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Abstract

Feeds and feeding constitute the major part of costs in intensive aquaculture. Any action to reduce feeding costs without negatively affecting fish production parameters and flesh quality would improve profitability of farming. Therefore, we studied the effects of feeding frequency on production parameters, stomach capacity and nutritional value of muscle in juvenile rainbow trout (Oncorhynchus mykiss) in an experiment with two stages. First, during the nine-week “starvation period” we fed rainbow trout (initial weight c. 40 g) with four different feeding protocols in an attempt to adapt the fish to a progressive decrease in the number of feeding days. During the second stage, a four-week “re-feeding period”, all fish were fed in excess on weekdays. Fish growth, feed intake, stomach size, and biomolecule content of muscle were monitored as response variables. During the starvation period, feed intake and growth decreased along with the number of feeding days. Compensatory growth during the refeeding was either only modest or absent. The fish in the starved groups were unable to significantly increase their stomach capacities. Starvation and re-feeding had only a slight effect on muscle fatty acid and amino acid composition. The used feeding protocols did not affect important production parameters (e.g. feed conversion or size variation). Our results suggest that despite differences in fish growth starvation and re-feeding hardly affect the nutritional value of fish. It is possible that several decades in captivity have made rainbow trout incapable to adjust their stomach size in respect to feeding frequency.

Keywords	Compensatory growth, stomach volume, salmonids, fatty acids, amino acids, feed conversion ratio
Taxonomy	Animal Nutrition, Aquatic Biology
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Research Data Related to this Submission

There are no linked research data sets for this submission. The following reason is given:
Data will be made available on request

Dear Editor

We have gone carefully through the referees' comments and corrections and made the changes as suggested. However, we did not make every single suggestion of the reviewer 2 what s/he had made in the edited ms version, as we regarded some of those as opinions that can be taken into account.

-Reviewer 1

- The manuscript was remarkably improved by the authors by including polynomial contrasts, but there are still some minor linguistic issues. I tried to edit them in the main text in track changes mode. My only suggestion is that the authors should benefit from the power of trend analysis in discussion section rather than relying on ANOVA results. Then, the manuscript is acceptable after a minor revision in my opinion.

We have made all suggested linguistic corrections as suggested by the referee. We have modified discussion by utilizing the results of the polynomial contrast analyses.

-Reviewer 2

- Dear Editor,

The manuscript titled "Effects of progressive decrease of feeding frequency and re-feeding on production parameters, stomach capacity and muscle nutritional value in rainbow trout (Oncorhynchus mykiss)" is an original study of practical interest for fish farming.

According to the previous revision, the manuscript has been improved in many aspects.

As was previously commented, results obtained are not the expected but experimental design and methodology is consistent being results suitably discussed. Although the discussion may seem initially extensive, it is read fluently and is well supported by bibliographic references.

According to not expected results, maybe the authors should consider a possible trout adaptation to a slow progressive decrease of fasting days? Some studies with and immediate and absolute fasting during some weeks have manifested a later compensatory growth in a re-feeding period.

We have now added a sentence into discussion showing an example of this kind of late compensatory growth, L 373-376.

By other hand, one of the main found in this study is that fatty acids and amino acids profile of flesh is not affected by fasting, being this aspect of interest for rainbow trout culture.

Anyway, authors should improve some minor aspects related to manuscript, to consider for publication.

According to sections of abstract and introduction, they are suitable and well supported. Some suggestions are given in Word document. Focus of the study should be more summarized and concrete.

We think that it is always good to present testable hypotheses in an article. However, we changed the wording of the second hypothesis to some extent (L 98-99).

Referring to Material and methods some minor consideration have to be in count. Results are suitably described, but some data in text has to be contrasted with the tables of supplement 1 and 2.

We have made the corrections as suggested in the file provided.

In tables and figures, some suggestions are given regarding to title and footnotes as well as description of experimental groups and fasting periods in head of tables.

Changes have been made as suggested

In discussion of data, all comments support the (not expected) results, but some sentences that in my opinion can results too speculative.

We have deleted some parts of the text the reviewer regard too speculative. However, we do not think that the suggested future research (comparison of wild fish to hatchery fish) is speculation but an important question that rose based on our results (L 356-360).

These general comments are detailed in the Word document in order to help the authors to improve this interesting document.

Highlights

- Rainbow trout were unable to adapt to progresive decrease of feeding frequency in 63 days.

- 28-day re-feeding period did not elicit compensatory growth.

- Starvation and re-feeding did not affect nutritional value of flesh.

Effects of progressive decrease of feeding frequency and re-feeding on production parameters, stomach capacity and muscle nutritional value in rainbow trout (*Oncorhynchus mykiss)*

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ABSTRACT

Feeds and feeding constitute the major part of costs in intensive aquaculture. Any action to reduce feeding costs without negatively affecting fish production parameters and flesh quality would improve profitability of farming. Therefore, we studied the effects of feeding frequency on production parameters, stomach capacity and nutritional value of muscle in juvenile rainbow trout (*Oncorhynchus mykiss*) in an experiment with two stages. First, during the nine-week "starvation period" we fed rainbow trout (initial weight c. 40 g) with four different feeding protocols in an attempt to adapt the fish to a progressive decrease in the number of feeding days. During the second stage, a four-week "re-feeding period", all fish were fed in excess on weekdays. Fish growth, feed intake, stomach size, and biomolecule content of muscle were monitored as response variables. During the starvation period, feed intake and growth decreased along with the number of feeding days. Compensatory growth during the re-feeding was either only modest or absent. The fish in the starved groups were unable to significantly increase their stomach capacities. Starvation and re-feeding had only a slight effect on muscle fatty acid and amino acid composition. The used feeding protocols did not affect important production parameters (e.g. feed conversion or size variation). Our results suggest that despite differences in fish growth starvation and re-feeding hardly affect the nutritional value of fish. It is possible that several decades in captivity have made rainbow trout incapable to adjust their stomach size in respect to feeding frequency.

Key words: compensatory growth, stomach volume, salmonids, fatty acids, amino acids, feed conversion ratio

42 **1. Introduction**

43 Feeds and feeding constitute the major part of costs in intensive aquaculture operations. Thus,
44 any decrease in these costs through feed development or rationalization of feeding practices
45 would improve the profitability of farming. In aquaculture research dealing with fish nutrition
46 and husbandry, the focus is typically on growth and feed utilization, but also the composition
47 of the flesh is important from the human nutrition point of view. One option for
48 rationalization of feeding is optimization of feeding rhythmicity or frequency, and this has
49 attracted a lot of attention from aquaculture researchers.

50 In studies dealing with feeding frequency or starvation and consequent re-feeding, the fish
51 are typically fed with the same predetermined frequency throughout the experiment.
52 However, if the time intervals between feedings are several days or weeks, it may take quite
53 a long time for the fish to become acclimated to such sparse feeding regimens (Pirhonen and
54 Forsman, 1998; Nikki et al., 2004; Blake et al., 2006). Acclimation in this case means an
55 increase in the capacity to ingest feed, as the fish are known to adjust their stomach capacities
56 according to the meal size (Ruohonen et al.,1997), and a decrease in feeding frequency leads
57 to an increase in the amount of feed ingested per feeding and consequent increase in stomach
58 volume (Känkänen and Pirhonen, 2009; Mattila et al., 2009).

59 After a period of starvation or feed restriction animals typically show a growth spurt when
60 supplied food in excess. During re-alimentation animals can show maximal growth rates
61 which are not otherwise observed (Metcalfe and Monaghan, 2001; Ali et al., 2003) as they
62 attempt to regain the lost growth. This phenomenon is referred to as compensatory growth
63 and it has been investigated widely also with fishes in order to be exploited in commercial

64 farming to obtain improved feed efficiency without compromising weight gain or muscle
65 nutritional quality. However, research results are variable in this respect, and compensation
66 is often induced by hyperphagia rather than by improved feed efficiency (Ali et al., 2003; Fu
67 et al., 2007; Huang, 2008; Känkänen and Pirhonen, 2009; Mattila et al., 2009). A decrease in
68 feeding frequency or starvation can also decrease the relative liver size and visceral fat
69 accumulation (Weatherley and Gill, 1981; Nikki et al., 2004; Känkänen and Pirhonen, 2009;
70 Mattila et al., 2009; Güroy et al., 2011), but a clear feeding frequency related decrease can
71 also be absent due to large individual variation (Nikki et al., 2004). Starvation can also alter
72 physiological responses seen as changes in hematocrit or plasma ions (Einarsdóttir and
73 Nilssen, 1996; Falahatkar, 2012; Caruso et al., 2011, 2012).

74 The dietary availability of long-chain ω-3 polyunsaturated fatty acids (PUFA) influences the
75 growth and development of fish (Tocher, 2010; Glencross et al., 2014), but also the
76 nutritional value of fish (Sargent et al., 1995; Jobling, 2003). Previous studies have shown
77 that low dietary content of docosahexaenoic acid (DHA, 22:6ω3) results in lower growth
78 rates compared to fish feed rich in this fatty acid (Murray et al., 2014; Taipale et al., 2018).
79 However, fish physiology and metabolism can also influence fatty acids (FA) composition
80 in fish tissues since fish have the ability to synthesize long chain ω-3 PUFA, DHA and
81 eicosapentaenoic acid (EPA, 20:5ω3), from precursors such as α-linolenic acid (ALA,
82 18:3ω3; Tocher, 2003; Murray et al., 2014,2015). Nevertheless, the ability for conversion
83 varies by fish species and age (Tocher, 2010). For example, adult and juvenile rainbow trout
84 (*Oncorhynchus mykiss*) are reported to be able to synthesize EPA and DHA from high
85 concentration of dietary ALA (Gregory and James, 2014), but the direct dietary source of
86 DHA is crucial for larvae (Wirth et al., 1997; Taipale et al., 2018). On the other hand, it is

87 known that, in addition to long chain PUFA, proteins and amino acids are required for growth
88 and development of fish (Wilson and Halver, 1986; Rønnestad et al., 1999), and restricted
89 availability of essential amino acids (EAA) causes growth retardation and fin rot (Ketola,
90 1982). A study with juvenile rainbow trout (Taipale et al., 2018) showed ability of this
91 species to compensate for the low EAA but not low DHA content of diet.

