, J.R., 2001.
571 Hepatocyte fatty acid desaturation and polyunsaturated fatty acid

composition of liver in salmonids: effects of dietary vegetable oil. Comp. Biochem. Physiol. 130B, 257-270.

Tocher, D.R., 2003. Metabolism and Functions of Lipids and Fatty Acids in Teleost Fish. Rev. Fisheries Sci. 11, 107-184.

Torstensen, B., Lie, O., Froyland, L., 2000. Lipid metabolism and tissue composition in Atlantic salmon (*Salmo salar* L.)- Effects of capelin oil, palm oil, and oleic acid-enriched sunflower oil as dietary lipid sources. Lipids 35, 653-664.

Torstensen, B.E., Bell, J.G., Rosenlund, G., Henderson, R.J., Graff, I.E., Tocher, D.R., Lie, O., Sargent, J.R., 2005. Tailoring of Atlantic Salmon (*Salmo salar* L.) Flesh Lipid Composition and Sensory Quality by Replacing Fish Oil with a Vegetable Oil Blend. Journal of Agricultural and Food Chemistry 53, 10166-10178.

Torstensen, B.E., Espe, M., Sanden, M., Stubhaug, I., Waagbo¸, R., Hemre, G.I., Fontanillas, R., Nordgarden, U., Hevroy, E.M., Olsvik, P., Berntssen, M.H.G., 2008. Novel production of Atlantic salmon (*Salmo salar*) protein based on combined replacement of fish meal and fish oil with plant meal and vegetable oil blends. Aquaculture 285, 193-200.

Turchini, G.M., Torstensen, B.E., Ng, W.K., 2009. Fish oil replacement in finfish nutrition. Rev. Aquaculture 1, 10-57.

Turnbull, J., Bell, A., Adams, C., Bron, J., Huntingford, F., 2005. Stocking density and welfare of cage farmed Atlantic salmon: application of a multivariate analysis. Aquaculture 243, 121-132.

Van Weerd, J.H., 1995. Nutrition and growth in *Clarias* species - a review. Aquatic Living Res. 8, 395-401.

Waagbó, R., Hemre, G.I., Holm, J.C., Lie, O., 1995. Tissue fatty acid composition, haematology and immunity in adult cod, *Gadus morhua* L., fed three dietary lipid sources. J. Fish Dis. 18, 615-622.

Worm, B., Barbier, E.B., Beaumont, N., Duffy, J.E., Folke, C., Halpern, B.S., Jackson, J.B.C., Lotze, H.K., Micheli, F., Palumbi, S.R., Sala, E., Selkoe,

602 K.A., Stachowicz, J.J., Watson, R., 2006. Impacts of Biodiversity Loss on
603 Ocean Ecosystem Services. Science 314, 787-790.

604 Zdunczyk, Z., Minakowski, D., Frejnagel, S., Flis, M., 1999. Comparative study of
605 the chemical composition and nutritional value of pumpkin seed cake,
606 soybean meal and casein. Die Nahrung 43, 392-395.-622

607 Zheng, X., Torstensen, B.E., Tocher, D.R., Dick, J.R., Henderson, R.J., Bell,
608 J.G., 2005. Environmental and dietary influences on highly unsaturated
609 fatty acid biosynthesis and expression of fatty acyl desaturase and
610 elongase genes in liver of Atlantic salmon (*Salmo salar*). Biochim. Biophys.
611 Acta 1734, 13-24.

612

613

614 **Table and figure caption**

615 **Table 1**

616 Feed components (in %) of the gradual decrease in fish meal and increase in

617 pumpkin kernel cake and rapeseed oil from feeds F1 to F4.

618 Table 2

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

620 **Table 3**

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

622 **Table 4**

623 Total lipid and fatty acid contents (mg FAME/g dw) of dorsal and ventral muscle

624 tissue from fish fed the different diets (F1 – F4).

625 **Table 5**

626 Fatty acid retention ratios of dorsal and ventral muscle tissue from fish fed 4

627 different diets (F1 – F4).

