

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

NOTICE: this is the author's version of a work that was accepted for publication in Aquaculture. Changes resulting from the publishing process, such as peer review, editing, corrections, structural formatting, and other quality control mechanisms may not be reflected in this document. Changes may have been made to this work since it was submitted for publication. A definitive version was subsequently published Aquaculture, [VOL 431 (2014)] DOI: <http://dx.doi.org/10.1016/j.aquaculture.2014.03.039>

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

5 D.S. Murray^a, H. Hager^a, D.R. Tocher^b, M.J. Kainz^a

6 ^a *WasserCluster - Biologische Station Lunz, 3929 Lunz am See, Austria.*

7 ^b *Institute of Aquaculture, University of Stirling, Stirling, FK9 4LA, Scotland, UK.*

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

10 Corresponding author: David S. Murray

11 Office phone number: (+43) 7486-20060

12 Mobile number: (+43) 6802-202422

13 Fax number: (+43) 7486-2006020

14

15 **Abstract**

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

38

39 **Keywords:** fatty acids; physiology; tissue; retention.

40

41 1. Introduction

42 The availability, cost and environmental sustainability of feed fish are some of
43 the main bottlenecks preventing the expansion of aquaculture industry (Tocher
44 2009; Worm *et al.*, 2006). Farmed carnivorous fish are traditionally fed diets
45 containing large amounts of marine fish meal (FM) and fish oil (FO) (Torstensen
46 *et al.*, 2008). Fish meal is the major protein source in feeds, while FO provides
47 the major source of lipids, including omega-3 long-chain polyunsaturated fatty
48 acids (n-3 LC-PUFA). Both proteins and lipids derived from FM and FO serve a
49 variety of important biological functions in fish and are important in human
50 nutrition (Drevon 1992; Nyina-Wamwiza *et al.*, 2010). On the basis of increasing
51 global FM and FO costs, alternative protein and lipid sources are required to
52 ensure the economic and environmental viability of the aquaculture industry
53 (Tacon *et al.*, 2006; Turchini *et al.*, 2009).

54 Fish oil contains high amounts of n-3 LC-PUFA, such as eicosapentaenoic acid
55 (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) (Kaushik *et al.*, 1995;
56 Turchini *et al.*, 2009) that are highly retained in farmed fish (Bell *et al.*, 2003;
57 Torstensen *et al.*, 2004). Despite lacking n-3 LC-PUFA, vegetable oils (VO) have
58 been proposed as sustainable alternatives to dietary FO (Torstensen *et al.*, 2005)
59 with various studies finding no deleterious impact on the health or growth rate
60 of farmed fish when FO was replaced with VO (Bell *et al.*, 2001; Seirestad *et al.*,
61 2005; Torstensen *et al.*, 2000, Waagbo *et al.*, 1991). However, it is widely
62 accepted that complete or partial replacement of FO with VO reduces
63 particularly the n-3 LC-PUFA content of fish tissues (Bell *et al.*, 2003, 2004;
64 Mourente and Bell, 2004; Torstensen *et al.*, 2005), which is a concern for the
65 general fish condition and nutritional value to the consumer.

66 Although tissue fatty acid compositions are closely correlated with those of
67 dietary supply, many fish, including Atlantic salmon (*Salmo salar*) and brown
68 trout (*Salmo trutta*) can convert α -linolenic acid (ALA; 18:3n-3) to EPA and DHA,
69 albeit rather inefficiently (Tocher 2003). Understanding and utilising this
70 biosynthetic pathway through the provision of VO-derived precursors may enable
71 farmed fish to meet their physiological n-3 LC-PUFA requirements, even if these
72 n-3 LC-PUFA are not sufficiently supplied within the diet (Tocher 2003).
73 Rapeseed oil appears to be a particularly effective alternative due to its lower

74 cost, but higher sustainability and relatively high amounts of the essential n-3
 75 LC-PUFA precursor ALA (Bell *et al.*, 1997, 2001; Tocher *et al.*, 2001; Turchini *et*
 76 *al.*, 2009).