92 To the best of our knowledge there are no earlier studies looking at the effects of a progressive
93 decrease in feeding frequency in fishes. This study was planned to investigate these effects
94 with a two-part approach. In the first part of the experiment (starvation period) we tested a
95 hypothesis that rainbow trout would be able to increase feed intake by increasing stomach
96 volume without significant influence on growth rate when the feeding frequency is decreased
97 progressively. Our second hypothesis was that starved fish would be able to full growth
98 compensation during the re-feeding period (second part of the experiment). In addition to
99 responses in growth and feed conversion, we measured treatment effects on the liver, viscera,
100 stomach capacity, body composition and muscle biochemistry.

101 **2. Materials and methods**

102 **2.1. Animals and experimental conditions**

103 The experiment was carried out on 0+ age all-female rainbow trout between March 27 and
104 June 26, 2017. On March 7, the fish were transported from a commercial fish farm to the
105 laboratory at the University of Jyväskylä where they were held in a stock tank (0.5 m^3) until
106 2 weeks prior to the start of the experiment. At this time, 240 fish (39.5 ± 7.8 g), were taken
107 from the stock tank and placed in twelve 360 L flow-through stainless steel tanks (20
108 individuals per tank) supplied with flowing (1 L min^{-1}) well water. Each tank was aerated

through an air stone. Dissolved oxygen concentration and pH were c. 9.0 mg L^{-1}, and 6.4 respectively. Experimental fish were exposed to a 12L: 12D photoperiod.

2.2. Experimental design

The experiment consisted of two periods. The first period (starvation period) lasted for 9 weeks and tested the possible adaptation to the decrease in feeding frequency. Four treatments were designated: control (fed every weekday, Monday to Friday); T1 (fed three days a week: Mon., Wed., Fri.), T2 (fed three days a week for the first two weeks, and then twice, Mon., Fri.) and T3 (fed three days a week for the first two weeks, then twice the following two weeks and thereafter once a week, Mon.). After these starvation periods, the fish were subjected to a 4-week feeding period, when all the fish were fed from Monday to Friday (Fig. 1). The number of days the experimental fish were fed during the first period were 45 (control), 27 (T1), 20 (T2) and 15 (T3). During the second period all the fish were fed for 20 days (Fig. 1). During the first 4 weeks of the starvation period (as well as during the 2-week acclimation), the fish were hand-fed with commercial trout pellets (Biomar Efico Enviro 920 Advance 3 mm; proximate composition according to manufacturer was fat 31.5%, protein 44.5%, fiber 1.6%, ash 5.5%, energy 25 MJ kg^{-1}) and thereafter fish were fed with bigger pellets (Royal Plus 3.5 mm, Raisio Ltd, Raisio, Finland; proximate composition according to manufacturer was fat 28%, protein 43%, fiber 1.1%, ash 6.5%, energy 24.4 MJ kg^{-1}). Fish were fed twice per day between 08.00–09.00 and 15.00–16.00 h. Fish were always fed *ad libitum* (as much as they were willing to eat), and eaten feed was recorded considering uneaten feed siphoned out from the tanks after feeding. Five individuals died during the experiment for unknown reasons (T1: three and T3: two individuals) and four fish (in one of the control group tanks) died because of a technical failure in the aeration system (day 56).

132 **2.3. Sampling procedure**

133 Every two weeks (on Mondays) fish were sampled. During weighing days, the fish were not
134 fed in the morning. Fish were anesthetized using clove oil: ethanol mixture (1:10, clove oil
135 concentration 40 mg L^{-1}) and measured for weight and length. Five animals were netted out
136 and killed with a sharp blow on the head. From these fish blood samples were taken from the
137 caudal vessel of five individuals per tank with a heparin-coated syringe at the end of both
138 periods. Hematocrit was analyzed right away and the remaining blood was centrifuged (7000
139 rpm) to separate plasma (frozen at -20°C) for chloride assays (Sherwood Chloride Analyzer,
140 Model 926S).

141 Digestive tract was removed and, when all visible visceral fat and liver had been separated
142 and weighed, was frozen (-20°C) for later stomach weight and volume measurement. A piece
143 of muscle (two fish per tank) was excised from under the dorsal fin, and separated into two
144 microtubes, one for the analysis of lipids, fatty acids and amino acids (frozen at -80°C) and
145 the other for the water content (dried at 75°C).

146 **2.4. Growth performance indices**

147 Specific growth rate was calculated as SGR (% day^{-1}) $= 100(\ln W_2 - \ln W_1)*t^{-1}$,
148 where W_1 and W_2 were weights (g) at the start and end of the measuring period and t was the
149 period in days. Condition factor (CF) was calculated as $100W*L^{-3}$, where L was the total
150 length (cm).

151 Relative feed intake $feeding^{-1}$ was calculated as I_R = (total intake (g) * number of
152 $feedings^{-1}$) * W^{-1}, where W was the average weight (g) of the fish. Feed conversion ratio was
153 calculated as FCR = intake (g) * gain $(g)^{-1}$. Possible compensation (for weight gain and

154 intake) in the treatment groups during the two periods was estimated by a compensation
155 coefficient which was calculated as CC = $\Delta T * \Delta C^{-1}$, where ΔT was the average weight gain
156 or intake (g) in the treatment group tanks divided by the number of feeding days and ΔC was
157 the average weight gain and intake (g) in the control group tanks divided by the number of
158 feeding days; thus, CC > 1.0 would indicate compensation. Hepatosomatic index was
159 calculated as HIS (%) = 100 $W_L * W^{-1}$, where W_L was liver weight (g). The visceral-somatic
160 index was calculated as VSI (%) = 100 $W_V * W^{-1}$, where W_V was visceral weight (g).

161 **2.5. Stomach measurement**

162 Stomach capacity (volume and weight) was measured at three sampling points: on days 28,
163 63, and 91. For the measurement of stomach volume, a string was tied around the pyloric
164 sphincter and the esophagus was tied to a 50 cm (=50 mL) burette. Stomach volume was
165 estimated as the volume of water required to dilate the stomach under a pressure head of
166 50 cm water (Jobling et al., 1977), i.e. the amount of water added to the burette (to keep it at
167 50 cm) was regarded as the volume (to 0.1 mL) of the stomach. After the volume
168 measurement, the stomach was separated from the intestine and weighed (to 0.01 g).

169 **2.6. Lipids and fatty acids assays**

170 Approximately 1-5 mg of freeze dried muscle samples (two fish per tank, 3 tanks per
171 treatment) were analyzed for lipids. This assay was also carried on feeds (Supplement 1 and
172 2). Total lipids were extracted with chloroform:methanol:water mixture (2:1:0.75). For the
173 formation of fatty acid methyl esters (FAME), mild acidic methylation (1% sulphuric acid-
174 methanol solution) was used and samples were incubated in a 90 °C water bath for 1.5 h.
175 FAMEs were run by a coupled gas chromatography-mass spectrometry (GC-MS, Shimadzu

176 Ultra, Kyoto, Japan) using an Agilent® DB-23 column (30 m × 0.25 mm × 0.25 μm) as
177 previously published temperature ramp (Taipale et al. 2016). The identification of FAMEs
178 was based on retention times and their specific mass ions (Taipale et al. 2016). For
179 quantification of FAMEs we used 566c fatty acid mixture (Nu chek Prep) and specific ions
180 following the protocol of Taipale et al. (2016).

181 **2.7. Proteins and amino acids assays**

182 Freeze-dried muscle samples (two fish per tank, 3 tanks per treatment) and feeds (Supplement
183 1 and 2) were pulverized using a mortar and pestle, and 0.1-0.6 mg of homogeneously mixed
184 sample was weighed in tin cups for the analysis of elemental nitrogen (Carlo Erba Flash EA
185 1112 elemental analyzer). Two replicates of the dried white muscle of pike *Esox lucius* L.,
186 as an internal working standard, were analyzed after every 10 samples in each sequence.
187 Total protein content was analyzed by multiplying elemental nitrogen content with a
188 coefficient of 6.25 (Mariotti et al., 2008).

189 For amino acid analysis we used 0.5–1 mg of diets or freeze dried muscle tissue of the same
190 two fish per tank as for the FA analyses. Proteins were hydrolyzed with 1 mL of 6 M HCl at
191 110 °C for 20 h and the solvent evaporated to dryness overnight. Amino acids were run as
192 their propyl chloroformates using EZ:faast kit for preparation (Phenomenex) and a GC-MS
193 using ZB-AAA column (9.5 m x 0.25 μm x 0.25 mm) with a previously published protocol
194 (Taipale et al. 2018). Amino acid identification was based on specific ions included by the
195 EZ:faast library. For quantification, we used Sigma-Aldrich AA-18 standard mix of which
196 we made four-point calibration curve (0.005 μg μl^{-1}; 0.05 μg μl^{-1}; 0.1 μg μl^{-1}; 0.2 μg μl^{-1})
197 which was derivatized using EZ:faast kit. Eight essential amino acids (valine, leucine,

198 isoleucine, threonine, methionine, phenylalanine, lysine and histidine) were analyzed, but not
199 arginine or tryptophan. Also, two conditionally essential amino acids (glycine and proline)
200 and seven non-essential amino acids (alanine, serine, asparagine, glutamic acid, ornithine,
201 glycine-proline and tyrosine) were quantified.

202 **2.8. Statistical analyses**

203 Statistical analyses were performed using Minitab 18 for Windows and SPSS 24.0. Possible
204 differences in weight, length and condition factor (CF), SGR, feed intake, weight gain, FCR,
205 were tested using one-way ANOVA and the tank average value as an observational unit
206 (i.e. $n = 3$). A one-sample *t*-test was used to test the possible difference of average CC of the
207 treatment groups from the control group value (1, expected value when no
208 compensation). *Post-hoc* comparisons were tested by Tukey's test. $P = 0.05$ was taken as the
209 level of significance. Polynomial contrasts (linear, quadratic and cubic) were used to detect
210 possible significant trends in responses during the starvation and re-feeding periods by using
211 the number of feeding days during the starvation period (45, 27, 20 and 15 days in the control,
212 T1, T2 and T3, respectively) as model effects. In the case of significant ($P < 0.05$) trend(s),
213 we report the most significant one of the three (linear, quadratic or cubic). Permutational
214 multivariate analysis of variance (PERMANOVA, Anderson et al., 2008) was used to test
215 significant differences in amino acids and fatty acids between treatments were significant.
216 PERMANOVA was run with unrestricted permutation of raw data and type III sums of
217 squares. All the multivariate analyses were operated on Bray-Curtis distances of
218 untransformed data with the program PRIMER-E (v.7; Ivybridge, United Kingdom) and the
219 PERMANOVA+ add-on.