628

629 **Fig. 1.** Average biomass of Arctic charr fed diets containing decreasing
630 concentrations of FM and FO and increasing concentrations of pumpkin kernel
631 cake and rapeseed oil (F1 → F4).
632 **Fig. 2.** Principle components analysis of dorsal muscle tissue and dietary fatty
633 acid compositions.
634 **Fig. 3.** Principle components analysis of ventral muscle tissue and dietary fatty
635 acid compositions.
636 **Fig. 4.** Production (mean ± SD) of EPA and DHA from ^{14}C-labeled ALA by isolated
637 hepatocytes from Arctic charr fed diets containing decreasing amounts of fish
638 meal and fish oil.

639 Table 1

	F 1	F 2	F 3	F 4
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

640

641

642

643

644

645

646

647

648

649

650 Table 2

	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

651

652

653

654

655

656

657

658

659

660

661

662

663

664

665 Table 3

666

Fatty Acids	F1	F2	F3	F4
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
Total saturated fatty acids (SAFA)[a]	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-9)	54.5±11.4	54.414.1	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
Total monounsaturated fatty acids (MUFA)[b]	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
Total (n-6) polyunsaturated fatty acids (PUFA)[c]	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[d]	26.5±5.1	23.4±7.7	17.8±2.9	16.7±3.7

667

668 Values are means of two replicate measurements.

669 a Includes 12:0, 15:0, 20:0, 22:0 and 24:0

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

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

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

673

674 Table 4

675

	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

676 Values are mean ± S.D. Values in the same row with different superscript letters are significantly different ($P<0.05$).
677

678

679

680

681

682 Table 5

683

	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}

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

Fig. 1

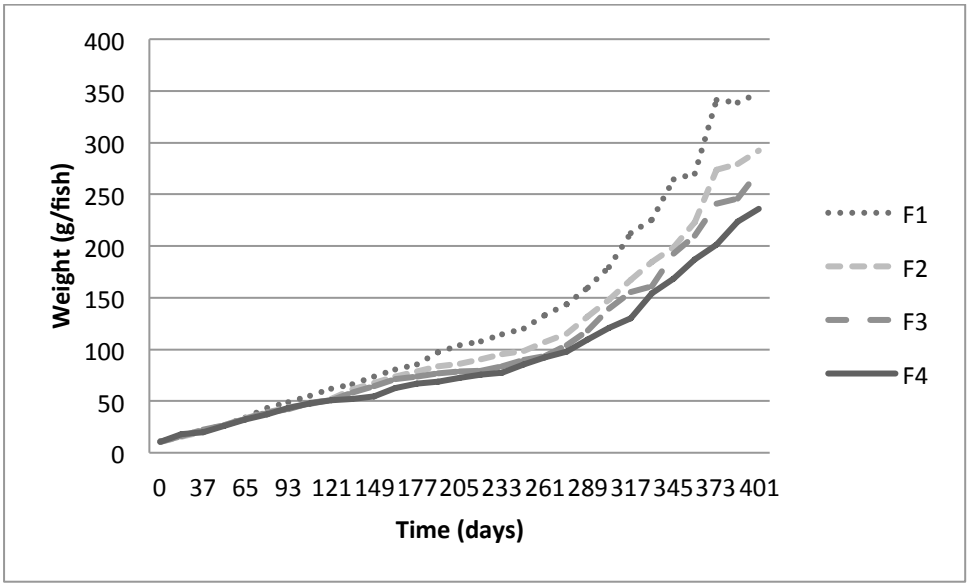

400
350
300
250
200
150
100
50
0
Weight (g/fish)
0 37 65 93 121 149 177 205 233 261 289 317 345 373 401
Time (days)
F1
F2
F3
F4