77 Sustainable alternatives to FM include vegetable meals containing 20-50% crude
 78 protein, which can approach the levels found in FM typically fed to intensively
 79 reared fish (Hertrampf and Pascual, 2003; Van Weerd 1995). Fish meal can be
 80 partially or totally replaced with alternative plant protein sources without
 81 affecting the survival or growth rate of farmed fish (Fagbenro 1999; Gomes *et*
 82 *al.* 1995; Kaushik *et al.* 1995; Nyina-Wamwiza *et al.* 2010). However, the use of
 83 plant derived protein sources as feed ingredients is limited by the presence of
 84 anti-nutritional factors (ANFs) that inhibit specific metabolic pathways,
 85 decreasing digestibility and nutrient absorption (Francis *et al.*, 2001).

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

98 While many previous investigations identified how FM or FO replacements
 99 affected a variety of physical and biochemical variables, less is known about how
 100 dual replacement of both marine proteins and lipids with terrestrial alternatives
 101 affects the growth rate and fatty acid composition of farmed fish (Torstensen *et*
 102 *al.*, 2008; Turchini *et al.*, 2009). In addition, the use of pumpkin kernel cake as
 103 the main source of protein in feed has never been examined in farmed
 104 freshwater salmonids, such as Arctic charr (*Salvelinus alpinus*) that is
 105 increasingly farmed (FAO 2010). Therefore, in the current study we address this
 106 question directly by examining the effect of partial replacement of dietary FM

107 and FO with graded amounts of pumpkin kernel cake and rapeseed oil on the
 108 growth rate, tissue fatty acid profiles and metabolism in consumer-sized Arctic
 109 charr. Our null hypothesis was that there is no difference in the growth rate or
 110 tissue fatty acid profiles among the fish feeding on the different diets. Thus, our
 111 underlying assumption was that pumpkin kernel cake and rapeseed oil in fish
 112 feeds can fully replace commonly used FM and FO resulting in equal fish growth
 113 rates. In addition, fish provided with dietary rapeseed oil will endogenously
 114 convert dietary ALA to the n-3 LC-PUFA EPA and DHA and thus prevent any
 115 discernable differences in tissue fatty acid profiles compared to fish fed feeds
 116 containing typically high contents of FM and FO.

117

118 2. Materials and methods

119 2.1 Fish, husbandry and experimental diets

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

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

135 Four isocaloric fish feeds were formulated (GarantTM, Austria) to provide
 136 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 gravimetical 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

202 10 ml medium 199 containing 10mM HEPES. A 100 μ L aliquot of cell suspension
 203 was retained for protein determination using the modified Bradford assay
 204 (Bradford 1976) described by Murray *et al.* (2013).

205 2.6. Assay of hepatocyte fatty acyl desaturation/elongation activities

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

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

227 2.7. Data analysis

228 Principle components analysis (PCA) was used to reduce the number of individual
 229 FA into a single FA composition score (Adams *et al.*, 2007; Turnbull *et al.*, 2005)
 230 and used to analyse the difference between dietary and tissue FA compositions.
 231 Significant differences between dietary treatments were determined by one-way
 232 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 $\text{g fish}^{-1} \pm \text{SD}$) 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 ($236.3 \pm 17.0 \text{ g fish}^{-1}$), higher for F2 ($291.9 \pm 12.5 \text{ g fish}^{-1}$) and highest for F1 ($350.0 \pm 22.8 \text{ g fish}^{-1}$) (Fig. 1). Biomass of fish fed F3 ($270.3 \pm 8.0 \text{ g fish}^{-1}$) 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

262 %) and decreased gradually in fish fed F2 (0.83 ± 0.01 %), F3 (0.81 ± 0.02 %), and
 263 F4 (0.78 ± 0.02 %). Fish fed F4 had significantly lower SGR than F1 and F2 fish
 264 ($F_{[3-11]} = 8.19$; $R^2 = 0.66$; $p = 0.008$), but not significantly different than F3 fish.