220 **3. Results**

221 **3.1. Intake and growth performance**

222 At day 63 (end of the 9-week starvation period), fish weight and total length were
223 significantly smaller in the treatments T1, T2 and T3 than in the control group, and condition
224 factor (CF) was significantly smaller in T3 than in the controls, and the decreasing linear
225 trend was significant (Table 1). At day 91 (end of the 4-week re-feeding period), fish in the
226 T2 and T3 groups were still significantly smaller than the controls and there was still a
227 significant decreasing linear trend in CF. The coefficient of variation of fish weight exhibited
228 a significant increasing linear trend along with the decrease of feeding even if there were no
229 significant differences between treatment averages (Table 1).

230 Weight gain (%) and SGR decreased along with the decrease of the feeding frequency, and
231 all treatments were significantly different from each other at the end of the starvation period
232 (Table 1). During the re-feeding period the trends were opposite: the less the fish had grown
233 during the first period the more they tended to grow during the second period. However,
234 weight gain (%) and SGR were significantly larger only in T3 than in the controls (Table 1).

235 In respect to feed intake during the starvation period, control fish ate significantly more than
236 the fish in the other groups, and T3 fish consumed significantly less feed than the fish in T1
237 or T2, and a negative linear trend was significant (Table 2). During the re-feeding period,
238 there were no significant differences in feed consumption between the treatments, and no
239 trend was observed. On the other hand, relative feed intake (% of body weight) increased
240 during both periods along with the increase in the length of starvation of the first period, and
241 a positive linear trend was significant (Table 2). Feed conversion ratio (FCR) did not differ

242 between treatments (T1: 0.99; T2: 0.98 and T3: 1.17) and control (1.10) during the starvation,
243 re-feeding and total experimental periods. However, there was a significant trend in FCR
244 during the starvation period (quadratic contrast): FCR decreased from the controls (1.16) to
245 T2 (0.95) and then increased in T3 (1.39).

246 The possible compensation of the fish in the treatment groups (T1, T2, T3) was estimated by
247 comparing intake and weight gain to those of the control fish during the days when the fish
248 were fed. Compensation coefficient of feed intake (CC_{intake}) during the first period was in
249 T2-group 1.17 ± 0.05 (Fig.2a) and of weight gain (CC_{gain}) 1.38 ± 0.05 (Fig. 2b), which were
250 significantly higher than 1 but the significant difference disappeared during the second
251 period. In the other two treatments (T1 and T3) CC did not differ from 1 either in terms of
252 intake or weight gain (Fig. 2).

253 **3.2. Stomach capacity**

254 According to stomach capacity measurements in absolute terms (weight g, volume mL) there
255 were no significant differences between the treatments or trends (Table 3). In relative terms
256 stomach weight (% of body weight) was significantly smaller in the controls (0.77 ± 0.09)
257 than in the group T3 (1.04 ± 0.05) at the end of the experiment while T1 and T2 did not differ
258 from the other groups, and there was also a positive linear trend. Also in relative stomach
259 volume, there was a linear positive trend at the end of the first period. The stomach weight
260 to volume -ratio was significantly higher in the controls than in the groups T1 and T2 at the
261 end of the first period, and there was a significant decreasing linear trend, but there were no
262 significant differences or trends at the end of the experiment (Table 3).

263 **3.3. Hematocrit and plasma chloride**

264 At the end of the starvation period (day 63) hematocrit values varied between 44.5 and 50.5
265 and at the end of the experiment (day 91) between 40.6 and 44.5, not displaying statistical
266 differences between feeding regimes. However, there was a significant decreasing trend in
267 hematocrit along with the decrease in feeding frequency at the end of the first period. Plasma
268 chloride varied between 121.3 and 126.3 mmol/L at the end of the first period and at the end
269 of the experiment between 124.7 and 128.6 mmol/L without being significantly different
270 between the treatments.

271 **3.4. Liver and visceral indices and composition**

272 The absolute liver size was significantly smaller in T3 fish than in the other groups at the end
273 of the first period (day 63), and livers also in T1 and T2 groups were smaller than in the
274 controls (Table 4). At the end of the re-feeding period, there were no differences in liver
275 weight between the treatments. Relative liver size (HSI) or liver water content were not
276 significantly different at any sampling points between the treatments, but in the group T3
277 HSI was significantly smaller on day 63 than on the other two sampling points (Table 4).

278 Visceral somatic (VSI) and visceral fat somatic (VFSI) indices did not differ significantly
279 between the treatments but VFSI increased in all the treatments during the experiment (Table
280 4). There was an apparent decreasing linear trend in VFSI along with the decrease of feeding
281 on days 63 (after starvation) and 91 (after re-feeding).

282 Muscle water content increased along with the decrease in feeding frequency at the end of
283 the first (day 63) and second (day 91) periods (significant linear trends), and the control group
284 had significantly lower water content than the fish in T3 on both sampling times (Table 5).
285 Lipids tended to decrease along with the decrease in the feeding frequency, but the trend was

286 significant only on day 63 and the treatment effects were significant only on day 28. Muscle
287 protein decreased along with the feeding frequency at the end of the starvation period (day
288 63) (Table 5).

289 **3.5. Fatty acid and amino acid profiles in muscle**

290 Before the experiment ω-3 and ω-6 PUFA contributed 29.5±6.4% and 13.2±7.4% of all FA
291 of rainbow trout muscle, respectively, and DHA was the major constituent of (22.1±5.0%)
292 ω-3 PUFA (Supplement 2). Total FA content of fish muscle prior to the experiment was
293 41±14 µg mg^{-1} dry weight (DW) and remained at a similar level after the period 1 (treatment
294 averages varied between 34 and 47 µg mg^{-1} DW^{-1}, Supplement 2), but increased slightly after
295 the period 2 (varied between 47 and 83 µg mg^{-1} DW^{-1}). Two factor (treatment x period)
296 PERMANOVA for the contents of ALA, LIN (linoleic acid; 18:2ω6), SDA (stearidonic acid;
297 18:4ω3), ARA (arachidonic acid; 20:4ω6) (Fig. 3c), EPA and DHA in fish muscle (Fig. 3d)
298 revealed that treatments explained only 7.3% of variances (Pseudo-$F_{3, 42}$=1.32, p=0.275),
299 whereas period explained 18.4% of variances (Pseudo-$F_{1, 42}$=10.06, p=0.001). Treatments did
300 not differ from each other whereas periods 1 (day 63) and 2 (day 91) differed statistically
301 from each other. PERMANOVA for fish muscle content of LIN and ARA separately for the
302 periods 1 and 2 resulted in slightly higher explanation percentages for treatments, 24.6% and
303 18.2%, however, the difference between treatments was not statistically significant
304 (PERMANOVA, $F_{(3, 23)}$ = 1.49-1.63, p=0.2-0.211). Correspondingly, treatments
305 explained 15.4% and 23.3% of ALA, SDA, EPA and DHA for the periods 1 and 2,
306 respectively, and the treatments did not differ (PERMANOVA, $F_{(3, 19)}$ = 0.91-2.02, p=0.13-
307 0.46) in their ω-3 contents.

308 At the beginning of the experiment, essential amino acids (EAA, Supplement 2) formed
309 64.3±2.5% of all AA in fish muscle and remained similar in control and treatment groups
310 after the first (64.3±2.5% of all AA) and second periods (64.1±3.2% of all AA). Total AA
311 content of fish muscle was 482±73 µg AA mg^{-1} DW at the beginning of the experiment and
312 did not differ between control and treatment groups after the first or second periods
313 (Supplement 1). Leucine (21.7±1.7% of all AA) and lysine (15.4±1.4% of all AA) were the
314 most abundant essential amino acids (Fig. 3a) and alanine (10.0±0.9% of all AA) was the
315 most abundant non-essential amino acid (NEAA) (Fig. 3b) in fish muscle throughout the
316 experiment. Two-factor PERMANOVA showed that treatments explained only 5.3%
317 (Pseudo-$F_{3, 44}$=0.94, p=0.485) and period 22.6% (Pseudo-$F_{1, 44}$=12.095, p=0.002) of variance
318 of NEAA content of fish muscle and whereas there were no differences in treatments but
319 between periods. Correspondingly, treatments explained 9.2% (Pseudo-$F_{3, 44}$=1.55, p=0.21)
320 and periods 14.2% (Pseudo-$F_{1, 44}$=7.16, p=0.011) of the variance of EAA content of fish
321 muscle, and there were similarly no differences in treatments but between periods. When the
322 periods 1 and 2 were separately tested, treatments explained only 12.1% and 15.9% of EAA
323 (PERMANOVA, $F_{(3, 19)}$ = 0.83-1.20, p=0.31-0.53) and 8.5% and 10.8% of NEAA
324 (PERMANOVA, $F_{(3, 19)}$ = 0.55-0.77, p=0.59-0.77) of variance of fish muscle. EAA and
325 NEAA content of fish muscle did not differ among treatments.

326 **4. Discussion**

327 In this experiment, the growth response in the form of compensation to the decrease in
328 feeding frequency was actually much less than expected. We increased the number of days
329 of starvation during the course of the experiment as an attempt to acclimate the fish to the
330 sparse feeding rhythm. Rainbow trout is a predatory fish and opportunistic feeder, and such

331 qualities could be expected to allow growth of the stomach in terms of weight and/or volume
332 to enable the fish to eat large amount at a single feeding bout. Enlargement of the stomach
333 volume has been reported to occur in rainbow trout as a consequence of an increase in the
334 food water content (fish fed with chopped herring, *Clupea harengus*, vs. fish fed with dry
335 feed) (Ruohonen and Grove 1996) and also as a response to sparse feeding frequency (Nikki
336 et al., 2004). In the current study feeding rhythms of the treatment groups elicited some
337 changes in the stomach, and significant trends related to the severity of starvation were seen
338 in those stomach variables which were relative of fish size (Table 3). However, the lack of
339 stronger compensatory growth may be related to the duration of the first experimental period
340 (63 days) and the fish may not have had sufficient time to become fully adjusted to the sparse
341 feeding protocols. For example, brown trout (*Salmo trutta*) needed over two months before
342 showing compensatory growth when fed only twice a week (Pirhonen and Forsman, 1998).
343 On the other hand, pikeperch (*Sander lucioperca*) fed chopped fresh fish flesh clearly became
344 adapted to the sparse (fed every fourth or every seventh day) feeding frequency in the latter
345 half of the 58-day experiment (Mattila et al., 2009).