Fig. 2

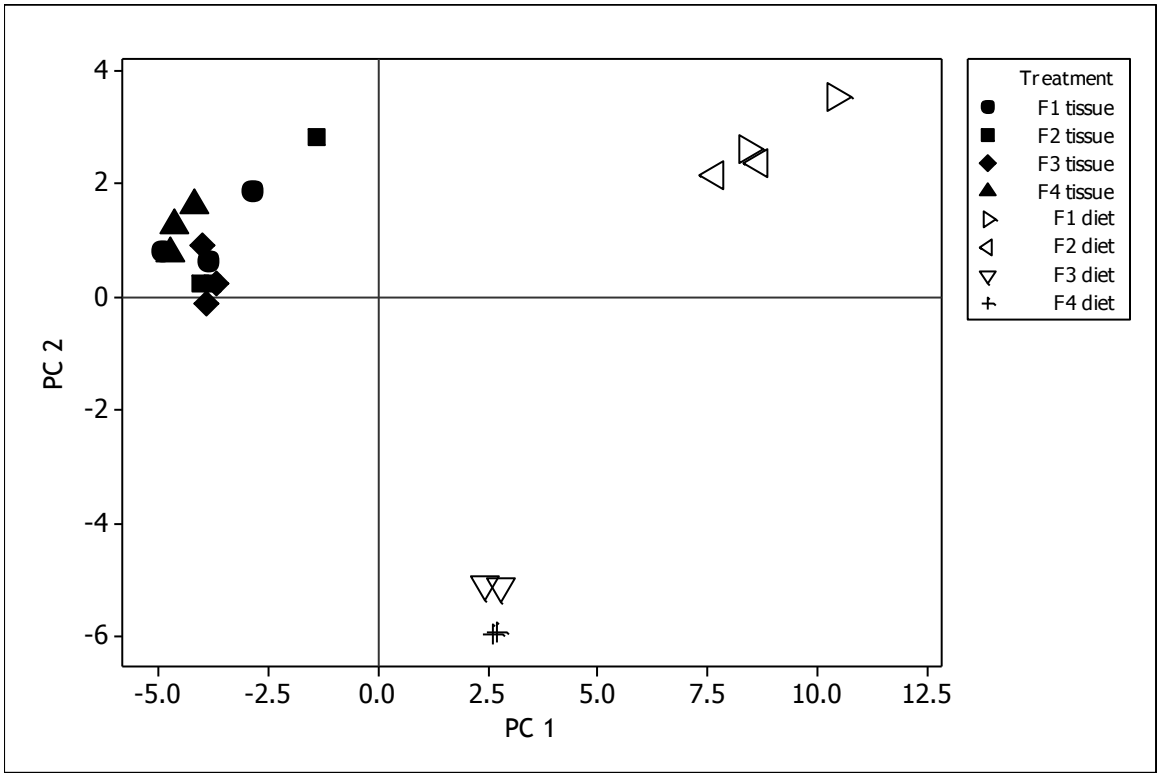

Treatment
F1 tissue
F2 tissue
F3 tissue
F4 tissue
F1 diet
F2 diet
F3 diet
F4 diet
PC 2
PC 1
4
2
0
-2
-4
-6
-5.0
-2.5
0.0
2.5
5.0
7.5
10.0
12.5