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

269 3.3. Total lipid content and fatty acid composition

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

278 In dorsal muscle, F3 and F4 fish retained more DHA compared to F1 and F2 fish
 279 ($F_{[3-11]} = 23.73$; $R^2 = 0.861$; $P < 0.001$; Table 5). Retention of EPA in dorsal muscle
 280 was also higher in F3 and F4 fish compared to F1 fish ($F_{[3-11]} = 11.79$; $R^2 = 0.746$; P
 281 $= 0.003$) (Table 5). Retention of DHA in ventral muscle of F3 and F4 fish was
 282 higher than that of F1 and F2 fish ($F_{[3-11]} = 33.96$; $R^2 = 0.900$; $P < 0.001$), F3 and
 283 F4 fish also retained more EPA in ventral muscle than F1 and F2 fish ($F_{[3-11]} =$
 284 22.19 ; $R^2 = 0.853$; $P < 0.001$) (Table 5). SAFA ($F_{[3-11]} = 4.95$; $R^2 = 0.650$; $P = 0.031$)
 285 ventral muscle retention ratios were higher in F3 fish compared to F1 and F2
 286 fish. F3 fish also retained more n-3 PUFA in their ventral muscle compared to F1
 287 and F2 fish ($F_{[3-11]} = 5.42$; $R^2 = 0.693$; $P = 0.025$) (Table 5).

288 3.4. Dietary versus muscle tissue FA compositions

289 The fatty acid compositions of muscle tissue did not fully reflect dietary fatty
 290 acid compositions. There was no significant linear relationship between muscle
 291 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

322 dietary biochemical quality had a negative effect on fish biomass accrual.
323 Differences in specific growth rates were also observed during individual time
324 points within the study. Fish fed F1, F2 and F3 diets had significantly higher
325 specific growth rates after 37 days compared to fish fed F4 diets. However,
326 these differences were not consistently observed and only identified again after
327 373 days, whereby only F1 fish had higher specific growth rates than F4 fish.
328 Nevertheless, these results suggest that the reduction of FM and/or inclusion of
329 rapeseed oil in F3 and F4 resulted in a variable reduction of specific growth rate
330 in Arctic charr compared to those fed F1 diet.

331 The majority of studies examining the effect of dietary VO on specific growth
332 rates of farmed fish were performed over relatively short periods of time
333 (Turchini *et al.* 2009). For example, studies reporting no significant differences
334 in specific growth rates between fish consuming commercial feeds and feeds
335 containing a wide span of rapeseed oil (14 - 100 % of added oil) were performed
336 for between 12 and 21 weeks (Bell *et al.*, 2001; Tocher *et al.*, 2000, 2001;
337 Torstensen *et al.*, 2000). Results of the current study are based on Arctic charr
338 grown to their harvest weight for 400 days, indicated that the impact of reduced
339 dietary FM and increased rapeseed oil on specific growth rates may be time
340 dependent. This argument is supported by a study by Bell *et al.* (2003), which
341 found that after 50 wks of feeding Atlantic salmon fed diets containing 100 %
342 rapeseed oil or 100 % VO blend (linseed oil/rapeseed oil, 2:1) had significantly
343 higher final weights compared to fish fed 100 % FO. The higher final weights of
344 fish reported by Bell *et al.* (2003) may have been caused by the relatively high
345 FM contained in the feeds used in the earlier trial compared to the current
346 study. Therefore, the lower growth rate in the present study was probably more
347 a consequence of the replacement of FM than the replacement of FO. Pumpkins
348 contain high contents of neutral detergent fibre and acid detergent fibre (Suara-
349 Calixto *et al.* 1983) that affect digestive functions by increasing intestinal flow
350 rates (Lienner 1980, Huisman *et al.* 1989, Krogdahl 1989; Nyina-Wamwiza *et al.*
351 2010), which may reduce the retention of dietary nutrients (Krogdahl 1989;
352 Meyer *et al.*, 1988). This suggests that a 2-fold increase in pumpkin kernel cake
353 in the present study may have affected nutrient absorption and general
354 metabolism resulting in the lower growth rates of Arctic charr fed F4 diets in
355 comparison to fish fed the higher FM.