346 It is plausible that when the fish are fed with dry pellets (water content c. 10 %) there is a
347 physiological limit to which they are capable to fill their stomachs, as the fish will need to
348 moisturize the feed both by increasing drinking and by excreting gastric juices before
349 digestion is possible (Ruohonen et al., 1997). Ruohonen et al., (1997) suggested that it is
350 actually the water availability to moisturize ingested dry feed which constraints feed intake
351 in rainbow trout rather than stomach capacity. Thus, the low feed water content may have
352 restricted the fish to eat and grow more than what they did in the treatment groups. On the
353 other hand, hatchery fish have been selected especially for fast growth, and it is known that

354 the fastest growing fish are the ones with the highest feed intake (e.g. Nikki et al., 2004), and
355 consequently probably with the largest stomachs. Some kind of upper level or plateau may
356 have been reached in their stomach capacities, and the hatchery fish may not be able for
357 further stomach volume increase in conditions when feed is not offered every day, thus
358 limiting their ability for expected compensatory growth. Therefore, it would be interesting to
359 compare feeding capacity of (semi-)wild fish to the domesticated rainbow trout.

360 Any decrease in feeding frequency during the first period affected fish growth (both in weight
361 and length) negatively (Table 1) although some compensation in feed intake and weight gain
362 were observed in the group T2 (Fig. 2). However, at their best the CC-values of the present
363 experiment were only modest (1.38) when compared to the CC-values in pikeperch (about
364 1.9; Mattila et al., 2009) but quite close to those observed in rainbow trout (about 1.5;
365 Taşbozan et al., 2016). It must be noted that if CC = 1 does not mean compensatory growth
366 but it indicates only that feed deprived fish have been able to eat or grow as much as the
367 controls during the feeding days. Therefore, the CC-values should be much higher than 1 in
368 the feed deprived groups if a full growth compensation was anticipated. We expected to see
369 compensatory growth especially during the second period in the treatment groups, but this
370 did not occur (Fig. 2), and the fish from T2 and T3 remained significantly smaller than the
371 controls despite the increasing trend in relative weight gain and SGR along with the decrease
372 in feeding during the first period (Table 1). Quinton and Blake (1990) observed in rainbow
373 trout clear growth compensation only on the third week of feeding after a three-week
374 starvation period, which suggests that rainbow trout may need several weeks before growth
375 rate starts to increase. Albeit fish from T1 were not significantly smaller than the controls,

the difference in size at the end of the experiment was about 15%, which is negative from the fish farmer's point of view, especially when there was no difference in FCR.

We decided to feed the fish in the control group only during the weekdays based on the results obtained in our laboratory (Nikki et al., 2004) with individually grown rainbow trout of similar size than in the current study. Nikki et al. (2004) exposed the trout to starvation periods of fixed length (from 2 to 16 days) in order to keep them hyperphagic, in comparison to controls, during the feeding days. In that research, the trout grew largest when feed was withdrawn for 2 days, and the hyperphagic response lasted typically for 5 days. Also Taşbozan et al. (2016) found in group-reared rainbow trout that when fasted for two days per week the fish grew significantly larger than the controls. Based on these earlier observations we can assume that the control fish of the present experiment grew at least as well as they would have grown if they had been feed every day. The other advantage of fasting the fish during the weekends is that when the weighing of the fish is on Monday, the fish have more or less empty stomachs (Grove et al., 1978), which in turn aids in getting rather standardized body weight for all individuals in each treatment.

For the fish farmer, size homogeneity within a tank is important as it will decrease the need for size selection and the end product will be of similar market size. The variability in size is commonly expressed by the coefficient of variation, CV, and the increase of CV is typically related to the competition for food and increase of aggressiveness between individuals (Jobling, 1995). In brown trout (*Salmo trutta*) it was observed that a sparse feeding (twice per week) significantly decreased CV of intake, i.e. the fish on the restricted group ate very homogeneously when compared to the controls, but however, that did not affect CV of weight (Pirhonen and Forsman, 1998). In contrast, in the present experiment there appeared a

399 significant trend for the CV to increase along with the increase of levels of starvation. The
400 fish in our experiment were not restricted for food when they were fed but all fish were
401 always fed to apparent satiation, and as such the increase in CV of weight can be interpreted
402 to reflect inter-individual variability in feed intake or feed efficiency rather than competition
403 for food or aggressive behavior.

404 Condition factor is a widely used morphometric index showing indirectly how lean or fat an
405 individual animal of a given population is, even though it is only an approximate indirect
406 index for actual body fat reserves in fishes (Rennie and Verdon, 2008; McPherson et al.,
407 2011; Sutton et al., 2011). At the end of the starvation period, the significant decreasing
408 trend in CF (Table 1) indicates that the increase of duration of starvation directly affect the
409 ability of the fish to gain surplus energy from the feed. However, the condition factor in T3
410 and also in the other groups had increased significantly from the initial value (0.99) by the
411 end of the starvation period. The T3-group fish were apparently slightly starving during the
412 first period because during the re-feeding period it was the only group with a significant
413 increase in condition factor (to 1.21), but however, the decreasing trend in CF was still
414 significant (Table 1). Pirhonen and Forsman (1998) found in brown trout that the fish which
415 were fed only twice a week exhibited a clear rise in condition factor after two months of
416 rearing and that coincided with a clear rise in SGR.

417 One of the primary objectives of the potential use of compensatory growth in hatcheries
418 would be to improve feed conversion ratio, and improvement in FCR has been indicated in
419 some investigations (Gaylord and Gatlin, 2001; Oh et al., 2013; Xiao et al., 2013; Gao et al.,
420 2015). However, this is not always the case and growth compensation has also been reached
421 only by a hyperphagic response (Wang et al., 2000; Yengkokpama et al., 2013; Xiao et al.,

422 2013). The present study did not find an indication of a significant change in FCR suggesting
423 that the little what the fish were able to compensate for the feed deprivation was mostly
424 achieved by hyperphagia. FCR was about 9 % less in the groups T1 and T2 than in the
425 controls during the first period which would account for savings in the feed costs but on the
426 other hand decrease in fish size at the end of the first period was over 20 % in the favor of
427 controls outweighing the benefit from feed saving. The significantly higher FCR in the T3
428 group, also seen as a quadratic trend (Table 2), is another indication of too strong feed
429 deprivation (Adakli and Taşbozan, 2015).

430 Liver water content, HSI and VSI were not significantly affected by the length of starvation.
431 This indicates that the liver and viscera are not easily affected by starvation, which is in
432 accordance with the findings in other fish species (Miglavs and Jobling, 1989; Rueda et al.,
433 1998; Ali et al., 2016). There was a significant linear trend of VFSI to decrease along with
434 the severity of starvation at the end of the first and second periods. However, VFSI increased
435 in all treatments towards the end of the experiment showing that even in the least fed group
436 (T3) the fish prefer to accumulate fat in the body cavity rather than converting this energy
437 into growth.

438 Starvation under certain circumstances can be considered as a stress factor (Blom et al.,
439 2000). In this study starvation and re-feeding periods did not affect significantly hematocrit
440 and plasma chloride values albeit an increasing trend in hematocrit in the end of the first
441 period was observed. The literature regarding the effect of starvation on hematocrit are
442 conflicting. The increase of hematocrit in response to starvation has been reported in
443 European eel (*Anguilla anguilla*) and beluga (*Huso huso*) (Johansson-Sjöbeck et al., 1975;
444 Falahatkar, 2012) being a possible response to starvation stress. On the other hand, a decrease

445 in hematocrit related to starvation has been reported in lake sturgeon (*Acipenser fulvescens*),
446 channel catfish (*Ictalurus punctatus*) and binni (*Mesopotamichthys sharpeyi*) (Gillis and
447 Ballantyne, 1996; Lim and Klesius, 2003; Najafi et al., 2015). No effect on the haematocrit
448 was found in European sea bass (*Dicentrarchus labrax*), blackspot seabream (*Pagellus*
449 *bogaraveo*), red porgy (*Pagrus pagrus*), olive flounder (*Paralichthys olivaceus)*, persian
450 sturgeon (*A. persicus)* and grey mullet (*Mugil cephalus*) (Caruso et al., 2011, 2012; Kim et
451 al., 2014; Yarmohammadi et al., 2015; Akbary and Jahanbakhshi, 2016). Also, a possible
452 difference in plasma chloride between the treatments would have suggested hydromineral
453 imbalance, due to stress (Waring et al., 1992; Einarsdóttir and Nilssen, 1996). Even if
454 changes in plasma hydromineral balance can be regarded as a secondary stress response
455 (Einarsdóttir and Nilssen, 1996; Barton, 2002) the absence of differences in plasma chloride
456 can be interpreted as lack of stress in our treatment groups.

457 Muscle lipid and protein content decreased and water content increased during the starvation
458 period along with the increase of days of starvation. A decrease in muscle lipids and an
459 increase in water were expected due to starvation (Shearer, 1994). When starved fishes can
460 also oxidize protein to produce energy directly or through gluconeogenesis (Walsh, 1998)
461 which can explain the decreasing trend in muscle protein content at the end of the first period.
462 On the other hand, muscle protein has also been observed to remain constant in fishes
463 regardless of starvation (Shearer, 1994; Mattila et al., 2009).

