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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 (Iluyimi *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,

137 2011). Fish in triplicate tanks were fed 1 of the 4 different diets that gradually
138 contained less FM (35%-10%) and FO (i.e., salmon oil; 18%-3%; Table 1). Diets
139 were dispensed daily into the tank by a clockwork belt feeder (Dryden Aqua Ltd)
140 over a 12 hr feeding period. The daily feed ration exceeded the recommended
141 feeding rate for salmonids for the prevailing water temperature.

142 2.2. *Sampling procedure*

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

158 2.3. *Proximate analysis*

159 The gross nutrient composition of the four experimental diets was determined as
160 below (Table 2). Moisture was determined by drying to constant weight in an
161 oven at 110°C for 24 h (Bell *et al.*, 2003). Sample weight was recorded before
162 drying and after removal from the oven. Process was repeated at 1 h intervals
163 until weight change was <5 mg. Total protein content in experimental diets was
164 determined by modified Bradford assay (Murray *et al.*, 2013) and total lipids by
165 solvent extraction and gravimetric determination (Heissenberger *et al.*, 2010).
166 Ash content was determined by placing pre-weighed diets in a muffle furnace at
167 550°C for 8 h or until white ash was obtained (Bell *et al.*, 2003) that was
168 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

233 identified as nonhomogeneous, using variance test, were subjected to log
234 transformation before applying the statistical tests. The Minitab®16 statistical
235 software package was used for data analysis. Fatty acid retention ratios were
236 determined as the quotient of fatty acids in fish muscle tissues (mg FA per unit
237 biomass) and fatty acids in the respective diet. We define retention as the
238 ability of fish to regulate and control ingested fatty acids.

239

240 3. Results

241 3.1. Diet composition

242 All feeds contained similar contents of total proteins (~43-45%), total lipids (~23-
243 25%), total ash (~8-10%), and moisture (~6-9%; Table 2). The contents (mg FA per
244 unit biomass) for total saturated fatty acids (SAFA) decreased 1.4-fold from diets
245 F1 to F4 (Table 3). There was a 1.6-fold decrease in total n-3 PUFA contents
246 between diets F1 and F4, specifically a 4.0 and 4.2-fold decrease in DHA and
247 EPA, respectively (Table 3). Alternatively, total monounsaturated fatty acids
248 (MUFA) contents increased 1.3-fold, n-6 PUFA by 1.4-fold and ALA by 1.6-fold
249 between diets F1-F4 (Table 3).

250 3.2. Biomass and specific growth rate

251 After 191 days of feeding, fish biomass started differing significantly among the
252 4 dietary treatments ($F_{[3-11]} = 11.03$; $R^2 = 0.805$; $P = 0.003$) (Fig. 1). Fish fed diets
253 F4 (69.2 ± 8.9) and F3 (77.8 ± 8.2) diets had a significantly lower biomass (mean
254 $\text{g fish}^{-1} \pm \text{SD}$) than fish fed diet F1 (97.6 ± 2.8). This trend continued to the end of
255 the experiment and fish biomass was significantly lower ($F_{[3-11]} = 26.09$; $R^2 =$
256 0.873 ; $P < 0.001$) for fish feeding on F4 ($236.3 \pm 17.0 \text{ g fish}^{-1}$), higher for F2
257 ($291.9 \pm 12.5 \text{ g fish}^{-1}$) and highest for F1 ($350.0 \pm 22.8 \text{ g fish}^{-1}$) (Fig. 1). Biomass
258 of fish fed F3 ($270.3 \pm 8.0 \text{ g fish}^{-1}$) was also significantly smaller than F1 tanks,
259 but not F2 or F4 tanks (Fig.1).

260 Specific growth rates for the entire feeding period (with water temperatures
261 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

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 ($F_{[1-3]}$
297 $= 123.88$; $R^2 = 0.976$; $P = 0.002$) 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

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, Kroghdahl 1989; Nyina-Wamwiza *et al.*
351 2010), which may reduce the retention of dietary nutrients (Kroghdahl 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; Skulason 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.