Fig. 3

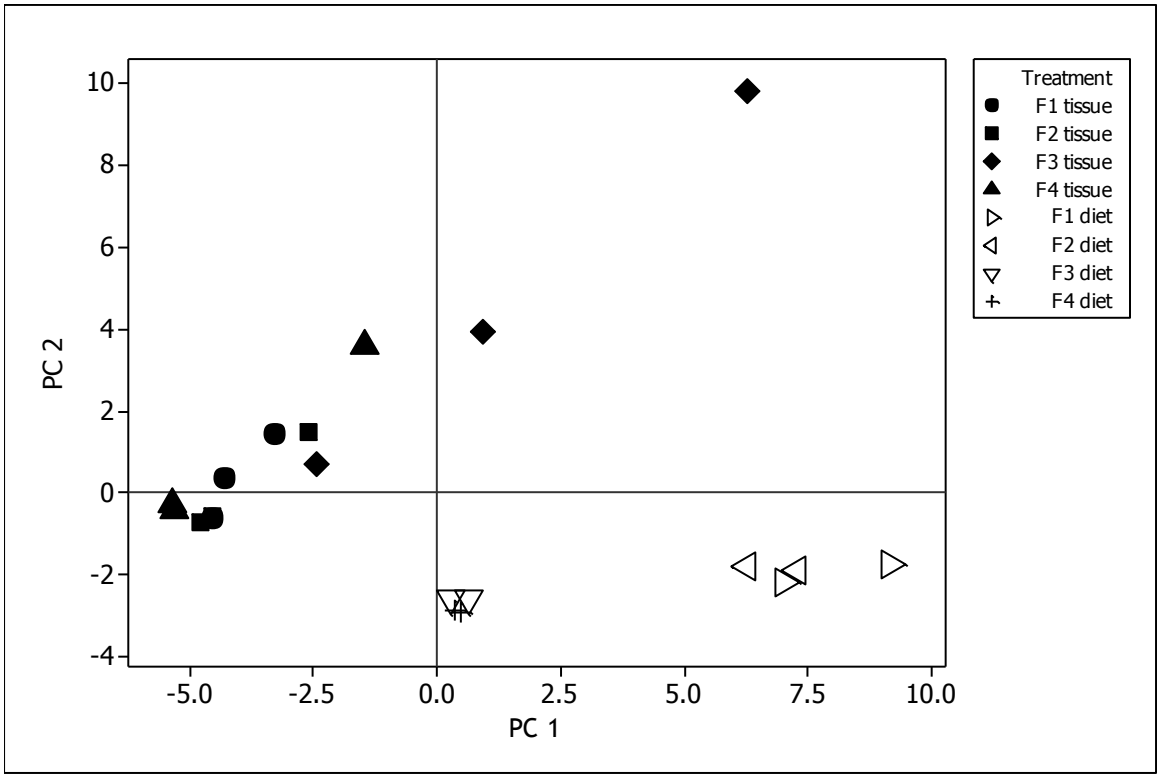
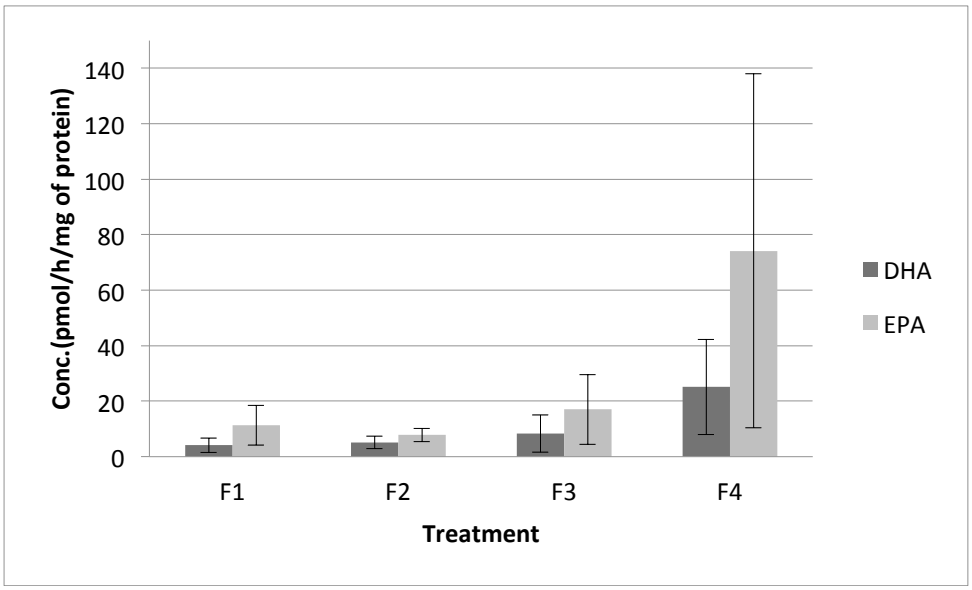

Treatment
F1 tissue
F2 tissue
F3 tissue
F4 tissue
F1 diet
F2 diet
F3 diet
F4 diet
PC 2
PC 1
10
8
6
4
2
0
-2
-4
-5.0
-2.5
0.0
2.5
5.0
7.5
10.0

Fig. 4


140
120
100
80
60
40
20
0
Conc.(pmol/h/mg of protein)
F1
F2
F3
F4
Treatment
DHA
EPA