464 DHA fatty acid and essential amino acids are crucial for optimal growth and development of
465 juvenile fishes (Wilson and Halver, 1986; Rønnestad et al., 1999; Tocher, 2010). The
466 decrease in growth in the treatment groups did not lower the contents of DHA and AA in fish
467 muscle. Previous studies have shown that fishes highly retain DHA (Glencross et al., 2003,

468 2014; Murray et al., 2014) and EAA and use protein-sparing strategy under nutrient limitation
469 (Cho and Kaushik, 1990). The fish in the starved groups most likely used dietary
470 carbohydrates and lipids for energy and spared AA and DHA for cell growth and optimal
471 performance. Conservation of AA and DHA show their essential roles in fish metabolism
472 (Tidwell et al., 1992; Rønnestad et al., 1994; Abi-Ayad et al., 2000). In the present research,
473 starvation did not influence the AA profile of muscle which is along with the previous
474 findings of the ability of juvenile rainbow trout to compensate for the low availability of
475 amino acids from their diet (Taipale et al., 2018). Altogether, our results showed that rainbow
476 trout sustained DHA and AA at the same level over the starvation period and thus did not
477 influence on nutritional value of fish.

478 **5. Conclusion**

479 The results obtained in this experiment failed to show that rainbow trout would be capable
480 to adapt to sparse feeding frequency by consuming enough during the feeding days to keep
481 growing with the pace of the control fish. Our first hypothesis about the stomach volume
482 increase and consequent increase in feed intake was not supported. The second hypothesis
483 that the increased stomach volume in previously feed restricted fish would allow full growth
484 compensation was supported only partly: although the fish from the treatment 1 (fed three
485 times per week during the period 1) were not significantly smaller than the controls in the
486 end, they weighed about 15 % less than the controls. Taken together, we did not find evidence
487 that progressive decrease in the feeding frequency would facilitate rainbow trout in getting
488 acclimated to the sparse feeding by expressing sufficient compensatory growth but these
489 tested feeding schedules severely decreased fish growth; however, none of these treatments
490 significantly altered the nutritional value of fish.

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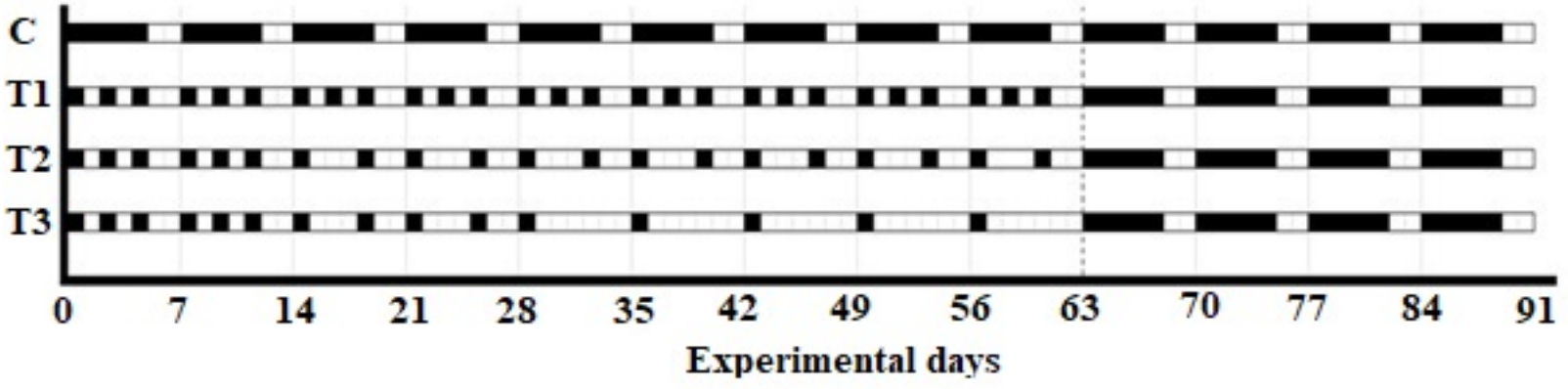
716 Figure 1. Feeding schedule in the experiment which first tested adaptation to starvation
717 (period 1, 63 days) and the responses to re-feeding (period 2, 28 days) in juvenile rainbow
718 trout (*Oncorhynchus mykiss*). C=Control (fed every weekday) and T1, T2 and T3 are
719 treatments 1-3. Black bars indicate feeding days.
720
721
722 Figure 2. Compensation coefficients of feed intake (a) and weight gain (b) of rainbow trout
723 (*Oncorhynchus mykiss*) fed according to different feeding regimes (treatment groups T1, T2,
724 T3) for 91 days under starvation (period 1, 63 days) and re-feeding (period 2, 28 days). Each
725 bar represents mean ± S.D, n = 3. CC>1 indicates compensation, and asterisk indicates a
726 significant difference from 1.
727
728
729 Figure 3. Amino acid and fatty acid content (mean±sd; µg AA/FA mg^{-1} DW^{-1}) of rainbow
730 trout (*Oncorhynchus mykiss*) muscle in the control group and different feeding treatments
731 (T1, T2, T3) under starvation (period 1, 63 days) and re-feeding (period 2, 28 days). A)
732 Essential amino acids (EAA: valine, leucine, isoleucine, threonine, lysine, phenylalanine,
733 methionine), B) Non-essential amino acids (NEAA: alanine, glycine, serine, proline, aspartic
734 acid and tyrosine), C) ω-6 polyunsaturated fatty acids (linoleic acid and arachidonic acid)
735 and D) ω-3 polyunsaturated fatty acids (alfa-linolenic acid, stearidonic acid,
736 eicosapentaenoic acid, docosahexaenoic acid).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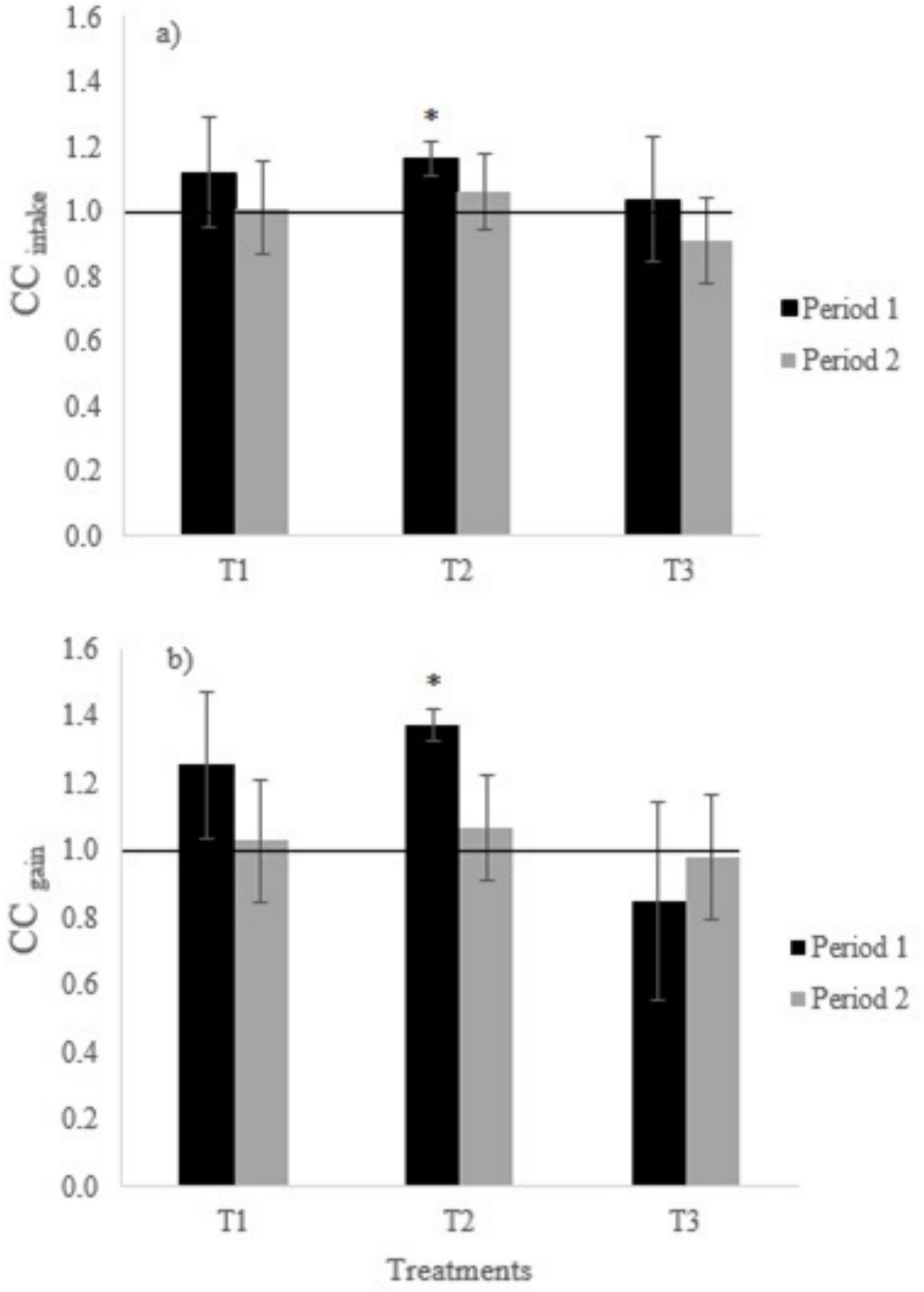
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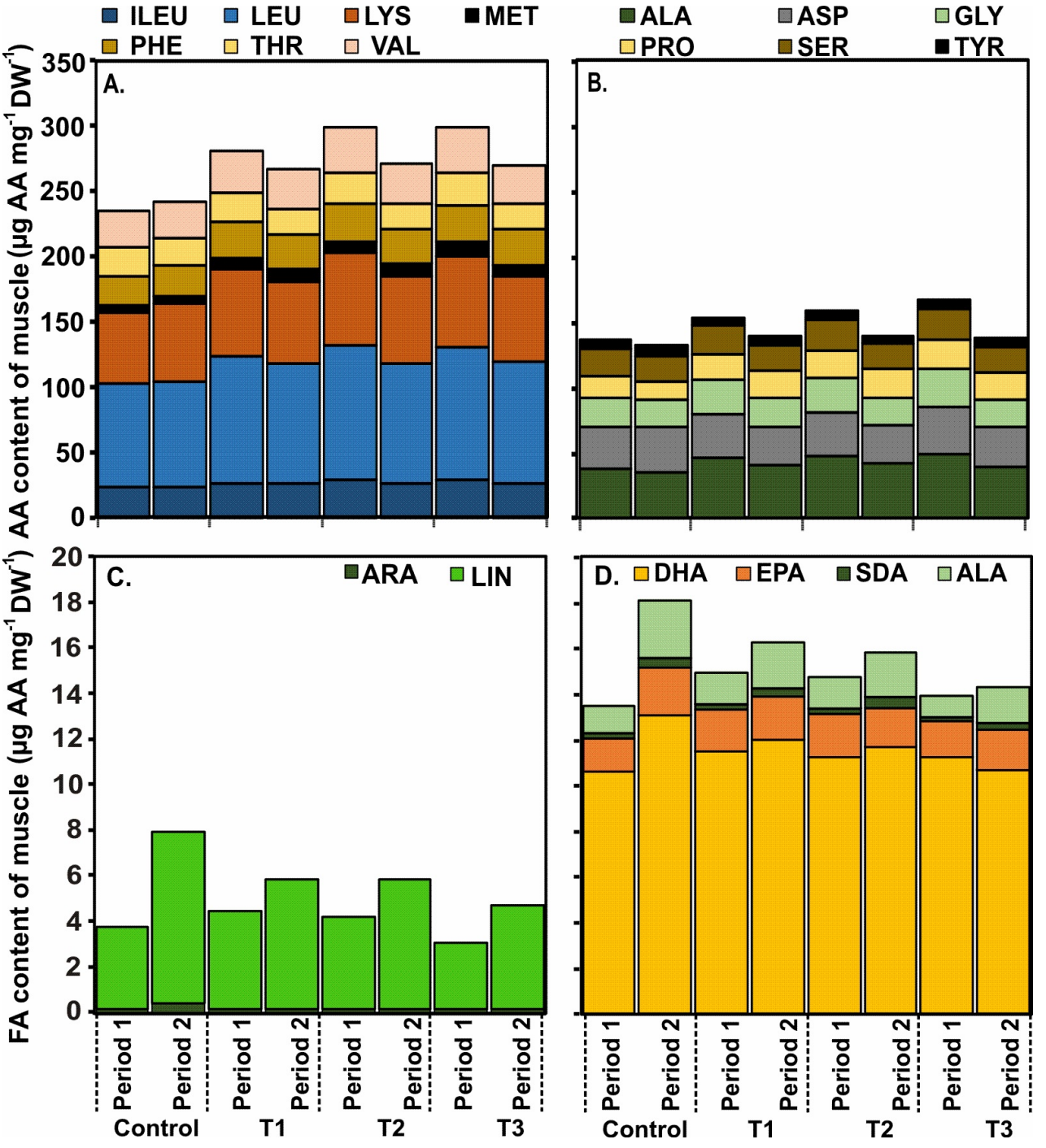
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AA content of muscle (µg AA mg-1 DW-1)
FA content of muscle (µg AA mg-1 DW-1)
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Period 1
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Control
T1
T2
T3