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

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

383 Diet is known to directly affect desaturase enzyme activity in mammals (Brenner
384 1981). Previous studies have shown that increasing dietary content of VO and VO
385 blends, increased desaturation and elongation activity in salmonid hepatocytes
386 (Bell *et al.*, 1997; Leaver *et al.*, 2011; Tocher *et al.*, 1997; 2000). In the present
387 study, there was also a trend for increased hepatic conversion of ALA to DHA by
388 partially replacing FO with rapeseed oil. However, there was also a large amount
389 of individual variation within treatments that prevented the results from being

390 significantly different, suggesting that the ability to convert ALA to DHA is not
391 entirely driven by dietary VO concentrations. Previous studies have reported
392 that Arctic charr populations are highly variable with many intra-population life-
393 history strategies, phenotypic plastic traits and an increased potential for
394 sympatric morphological divergence (Adams *et al.*, 2003; Alexander and Adams,
395 2000; Skúlason and Smith, 1995). In addition, Morais *et al.* (2011) found that
396 expression of genes associated with LC-PUFA metabolism were differentially
397 affected by diet but that genetic background of the fish was also a strong
398 influencing factor. In the current study, genotypic factors, such as gene
399 regulation of desaturases (Morais *et al.*, 2011; Zheng *et al.*, 2005), may have
400 influenced the ability of individual Arctic charr to convert ALA to DHA,
401 irrespective of dietary rapeseed oil concentrations.

402 In summary, the present study suggests that inclusion of 25 % pumpkin kernel
403 cake and 15 % rapeseed oil with 10 % FM and 3% FO in the diets of Arctic charr,
404 over an entire life-cycle, reduces their growth rate and biomass. However,
405 dietary inclusion of 12.5 % pumpkin kernel cake produced fish with similar
406 specific growth rates and biomass compared to fish fed with standard
407 commercial diets containing mainly FM and FO. Although there was a downward
408 trend, the inclusion of 15 % rapeseed oil with a 6-fold reduction in FO in diets for
409 Arctic charr did not significantly reduce EPA and DHA contents in muscle tissues,
410 which clearly points to selective retention of DHA and, to a certain extent, EPA.
411 Combined with an observed trend in generally increased hepatic conversion of
412 ALA to EPA and DHA in fish fed diets containing rapeseed oil, the results
413 indicated that the nutritional benefits of n-3 LC-PUFA in Arctic charr supplied
414 with pumpkinseed kernel cake and rapeseed oil will not be considerably reduced
415 and thus the fish will retain health benefits for human consumers.

416 **Acknowledgements**

417 This work received financial support by the Austrian Ministry of Life (project Nr.
418 100837; BMLFUW-LE.1.3.2/0051-II/1/2012) and GARANT Tiernahrung Austria. We
419 are grateful for technical assistance and support from Eduard Schneeberger, and
420 veterinary supervision by Heinz Heistinger. We also thank Katharina Drucker,
421 Katharina Hader, Katharina Winter, and Zahra Changizi for their laboratory
422 assistance.