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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

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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

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665 Table 3

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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±11.1	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

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Values are means of two replicate measurements.

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

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

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

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

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674 Table 4

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	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

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

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582 Table 5

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	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

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

Fig. 1

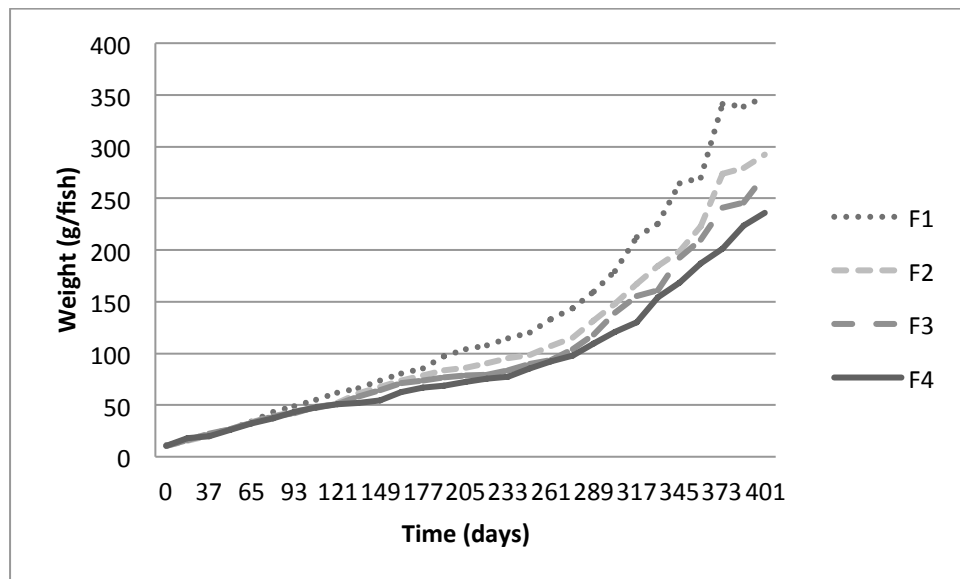


Fig. 2

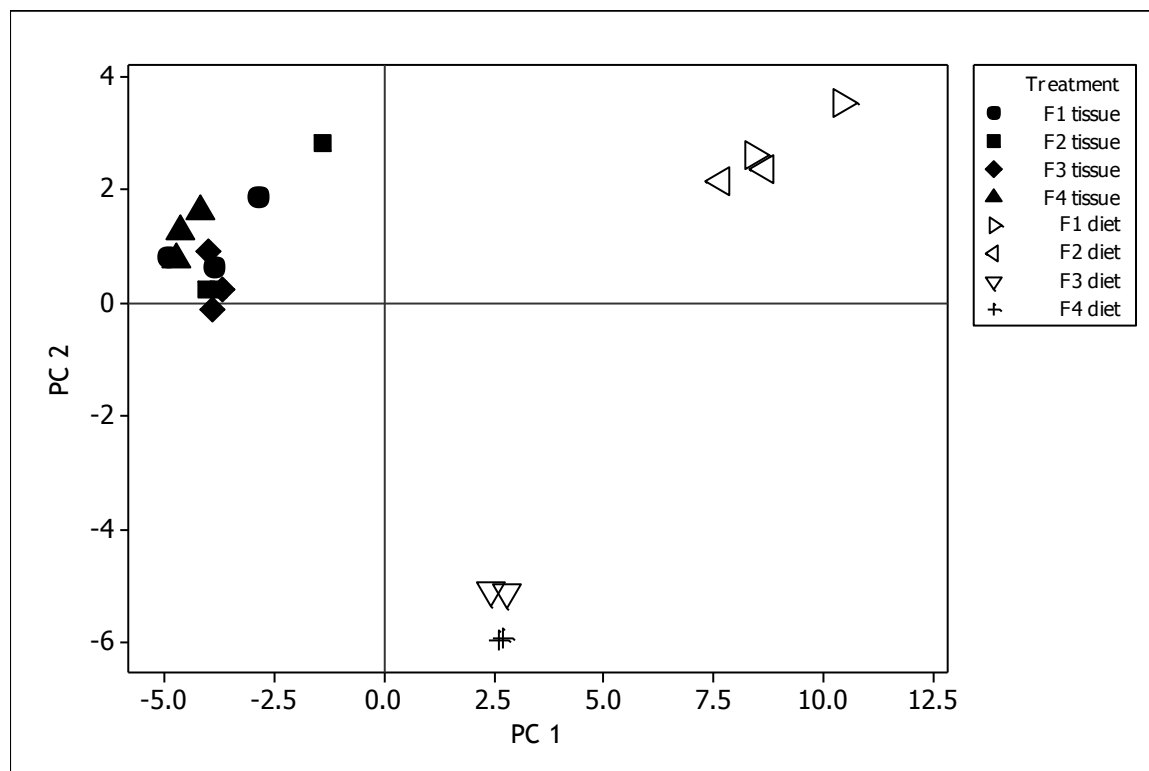


Fig. 3

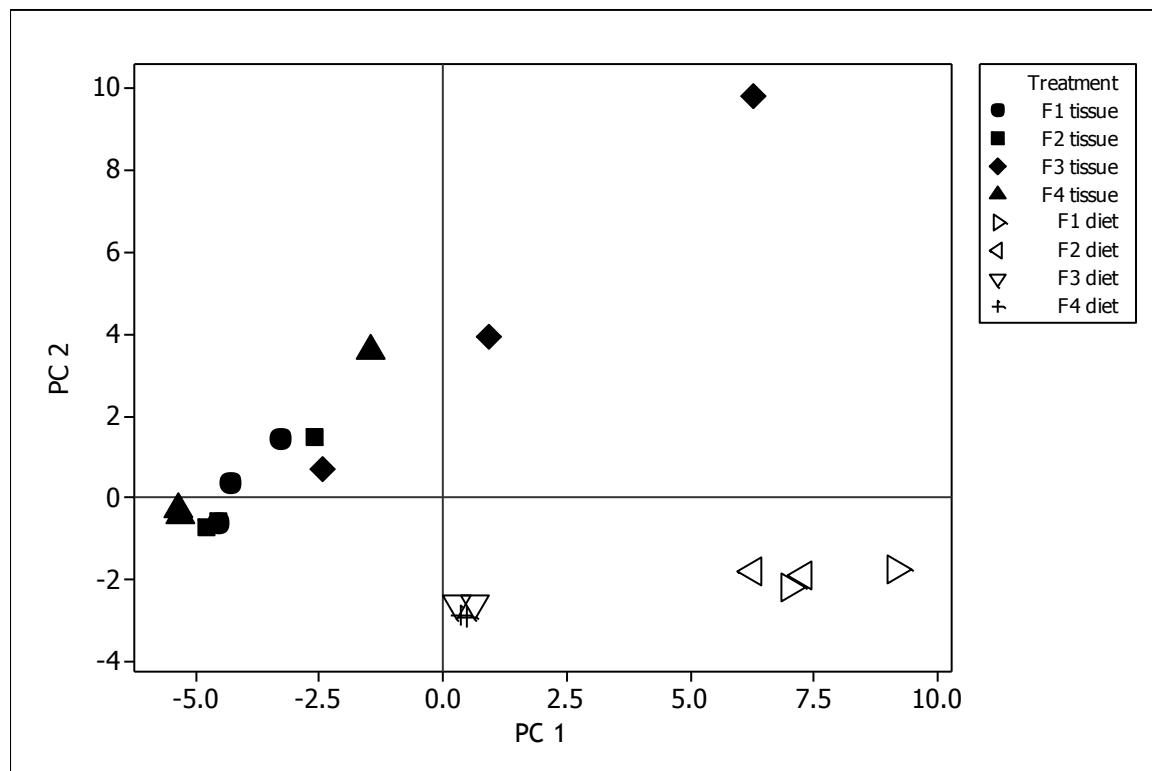


Fig. 4