Table 1. Growth performance of rainbow trout (*O. mykiss*) during the experiment of different feeding regimes under starvation (days 0-63) and re-feeding (days 64-91) periods.

	Control	T1	T2	T3	P.C.
Weight (g)					
Day 0	40.97 ± 1.59^{A}	37.97 ± 1.27^{A}	39.60 ± 0.78^{A}	39.37 ± 2.70^{A}	N.S.
Day 63	173.47 ± 13.60^{cB}	134.17 ± 8.82^{bB}	114.03 ± 3.52^{bB}	81.17 ± 10.70^{aB}	L
Day 91	235.33 ± 27.84^{cC}	200.87 ± 12.66^{bcC}	174.13 ± 9.81^{abC}	134.70 ± 24.50^{aC}	L
Length (cm)					
Day 0	16.07 ± 0.21^{A}	15.77 ± 0.06^{A}	15.90 ± 0.20^{A}	15.97 ± 0.25^{A}	N.S.
Day 63	23.87 ± 0.40^{cB}	22.37 ± 0.49^{bB}	21.10 ± 0.30^{bB}	19.50 ± 0.70^{aB}	L
Day 91	26.43 ± 0.83^{cC}	25.23 ± 0.65^{bcC}	24.27 ± 0.40^{abC}	22.27 ± 1.15^{aC}	L
Condition Factor					
Day 0	0.99 ± 0.01^{A}	0.97 ± 0.04^{A}	0.99 ± 0.02^{A}	0.99 ± 0.03^{A}	N.S.
Day 63	1.27 ± 0.04^{bB}	1.20 ± 0.10^{abB}	1.21 ± 0.03^{abB}	1.09 ± 0.03^{aB}	L
Day 91	1.27 ± 0.03^{B}	1.25 ± 0.03^{B}	1.22 ± 0.01^{B}	1.21 ± 0.04^{C}	L
CV of fish weight					
Day 0	17.44 ± 1.87	20.43 ± 0.58	19.89 ± 3.65	20.87 ± 1.03	N.S.
Day 63	17.20 ± 2.37	23.77 ± 1.88	25.23 ± 4.19	26.71 ± 5.32	L
Day 91	16.96 ± 3.93	20.80 ± 1.25	24.02 ± 4.68	23.49 ± 4.25	L
Weight gain (%)					
Starvation period	323.02 ± 16.94^{dB}	253.25 ± 16.06^{cB}	187.98 ± 7.91^{bB}	105.86 ± 18.74^{aB}	L
Re-feeding period	35.54 ± 10.27^{aA}	49.79 ± 5.24^{abA}	52.68 ± 6.57^{abA}	65.26 ± 8.51^{bA}	L
Total	473.85 ± 55.78^{c}	429.22 ± 31.83^{bc}	339.53 ± 16.67^{ab}	241.26 ± 49.30^{a}	L
SGR					
Starvation period	2.25 ± 0.06^{dB}	1.97 ± 0.07^{cB}	1.65 ± 0.04^{b}	1.12 ± 0.14^{aA}	L
Re-feeding period	1.04 ± 0.26^{aA}	1.39 ± 0.12^{abA}	1.46 ± 0.15^{ab}	1.73 ± 0.18^{bB}	L
Total	1.88 ± 0.11^{c}	1.79 ± 0.07^{bc}	1.59 ± 0.04^{b}	1.31 ± 0.15^{a}	L

Values are mean ± SD, n = 3. Different superscript lower case letters indicate significant differences between treatments and upper case letters refer to significant differences between measurement periods within each treatment during the experiment ($p < 0.05$). P.C.: Polynomial contrast analysis; N.S.: not significant; L: linear model was the most significant one. See Fig. 1. for feeding protocols of the treatments. CV = coefficient of variation.

Table 2. Absolute intake, relative feed intake and feed conversion ratio in rainbow trout (*O. mykiss*) of different feeding regimes under starvation (days 0-63) and re-feeding (days 64-91) periods.

	Control	T1	T2	T3	P.C.
Absolute intake (g fish^{-1})					
Starvation period	117.43 ± 7.54^{cA}	78.70 ± 11.00^{b}	60.17 ± 2.63^{b}	39.17 ± 7.25^{aA}	L
Re-feeding period	62.32 ± 8.46^{B}	66.58 ± 4.54	63.79 ± 7.18	57.68 ± 5.38^{B}	N.S.
Total	183.76 ± 13.13^{c}	145.28 ± 10.96^{b}	123.97 ± 7.88^{ab}	96.86 ± 12.61^{a}	L
Relative feed intake (%)					
Starvation period	0.44 ± 0.00^{aA}	0.61 ± 0.06^{bA}	0.71 ± 0.02^{bcA}	0.80 ± 0.07^{cA}	L
Re-feeding period	0.19 ± 0.05^{aB}	0.24 ± 0.02^{abB}	0.29 ± 0.02^{bcB}	0.34 ± 0.02^{cB}	L
Total	0.31 ± 0.02^{a}	0.43 ± 0.04^{b}	0.50 ± 0.01^{bc}	0.57 ± 0.03^{c}	L
Feed conversion ratio					
Starvation period	1.16 ± 0.32	0.99 ± 0.04	0.95 ± 0.01	1.39 ± 0.20^{A}	Q
Re-feeding period	1.04 ± 0.12	1.00 ± 0.03	1.01 ± 0.04	0.94 ± 0.04^{B}	N.S.
Total	1.10 ± 0.21	0.99 ± 0.03	0.98 ± 0.02	1.17 ± 0.12	Q

Values are mean ± SD, n = 3. Different superscript lower case letters indicate significant differences between treatments and upper case letters refer to significant differences between measurements periods within each treatment during the experiment ($p < 0.05$). P.C.: Polynomial contrast analysis; N.S.: not significant; L: linear model was the most significant one; Q: quadratic model was the most significant one. See Fig. 1. for feeding protocols of the treatments. Relative feed intake = (total intake (g) * number of feedings^{-1}) * average fish weight^{-1}.

Table 3. Absolute and relative stomach weight, stomach volume and stomach weight to volume -ratio in rainbow trout (*O. mykiss*) of different feeding regimes under starvation (days 0-63) and re-feeding (days 64-91) periods.

Stomach variable	Control	T1	T2	T3	P.C.
Weight (g)					
Day 28	1.32 ± 0.14^{A}	1.28 ± 0.24	0.97 ± 0.18^{A}	1.17 ± 0.18	N.S.
Day 63	2.19 ± 0.31^{B}	2.01 ± 0.65	1.71 ± 0.33^{B}	1.53 ± 0.40	N.S.
Day 91	1.98 ± 0.43^{AB}	2.19 ± 0.22	1.89 ± 0.21^{B}	1.60 ± 0.39	N.S.
Weight (% of wet weight)					
Day 28	1.46 ± 0.29^{B}	1.75 ± 0.12^{B}	1.35 ± 0.07^{B}	1.68 ± 0.10^{B}	C
Day 63	1.23 ± 0.17^{AB}	1.40 ± 0.42^{AB}	1.41 ± 0.29^{B}	1.55 ± 0.34^{AB}	N.S.
Day 91	0.77 ± 0.09^{aA}	0.97 ± 0.16^{abA}	0.89 ± 0.05^{abA}	1.04 ± 0.05^{bA}	L
Volume (mL)					
Day 28	2.55 ± 0.86^{A}	2.61 ± 0.28^{A}	2.37 ± 0.54^{A}	2.01 ± 0.79^{A}	N.S.
Day 63	5.37 ± 0.65^{B}	6.44 ± 1.92^{B}	6.23 ± 0.99^{B}	4.40 ± 0.88^{AB}	N.S.
Day 91	7.21 ± 1.30^{B}	7.65 ± 0.13^{B}	6.03 ± 2.18^{B}	5.69 ± 2.07^{B}	N.S.
Volume (% of wet weight)					
Day 28	2.77 ± 0.78	3.66 ± 0.95	3.28 ± 0.49^{AB}	2.91 ± 1.04	N.S.
Day 63	3.01 ± 0.38	4.48 ± 1.19	5.10 ± 0.81^{B}	4.46 ± 0.75	L
Day 91	2.80 ± 0.15	3.35 ± 0.26	2.84 ± 0.97^{A}	3.72 ± 1.19	N.S.
Weight / Volume (g/mL)					
Day 28	0.56 ± 0.19^{B}	0.50 ± 0.12^{B}	0.42 ± 0.05	0.63 ± 0.21	N.S.
Day 63	0.41 ± 0.01^{bAB}	0.31 ± 0.03^{aAB}	0.27 ± 0.02^{a}	0.35 ± 0.05^{ab}	L
Day 91	0.27 ± 0.02^{A}	0.29 ± 0.03^{A}	0.35 ± 0.14	0.30 ± 0.09	N.S.