423 References

- 424 Adams, C.E., Woltering, C., Alexander, G., 2003. Epigenetic regulation of
425 trophic morphology through feeding behaviour in Arctic charr, *Salvelinus*
426 *alpinus*. Biological Journal of the Linnean Society 78, 43-49.
- 427 Adams, C.E., Turnbull, J.F., Bell, A., Bron, J.E., Huntingford, F.A., 2007.
428 Multiple determinants of welfare in farmed fish: stocking density,
429 disturbance, and aggression in Atlantic salmon (*Salmo salar*). Can. J. Fish.
430 Aquatic Sci. 64, 336-344.
- 431 Bell, J.G., Tocher, D.R., Farndale, B., Cox, D., McKinney, R., Sargent, J., 1997.
432 The effect of dietary lipid on polyunsaturated fatty acid metabolism in
433 Atlantic salmon (*Salmo salar*) undergoing parr-smolt transformation.
434 Lipids 32, 515-525.
- 435 Bell, J.G., McEvoy, J., Tocher, D.R., McGhee, F., Campbell, P.J., Sargent, J.R.,
436 2001. Replacement of Fish Oil with Rapeseed Oil in Diets of Atlantic
437 Salmon (*Salmo salar*) Affects Tissue Lipid Compositions and Hepatocyte
438 Fatty Acid Metabolism. J. Nutr. 131, 1535-1543.
- 439 Bell, J.G., Henderson, R.J., Tocher, D.R., McGhee, F., Dick, J.R., Porter, A.,
440 Smullen, R.P., Sargent, J.R., 2002. Substituting Fish Oil with Crude Palm
441 Oil in the Diet of Atlantic Salmon (*Salmo salar*) Affects Muscle Fatty Acid
442 Composition and Hepatic Fatty Acid Metabolism. J. Nutr. 132, 222-230.
- 443 Bell, J.G., Tocher, D.R., Henderson, R.J., Dick, J.R., Crampton, V.O., 2003.
444 Altered Fatty Acid Compositions in Atlantic Salmon (*Salmo salar*) Fed Diets
445 Containing Linseed and Rapeseed Oils Can Be Partially Restored by a
446 Subsequent Fish Oil Finishing Diet. J. Nutr. 133, 2793-2801.
- 447 Bell, J.G., Henderson, R.J., Tocher, D.R., Sargent, J.R., 2004. Replacement of
448 dietary fish oil with increasing levels of linseed oil: Modification of flesh
449 fatty acid compositions in Atlantic salmon (*Salmo salar*) using a fish oil
450 finishing diet. Lipids 39, 223-232.
- 451 Bendiksen, E.Å., Johnsen, C.A., Olsen, H.J., Jobling, M., 2011. Sustainable
452 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 essential 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 lipides 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*).

- 482 Aquaculture 136, 351-362.
- 483 Izquierdo, M.S., Obach, A., Arantzamendi, L., Montero, D., Robaina, L.,
 484 Rosenlund, G., 2003. Dietary lipid sources for seabream and seabass:
 485 growth performance, tissue composition and flesh quality. *Aquacult. Nutr.*
 486 9, 397-407.
- 487 Jonsson, B., Jonsson, N., 2001. Polymorphism and speciation in Arctic charr. *J.*
 488 *Fish Biol.* 58, 605-638.
- 489 Kaushik, S.J., Cravedi, J.P., Lalles, J.P., Sumpter, J., Fauconneau, B., Laroche,
 490 M., 1995. Partial or total replacement of fish meal by soybean protein on
 491 growth, protein utilization, potential estrogenic or antigenic effects,
 492 cholesterolemia and flesh quality in rainbow trout, *Oncorhynchus mykiss*.
 493 *Aquaculture* 133, 257-274.
- 494 Kaushik, S.J., Coves, D., Dutto, G., Blanc, D., 2004. Almost total replacement of
 495 fish meal by plant protein sources in the diet of a marine teleost, the
 496 European seabass, *Dicentrarchus labrax*. *Aquaculture* 230, 391-404.
- 497 Kiessling, A., Pickova, J., Johansson, L., Åsgard, T., Storebakken, T., Kiessling,
 498 K.H., 2001. Changes in fatty acid composition in muscle and adipose
 499 tissue of farmed rainbow trout (*Oncorhynchus mykiss*) in relation to ration
 500 and age. *Fd. Chem.* 73, 271-284.
- 501 Krogdahl, Å., 1989. Alternative protein sources from plants contain antinutrients
 502 affecting digestion in salmonids, The current status of fish nutrition in
 503 aqua-culture. *Proceedings of the third international symposium on feeding*
 504 *and nutrition in fish.* August, pp. 253-261.
- 505 Leaver, M.J., Taggart, J.B., Villeneuve, L., Bron, J.E., Guy, D.R., Bishop, S.C.,
 506 Houston, R.D., Matika, O., Tocher, D.R., 2011. Heritability and
 507 mechanisms of n-3 long chain polyunsaturated fatty acid deposition in the
 508 flesh of Atlantic salmon. *Comp. Biochem. Physiol.* 6D, 62-69.
- 509 Leger, C., Fremont, L., Marion, D., Nassour, I., Desfarges, M.F., 1981. Essential
 510 fatty acids in trout serum lipoproteins, vitellogenin and egg lipids. *Lipids*
 511 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.I., 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

- 572 composition of liver in salmonids: effects of dietary vegetable oil. Comp.
573 Biochem. Physiol. 130B, 257-270.
- 574 Tocher, D.R., 2003. Metabolism and Functions of Lipids and Fatty Acids in
575 Teleost Fish. Rev. Fisheries Sci. 11, 107-184.
- 576 Torstensen, B., Lie, O., Froyland, L., 2000. Lipid metabolism and tissue
577 composition in Atlantic salmon (*Salmo salar* L.)- Effects of capelin oil,
578 palm oil, and oleic acid-enriched sunflower oil as dietary lipid sources.
579 Lipids 35, 653-664.
- 580 Torstensen, B.E., Bell, J.G., Rosenlund, G., Henderson, R.J., Graff, I.E., Tocher,
581 D.R., Lie, O., Sargent, J.R., 2005. Tailoring of Atlantic Salmon (*Salmo*
582 *salar* L.) Flesh Lipid Composition and Sensory Quality by Replacing Fish Oil
583 with a Vegetable Oil Blend. Journal of Agricultural and Food Chemistry 53,
584 10166-10178.
- 585 Torstensen, B.E., Espe, M., Sanden, M., Stubhaug, I., Waagbø, R., Hemre, G.I.,
586 Fontanillas, R., Nordgarden, U., Hevroy, E.M., Olsvik, P., Berntssen,
587 M.H.G., 2008. Novel production of Atlantic salmon (*Salmo salar*) protein
588 based on combined replacement of fish meal and fish oil with plant meal
589 and vegetable oil blends. Aquaculture 285, 193-200.
- 590 Turchini, G.M., Torstensen, B.E., Ng, W.K., 2009. Fish oil replacement in finfish
591 nutrition. Rev. Aquaculture 1, 10-57.
- 592 Turnbull, J., Bell, A., Adams, C., Bron, J., Huntingford, F., 2005. Stocking
593 density and welfare of cage farmed Atlantic salmon: application of a
594 multivariate analysis. Aquaculture 243, 121-132.
- 595 Van Weerd, J.H., 1995. Nutrition and growth in *Clarias* species - a review.
596 Aquatic Living Res. 8, 395-401.
- 597 Waagbø, R., Hemre, G.I., Holm, J.C., Lie, O., 1995. Tissue fatty acid
598 composition, haematology and immunity in adult cod, *Gadus morhua* L.,
599 fed three dietary lipid sources. J. Fish Dis. 18, 615-622.
- 600 Worm, B., Barbier, E.B., Beaumont, N., Duffy, J.E., Folke, C., Halpern, B.S.,
601 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 ¹⁴C-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	54.4±14.1	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

Table 4

	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).

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

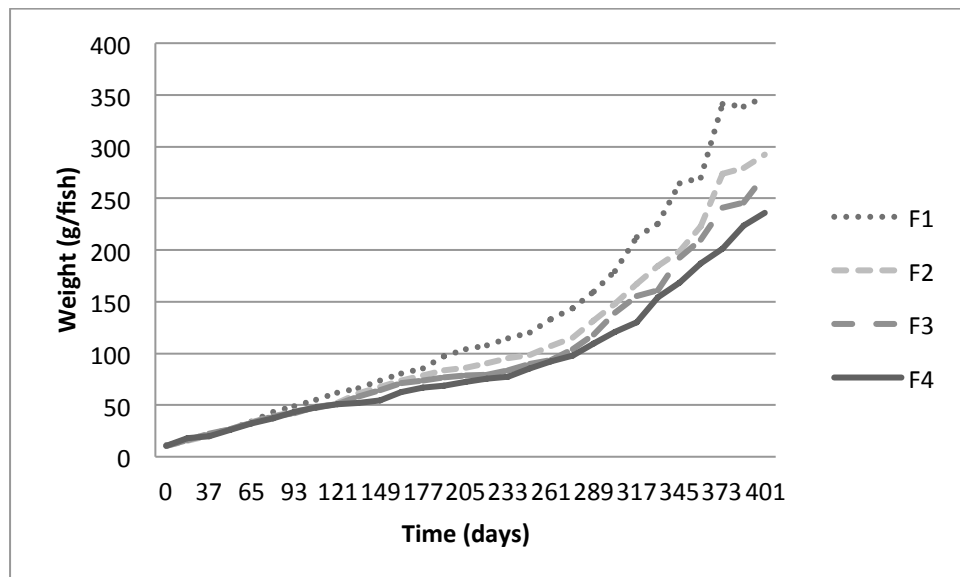


Fig. 2

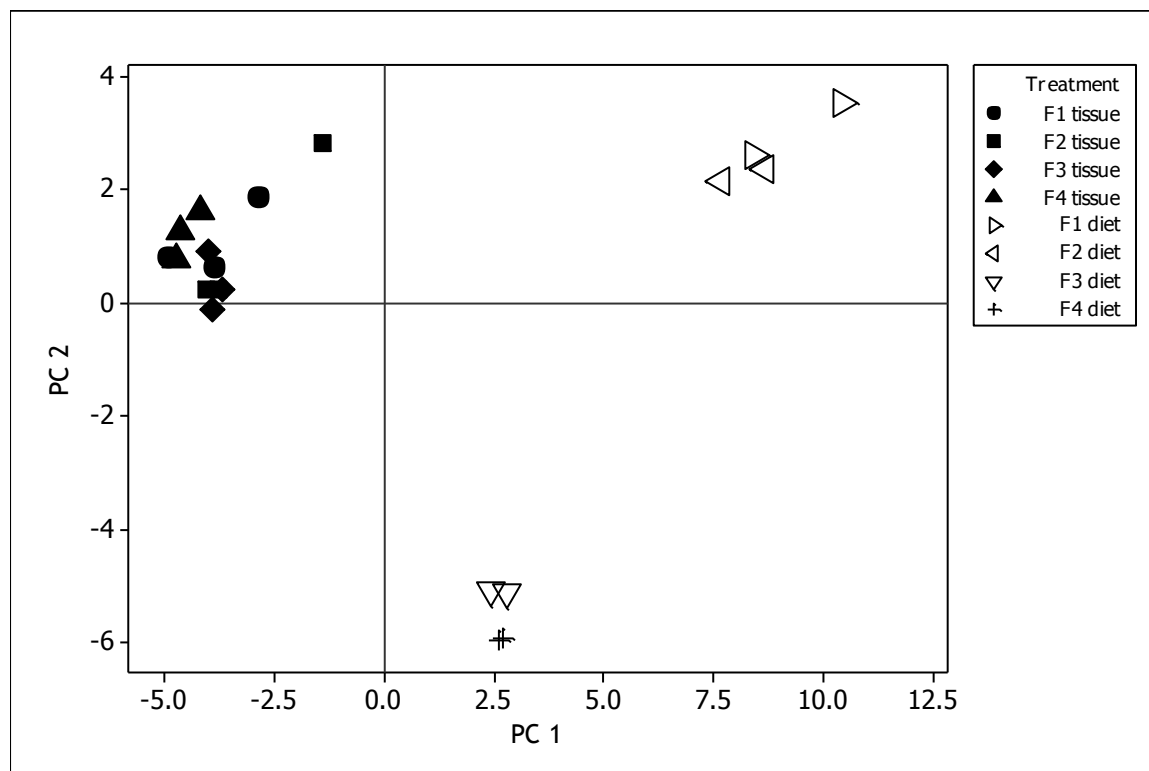


Fig. 3

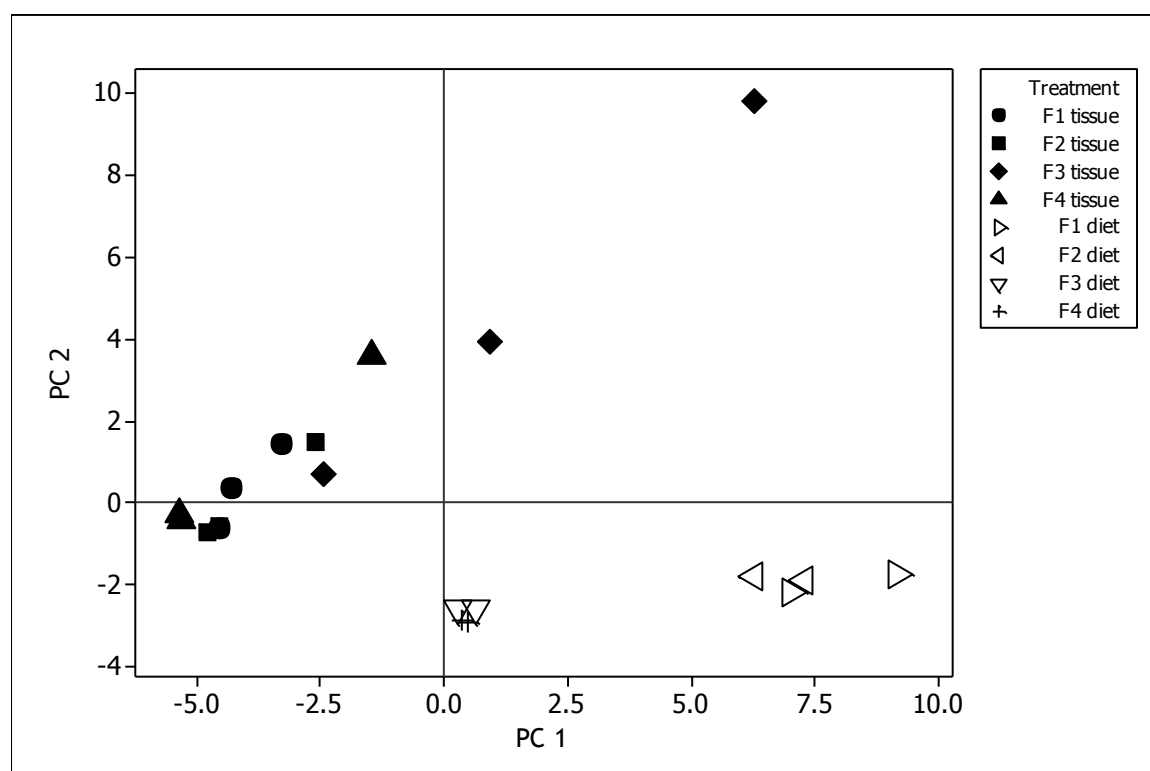


Fig. 4