Values are mean ± SD, n = 3. Different superscript lower case letters indicate significant differences between treatments and upper case letters refer to significant differences between measurements periods within each treatment during the experiment ($p < 0.05$). P.C.: Polynomial contrast analysis; N.S.: not significant; L: linear model was the most significant one; C: cubic model was the most significant one. See Fig. 1. for feeding protocols of the treatments.

Table 4. Liver and visceral parameters in rainbow trout (*O. mykiss*) of different feeding regimes under starvation (days 0-63) and re-feeding (days 64-91) periods.

	Control	T1	T2	T3	P.C.
Liver weight (g)					
Day 28	0.97 ± 0.19	0.84 ± 0.26	0.99 ± 0.26	0.80 ± 0.09	N.S.
Day 63	1.82 ± 0.13^{c}	1.44 ± 0.11^{b}	1.25 ± 0.14^{b}	0.80 ± 0.05^{a}	L
Day 91	2.29 ± 0.37	2.29 ± 0.37	2.05 ± 0.18	1.69 ± 0.38	N.S.
Liver water content (%)					
Day 28	75.43 ± 0.54	74.05 ± 1.05	74.80 ± 0.66^{B}	74.80 ± 0.64	N.S.
Day 63	73.70 ± 1.90	73.82 ± 0.91	72.61 ± 0.44^{A}	74.14 ± 2.02	N.S.
Day 91	73.38 ± 0.48	73.84 ± 0.62	73.69 ± 0.44^{AB}	74.24 ± 0.51	N.S.
HSI (% of weight)					
Day 28	1.06 ± 0.14	1.12 ± 0.13	1.35 ± 0.19^{B}	1.15 ± 0.05^{B}	N.S.
Day 63	1.02 ± 0.03	1.01 ± 0.11	1.03 ± 0.15^{AB}	0.82 ± 0.08^{A}	N.S.
Day 91	0.89 ± 0.05	1.01 ± 0.21	0.97 ± 0.03^{A}	1.10 ± 0.03B^{B}	N.S.
VSI (% of weight)					
Day 63	20.11 ± 0.62	21.89 ± 1.90	22.43 ± 1.89	20.39 ± 0.70	N.S.
Day 91	19.48 ± 1.33	17.34 ± 3.00	19.32 ± 1.44	20.50 ± 0.85	N.S.
VFSI (% of weight)					
Day 28	1.67 ± 0.69^{A}	1.39 ± 0.07^{A}	0.79 ± 0.41^{A}	1.20 ± 0.34^{A}	N.S.
Day 63	3.35 ± 0.72^{B}	3.21 ± 0.49^{B}	2.79 ± 0.61^{B}	2.06 ± 0.31^{AB}	L
Day 91	4.91 ± 0.15^{C}	4.23 ± 0.33^{C}	4.74 ± 0.40^{C}	3.45 ± 0.98^{B}	L

Values are mean ± SD, n = 3. Different superscript lower case letters indicate significant differences between treatments and upper case letters refer to significant differences between measurements periods within each treatment during the experiment ($p < 0.05$). HSI: Hepatosomatic index; VSI: Visceral somatic index; VFSI: Visceral fat somatic index. P.C.: Polynomial contrast analysis; N.S.: not significant; L: linear model was the most significant one. See Fig. 1. for feeding protocols of the treatments.

Table 5. Muscle composition of rainbow trout (*O. mykiss*) of different feeding regimes under starvation (days 0-63) and re-feeding (days 64-91) periods.

	Control	T1	T2	T3	P.C.
Muscle composition					
Water content (%)					
Day 28	74.66 ± 1.97	74.02 ± 1.30	75.53 ± 0.46	75.32 ± 0.61	N.S.
Day 63	75.58 ± 1.52[a]	76.78 ± 0.77[ab]	77.42 ± 0.46[ab]	78.86 ± 0.17[b]	L
Day 91	75.48 ± 0.08[a]	75.77 ± 0.29[ab]	75.95 ± 0.69[ab]	76.98 ± 0.61[b]	L
Lipids (%)					
Day 28	3.76 ± 0.52[ab]	3.67 ± 0.58[ab]	3.89 ± 0.76[b]	2.43 ± 0.25[a]	N.S.
Day 63	2.40 ± 1.33	1.90 ± 0.63	1.38 ± 0.46	0.86 ± 0.04	L
Day 91	2.05 ± 0.17	1.70 ± 0.17	1.65 ± 0.19	1.50 ± 0.81	N.S.
Protein (%)					
Day 28	19.43 ± 2.01	20.03 ± 1.45	18.61 ± 0.37	19.95 ± 0.65	N.S.
Day 63	20.72 ± 0.68[b]	19.84 ± 0.54[b]	19.56 ± 0.37[ab]	18.34 ± 0.43[a]	L
Day 91	20.56 ± 1.29	21.05 ± 0.39	21.19 ± 0.58	20.31 ± 0.31	N.S.

Values are mean ± SD, n = 3. Different superscript letters indicate significant differences between treatments ($p < 0.05$). P.C.: Polynomial contrast analysis; N.S.: not significant; L: linear model was the most significant one. See Fig. 1. for feeding protocols of the treatments.

Supplement 1. Amino acid content (µg AA mg^{-1} DW^{-1}) in two different feeds and muscle of rainbow trout (*O. mykiss*) of different feeding regimes at the end of the starvation (Day 63) and re-feeding (Day 91) periods.

	Feed 1	**Feed 2**	**Day 0**	**Control**		**T1**		**T2**		**T3**	
				Day 63	**Day 91**	**Day 63**	**Day 91**	**Day 63**	**Day 91**	**Day 63**	**Day 91**
Essential amino acids											
ALA	9.1 ± 0.6	9.1 ± 1.2	10 ± 1.0	10.5 ± 0.1	9.2 ± 1.2	10.7 ± 0.8	9.8 ± 0.6	10.4 ± 0.5	10.0 ± 0.6	10.3 ± 0.6	9.4 ± 1.0
ASN	0.1 ± 0.0	0.0 ± 0.1	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
ASP	10.6 ± 1.1	9.9 ± 1.2	7.3 ± 0.7	9.1 ± 2.7	8.4 ± 2.4	7.5 ± 0.9	7.0 ± 1.0	7.2 ± 0.9	7.1 ± 0.9	7.9 ± 1.3	7.2 ± 1.2
GLU	4.3 ± 1.9	3.4 ± 3.0	0.9 ± 0.1	1.0 ± 0.2	4.0 ± 5.4	0.9 ± 0.3	1.3 ± 0.3	0.9 ± 0.2	1.4 ± 0.4	1.1 ± 0.5	3.1 ± 4.1
GPR	0.2 ± 0.1	0.2 ± 0.1	0.0 ± 0.0	0.1 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
ORN	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
SER	3.4 ± 0.6	2.3 ± 1.2	1.1 ± 0.8	1.9 ± 0.4	1.9 ± 0.9	1.4 ± 0.4	1.5 ± 0.9	1.5 ± 0.4	1.4 ± 0.5	1.6 ± 0.8	1.5 ± 0.7
TYR	2.5 ± 0.4	2.7 ± 0.8	4.5 ± 0.9	4.3 ± 0.3	3.9 ± 1.2	4.5 ± 0.5	5.1 ± 0.4	4.5 ± 0.6	5.0 ± 0.4	4.6 ± 0.3	4.9 ± 1.1
Σ EAA	**30 ± 4.6**	**28 ± 7.5**	**24 ± 3.6**	**27 ± 3.9**	**27 ± 11.2**	**25 ± 3.0**	**25 ± 0.4**	**25 ± 0.3**	**25 ± 0.4**	**26 ± 0.4**	**26 ± 1.0**
Non-ssential amino acids											
GLY	6.7 ± 0.3	6.1 ± 0.5	6.5 ± 1.0	5.9 ± 0.2	5.4 ± 0.5	6.1 ± 0.3	5.4 ± 0.5	5.8 ± 0.3	5.3 ± 0.4	6.0 ± 0.2	5.0 ± 0.1
HIS	0.2 ± 0.0	0.4 ± 0.1	0.0 ± 0.0	0.2 ± 0.3	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
ILEU	4.4 ± 0.0	5.6 ± 0.5	6.3 ± 0.8	5.9 ± 1.2	6.1 ± 0.4	6.1 ± 0.3	6.4 ± 0.3	6.2 ± 0.2	6.2 ± 0.2	6.3 ± 0.5	6.1 ± 0.4
LEU	20.5 ± 1.6	19.7 ± 0.6	23.1 ± 2.6	20.0 ± 3.6	20.7 ± 2.5	21.9 ± 0.9	22.4 ± 1.2	22.2 ± 0.8	22.3 ± 0.6	21.5 ± 0.8	22.1 ± 1.2
LYS	9.3 ± 1.0	11.4 ± 1.0	14.1 ± 1.2	15.1 ± 2.5	15.7 ± 2.3	15.4 ± 1.2	15.3 ± 1.7	15.5 ± 1.4	15.8 ± 0.6	14.6 ± 0.6	15.6 ± 0.9
MET	0.7 ± 0.1	0.7 ± 0.3	1.9 ± 0.4	1.4 ± 0.7	1.5 ± 0.4	1.8 ± 0.4	2.2 ± 0.5	1.9 ± 0.5	2.2 ± 0.3	2.2 ± 0.4	2.1 ± 0.6
PHE	6.9 ± 0.5	6.4 ± 0.1	6.9 ± 1.3	5.8 ± 1.1	6.0 ± 0.7	6.4 ± 0.4	6.4 ± 0.4	6.3 ± 0.5	6.3 ± 0.2	6.1 ± 0.3	6.5 ± 0.4
PRO	8.9 ± 0.4	10.1 ± 1.0	5.2 ± 0.5	5.6 ± 0.6	4.8 ± 0.5	5.0 ± 0.2	4.8 ± 0.2	4.9 ± 0.2	4.8 ± 0.2	5.0 ± 0.2	4.6 ± 0.4
THR	4.6 ± 0.2	5.1 ± 0.3	5.0 ± 1.2	5.8 ± 0.5	5.3 ± 0.4	5.0 ± 0.3	4.8 ± 0.7	5.1 ± 0.4	4.9 ± 0.3	5.2 ± 0.4	4.7 ± 0.4
VAL	7.5 ± 0.0	6.8 ± 0.7	7.1 ± 0.6	7.4 ± 0.9	7.1 ± 0.5	7.3 ± 0.3	7.3 ± 0.4	7.4 ± 0.3	7.2 ± 0.4	7.3 ± 0.5	6.9 ± 0.4

Σ NEAA	**70 ± 0.5**	**72 ± 0.6**	**76 ± 1.1**	**73 ± 1.4**	**73 ± 0.9**	**75 ± 0.5**	**75 ± 0.7**	**75 ± 0.5**	**75 ± 0.4**	**74 ± 0.4**	**74 ± 0.6**
Total AA content (µg AA mg^{-1} DW^{-1})	**244 ± 32**	**171 ± 30**	**482 ± 73**	**436 ± 55**	**419 ± 121**	**440 ± 56**	**413 ± 49**	**463 ± 56**	**418 ± 54**	**473 ± 69**	**425 ± 67**

Supplement 2. Fatty acid content (µg FA mg^{-1} DW^{-1}) in two different feeds and muscle of rainbow trout (*O. mykiss*) of different feeding regimes at the end of the starvation (Day 63) and re-feeding (Day 91) periods.

	Feed 1	**Feed 2**	**Day 0**	**Control**		**T1**		**T2**		**T3**	
				Day 63	**Day 91**	**Day 63**	**Day 91**	**Day 63**	**Day 91**	**Day 63**	**Day 91**
Saturated fatty acids											
c14:0	2.8 ± 0.0	1.8 ± 0.0	0.8 ± 0.3	1.1 ± 0.2	1.5 ± 0.2	1.1 ± 0.2	1.3 ± 0.1	1.0 ± 0.1	1.2 ± 0.2	0.9 ± 0.2	1.2 ± 0.1
c15:0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.1	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
c16:0	10.8 ± 0.2	9.4 ± 0.1	15.0 ± 1.6	14.9 ± 1.2	13.6 ± 0.6	13.5 ± 0.4	13.7 ± 0.8	13.6 ± 0.9	14.4 ± 1.3	14.4 ± 0.7	14.4 ± 1.4
c17:0	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
c18:0	3.9 ± 0.1	3.8 ± 0.0	5.1 ± 0.8	4.0 ± 0.5	3.5 ± 0.1	3.8 ± 0.3	3.4 ± 0.2	3.9 ± 0.1	3.7 ± 0.3	4.0 ± 0.2	3.9 ± 0.2
c20:0	0.4 ± 0.0	0.8 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.1
c22:0	0.2 ± 0.0	1.4 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Σ SAFA	**18.5 ± 0.3**	**17.6 ± 0.2**	**21.5 ± 2.8**	**20.5 ± 2.0**	**19.4 ± 0.9**	**19.0 ± 0.9**	**19 ± 1.2**	**19.1 ± 1.1**	**20.0 ± 1.9**	**19.8 ± 1.1**	**20.1 ± 1.8**
Monounsaturated fatty acids											
16:1ω9	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.0	0.3 ± 0.1	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.1
16:1ω7	0.0 ± 0.0	0.0 ± 0.0	1.4 ± 0.3	1.5 ± 0.1	2.3 ± 0.3	1.4 ± 0.3	1.7 ± 0.3	1.1 ± 0.2	1.5 ± 0.2	1.0 ± 0.2	1.8 ± 0.3
18:1ω9c	35.8 ± 0.2	38.4 ± 0.4	24.3 ± 4.7	23.5 ± 2.2	32.0 ± 2.3	25.6 ± 2.9	28.4 ± 3.3	22.4 ± 4.2	26.1 ± 3.0	19.1 ± 1.8	26.3 ± 0.5
18:1ω7c	1.7 ± 0.2	1.6 ±0.2	2.3 ± 0.3	2.2 ± 0.1	2.2 ± 0.3	2.3 ± 0.1	2.4 ± 0.2	2.1 ± 0.2	2.3 ± 0.2	1.9 ± 0.1	2.3 ± 0.3
20:1ω9	0.3 ± 0.0	1.3 ± 0.0	1.6 ± 0.3	1.4 ± 0.1	1.5 ± 0.1	1.5 ± 0.2	1.3 ± 0.2	1.2 ± 0.3	1.3 ± 0.1	1.2 ± 0.2	1.3 ± 0.2
20:1ω7	4.8 ± 0.2	1.8 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
22:1ω9	1.3 ± 0.0	0.7 ± 0.0	0.2 ± 0.1	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.1
Σ MUFA	**43.9 ± 0.6**	**43.9 ± 0.8**	**30 ± 5.7**	**29 ± 2.6**	**38.6 ± 3.1**	**31.2 ± 3.6**	**34.4 ± 4.1**	**27.3 ± 5.0**	**31.8 ± 3.6**	**23.6 ± 2.4**	**32.2 ± 5.9**
ω-6 Polyunsaturated fatty acids											
18:2ω6c (LIN)	15.1 ± 0.0	15.5 ± 0.1	12.0 ± 2.8	9.7 ± 1.1	12.3 ± 0.9	10.8 ± 1.1	11.2 ± 1.2	9,9 ± 1.6	10.3 ± 1.3	8.5 ± 0.7	10.7 ± 1.6
18:3ω6	5.9 ± 0.1	6.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1
20:2ω6	0.6 ± 0.0	0.2 ± 0.0	0.6 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.5 ± 0.1	0.7 ± 0.1	0.6 ± 0.0	0.7 ± 0.1

20:3ω6	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.0	0.6 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.0	0.6 ± 0.1	0.5 ± 0.0	0.5 ± 0.1
20:4ω6 (ARA)	0.3 ± 0.0	0.2 ± 0.0	0.9 ± 0.2	0.7 ± 0.1	0.5 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.8 ± 0.1	0.7 ± 0.3
22:3ω6	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
22:5ω6	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.1
Σ ω-6 PUFA	**21.9 ± 0.2**	**22.2 ± 0.1**	**14.4 ± 3.3**	**12.3 ± 1.4**	**14.6 ± 1.2**	**13.2 ± 1.4**	**13.4 ± 1.6**	**12.1 ± 1.9**	**12.6 ± 1.6**	**10.8 ± 0.9**	**13.1 ± 2.2**
ω-3 Polyunsaturated fatty acids											
18:3ω3 (ALA)	6.0 ± 0.1	6.8 ± 0.0	2.8 ± 0.6	3.2 ± 0.5	4.3 ± 0.3	3.6 ± 0.5	3.9 ± 0.3	3.5 ± 0.3	3.6 ± 0.5	2.9 ± 0.3	3.6 ± 0.6
18:4ω3 (SDA)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.6 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	1.0 ± 1.0	0.5 ± 0.1	0.8 ± 0.1
18:5ω3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
20:3ω3	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
20:4ω3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
20:5ω3 (EPA)	3.8 ± 0.0	3.7 ± 0.0	3.7 ± 0.9	3.9 ± 0.4	3.0 ± 0.4	4.1 ± 0.8	3.6 ± 0.7	5.0 ± 0.9	3.6 ± 0.5	4.9 ± 0.4	4.1 ± 0.9
22:5ω3 (DPA)	1.4 ± 0.0	0.9 ± 0.0	1.6 ± 0.2	1.4 ± 0.2	1.4 ± 0.0	1.5 ± 0.2	1.4 ± 0.1	1.6 ± 0.3	1.5 ± 0.1	1.7 ± 0.1	1.6 ± 0.2
22:6ω3 (DHA)	4.3 ± 0.0	4.9 ± 0.1	25.9 ± 7.8	28.8 ± 2.7	17.7 ± 2.9	26.5 ± 3.8	23.3 ± 4.0	30.5 ± 5.2	25.8 ± 3.5	35.5 ± 3.0	24.4 ± 5.2
Σ ω-3 PUFA	**15.7 ± 0.2**	**16.4 ± 0.1**	**34.1 ± 9.5**	**38.2 ± 4.0**	**27.5 ± 3.8**	**36.6 ± 5.5**	**33.2 ± 5.3**	**41.5 ± 6.9**	**35.7 ± 5.6**	**45.7 ± 3.8**	**34.7 ± 7.1**
Σ PUFA	**37.6 ± 0.3**	**38.6 ± 0.2**	**48.5 ± 12.8**	**50.5 ± 5.4**	**42 ± 5.0**	**49.8 ± 6.9**	**46.6 ± 6.9**	**53.6 ± 8.8**	**48.3 ± 7.2**	**56.5 ± 4.8**	**47.7 ± 9.3**
ω-3 : ω-6 -ratio	**0.7 ± 0.0**	**0.7 ± 0.0**	**2.4 ± 1.1**	**3.1 ± 0.4**	**1.9 ± 0.3**	**2.8 ± 0.5**	**2.5 ± 0.5**	**3.4 ± 0.8**	**2.8 ± 0.5**	**4.2 ± 0.6**	**2.6 ± 0.7**
Total FA content (µg FA mg^{-1} DW^{-1})	**120 ± 15**	**130 ± 16**	**41 ± 14**	**37 ± 4.7**	**83 ± 26**	**47 ± 13.4**	**55 ± 11**	**39 ± 14.2**	**47 ± 13**	**33.9 ± 4.2**	**47 ± 15.3**

Declaration of interests

 The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: