



HAL
open science

Variable impacts of L-arginine or L-NAME during early life on molecular and cellular markers of muscle growth mechanisms in rainbow trout

Hélène Alami-Durante, Marianne Cluzeaud, Didier Bazin, Christiane Vachot, Sadasivam S. Kaushik

► To cite this version:

Hélène Alami-Durante, Marianne Cluzeaud, Didier Bazin, Christiane Vachot, Sadasivam S. Kaushik. Variable impacts of L-arginine or L-NAME during early life on molecular and cellular markers of muscle growth mechanisms in rainbow trout. *Comparative Biochemistry and Physiology - Part A: Molecular and Integrative Physiology*, 2020, 242, 10.1016/j.cbpa.2020.110652 . hal-02862783

HAL Id: hal-02862783

<https://hal.inrae.fr/hal-02862783>

Submitted on 21 Jul 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

1 Variable impacts of L-arginine or L-NAME during early life on molecular and cellular
2 markers of muscle growth mechanisms in rainbow trout

3

4 H el ene Alami-Durante*, Marianne Cluzeaud, Didier Bazin, Christiane Vachot, Sadasivam
5 Kaushik

6

7 INRA, Univ. Pau & Pays Adour, E2S UPPA, UMR 1419, Nutrition, M etabolisme,
8 Aquaculture, Saint P ee sur Nivelle, F-64310, France

9

10 * Corresponding author. Tel.: + 33 5 59 51 59 62; fax: + 33 5 59 54 51 52.

11 E-mail address: helene.durante-alami@inra.fr

12

13 **Abstract:**

14

15 Two experiments were conducted to test if manipulations of the Arginine-Nitric oxide
16 pathway during the early life of rainbow trout would act on its early myogenic process. In
17 experiment 1, trout embryos were immersed at 72 degree days post-fertilization (°dpf) or
18 104°dpf in water alone (control treatment, C) or containing 2mM/L L-Arg (treatment A) or
19 1mM/L of L-NAME, a NOS inhibitor (treatment N). We observed the beginning of
20 expression of myf5 and fmhc genes at 72°dpf and 96°dpf, respectively. “A” treatment
21 doubled the free Arg content of eggs but did not affect either the pattern of expression of
22 myf5 and fmhc, nor white muscle cross-sectional area and number of white muscle fibres at
23 hatching, nor embryo survival and fry growth. “N” treatment also did not affect these
24 markers. In experiment 2, trout fry were fed from first feeding onwards and during 20 days
25 either a control diet (C) or the same diet supplemented with L-NAME (0.1g/100g diet, N-
26 diet). In C-fed fry, distribution of a single meal after overnight fasting induced changes in
27 pcna, myod1, myog, fmhc, inos, nnos and cttd gene expressions. N-feeding decreased fry
28 growth but did not change their growth trajectory or survival. Twenty days of N-feeding led,
29 compared to C-feeding, to changes in kinetics of transcription of pcna, myod1, myog, fmhc,
30 inos, nnos, cttd genes and to decreased white muscle cross-sectional area, total number of
31 white muscle fibres, and number of large muscle fibres. L-NAME feeding thus decreased fry
32 muscle growth by altering both hyperplasia and hypertrophy.

33

34 **Keywords:**

35

36 Fish nutrition; Arginine; L-NAME; Myogenesis; Gene expression; Kinetic; Hyperplasia;

37 Hypertrophy.

38

39 **Highlights of the manuscript:**

40

41 • Myf5 and fmhc gene expressions were evidenced at 72°dpf and 96°dpf, respectively

42 • Embryonic arginine or L-NAME pulses do not affect muscle fibre number at hatching

43 • Feeding induces changes in transcription of myogenic and muscle growth-related
44 genes

45 • L-NAME feeding alters the kinetics of transcription of muscle growth-related genes

46 • L-NAME feeding decreases muscle growth, fibre recruitment and fibre hypertrophy

47

48 **Statement of relevance**

49

50 Knowledge on the effects of arginine on fish myogenesis will help maximising fish muscle

51 growth

52

53 **Abbreviations:**

54

55 Arg, arginine; Ctsd, cathepsin D; d, diameter of white muscle fibres; dpf, days post
56 fertilization; °dpf, degree days post fertilization; fMHC, fast myosin heavy chain; IAA,
57 indispensable amino acid; iNOS, inducible nitric oxide synthase; ISH, *in situ* hybridization;
58 L-NAME, N(ω)-nitro-L-arginine methyl ester; MRF, myogenic regulatory factor;
59 MRF4/Myf6, myogenic factor 6; Myf5, myogenic factor 5; MyoD1, myoblast determination
60 protein 1; Myog, myogenin; NO, nitric oxide; NOS, nitric oxide synthase; nNOS, neuronal
61 nitric oxide synthase; PCNA, proliferating cell nuclear antigen; TCSAWM, total cross-
62 sectional area of white muscle; TNWF, total number of white muscle fibres.

63

64 **1. Introduction**

65

66 Teleosts which reach a large adult body size, such as the rainbow trout (*Oncorhynchus*
67 *mykiss*), present the unique feature of producing new muscle fibres during a large part of the
68 life cycle. After the initial two muscle layers (deep fast and superficial slow) have been
69 formed from lateral and adaxial cells, increase in muscle fibre number (hyperplastic growth of
70 muscle) continues in two phases; stratified hyperplasia generates new fibres along a distinct
71 germinal layer, while during mosaic hyperplasia, new fibre production is disseminated across
72 the whole myotome (Rowlerson and Veggetti, 2001). The time window of stratified and
73 mosaic hyperplasia varies between fish species. In rainbow trout, both stratified hyperplasia
74 and mosaic hyperplasia begin early, before hatching (Xi et al., 2001). Hyperplasia is the major
75 process for the increase in muscle cross sectional area in rainbow trout fry, but, during
76 growth, its relative importance decreases at the expense of hypertrophy (increase in size of
77 existing fibres) (Stickland, 1983). These developmental-related changes in muscle cellular
78 phenotype result from temporal changes in expression levels of genes regulating specific
79 transition points in myogenesis, the myogenic regulatory factors (MRFs). Myoblast
80 determination protein (MyoD), myogenic factor 5 (Myf5) and myogenic regulatory factor 6
81 (Myf6/MRF4) function as myogenic determination factors, while myogenin (Myog) acts as a
82 differentiation factor, as do MRF4 and MyoD; myogenic progenitor cells can either activate
83 Myf5 and MyoD and differentiate or proliferate, providing a reserve cell population for
84 muscle growth during development (Buckingham and Rigby, 2014).

85 Although most of the knowledge on the nutritional regulation of muscle growth in fish
86 are based on studies undertaken with juveniles, different studies had shown that early
87 nutrition can have a profound effect on muscle growth. Changes in first-feed protein source
88 (Alami-Durante et al., 1997; Ostaszewska et al., 2008), protein/energy ratio (Alami-Durante

89 et al., 2014), protein level (Saavedra et al., 2016) and degree of hydrolysis of protein sources
90 (Canada et al., 2018) were successively proved to modify the pattern of early muscle growth
91 at cellular or/and molecular levels. Other studies, with first-feeds varying in both protein
92 sources and level of incorporation of several indispensable amino acids (IAA) (Canada et al.,
93 2016; Saavedra et al., 2016) showed that changes in IAA profile had consequences on muscle
94 cellularity and expression of muscle-growth related genes. The knowledge on the specific role
95 that each IAA might play *in vivo* during fish early muscle growth is scarce and limited to
96 lysine, which was found to alter the distribution of white fibre diameters in Nile tilapia larvae
97 albeit without any effect on fibre number and proliferating nuclei (Aguiar et al., 2005), to
98 phenylalanine, which also act on distribution of white muscle fibres in Nile tilapia larvae
99 (Yamashiro et al., 2016), and to methionine, which regulates the gene expression of myogenin
100 and fast myosin heavy chain in rainbow trout fry (Fontagné-Dicharry et al., 2017). The role
101 of other IAA on early myogenesis and muscle growth of fish remains to our knowledge to be
102 investigated.

103 Arginine (Arg) is an IAA in fish (NRC, 2011). Without metabolic processing, Arg is
104 reported to be a potent secretagogue of several pancreatic hormones in fish (Plisetskaya et al.
105 1991; Mommsen, 2001) but data suggested that changes in plasma insulin level occurred only
106 when fish are fed dietary arginine supplementation far exceeding the requirement levels (Lall
107 et al., 1994). Arg metabolism leads to the formation of γ -guanidinoacetate and then creatine
108 and creatine phosphate, which are essential for muscle function. Arg is also a precursor of
109 ornithine, glutamine, glutamate and proline, required for the *de novo* synthesis of protein, and
110 of putrescine, essential to the synthesis of polyamines which stimulate cell growth and
111 proliferation (Miller-Fleming et al., 2015). Arginine is also degraded by nitric oxide synthases
112 (NOS) into citruline and nitric oxide (NO), which regulates different skeletal muscle
113 functions such as force production, blood flow, respiration and glucose homeostasis (Stamler

114 and Meissner, 2001). It was demonstrated *in vitro*, by using NO donors (L-Arg, DETA-NO,
115 SNP, SNAP) and/or pharmacological inhibitors of NOS activity (L-NAME, NMMA), that
116 NO also mediates the activation (Anderson and Pilipowicz, 2002, Anderson and Wozniak,
117 2004; Betters et al., 2008; Tatsumi et al., 2002a,b, Wozniak and Anderson, 2007) and the
118 proliferation of muscle precursor cells (Buono et al., 2012; Long et al., 2006; Ulibarri et al.,
119 1999), as well as the fusion and differentiation of myoblasts (Lee et al., 1994; Long et al.,
120 2006; Li et al., 2016) in mammals and birds. *In vivo*, a decreased NO level leads to decreased
121 activation of muscle precursor cells (Anderson et al., 2000; Tatsumi et al., 2006) as shown in
122 mice and rats injected intraperitoneally with L-NAME, to decreased mean muscle fibre
123 diameter (Wang et al., 2001) and decreased myonuclear addition to mature muscle fibres
124 (Gordon et al., 2007) as shown in rats fed with L-NAME in drinking water, and to decreased
125 expression of myogenin and myosin (Carrazo et al., 2014) as shown in chick injected *in ovo*
126 with NPLA (a NOS inhibitor). Arg supplementation in birds led to increased mean myofibre
127 diameter (Fernandes et al., 2009) as shown in chick fed started diets supplemented with Arg,
128 and to increased expression of myod and myogenin (Subramaniyan et al., 2019) as shown in
129 chicken hatched from eggs injected with Arg. In mammals, Arg supplementation led to
130 increased formation of primary muscle fibres, to stimulation of myogenic proliferation and to
131 delay in muscular differentiation (Bérard and Bee, 2010; Kalbe et al., 2013) as shown in
132 foetuses from gilts fed diets supplemented with Arg during early gestation, and to increased
133 number of muscle fibres (Shi et al., 2018) as shown in growing-finishing pigs from sows fed
134 diets supplemented with Arg during early gestation. Studies on the effect of Arg
135 supplementation on fish muscle development are scarce. Available data only indicate that, *in*
136 *vivo*, dietary Arg supplementation modified the frequency distribution of muscle fibres in
137 juvenile Nile Tilapia (Neu et al., 2016) and had no effect on fibre density in juvenile Atlantic
138 salmon (Østbye et al., 2018). *In vitro*, supplementing with the NO-donor ISDN to the culture

139 medium of single muscle fibre cultures from adult zebrafish led to increased activation of the
140 satellite cells lying alongside them (Zhang and Anderson, 2014), and supplementing with Arg
141 the culture medium of satellite cells extracted from juvenile Atlantic salmon led to increased
142 myogenin and myosin light chain expressions (Østbye et al., 2018).

143 In light of the importance of NO in the early myogenesis of mammals and birds, we
144 hypothesized that manipulation of the Arginine-NO pathway during the early life of fish
145 would also act on their early myogenic process with, given the importance of the number of
146 muscle fibres formed during early life for subsequent fish growth potential (e.g. Alami-
147 Durante et al., 2007; Macqueen et al., 2008), potential long-term consequences. We thus
148 studied the effects of *in vivo* early supplementations (during embryogenesis or at first feeding)
149 with L-Arg or L-NAME on molecular and cellular markers of muscle growth mechanisms
150 (hyperplasia and hypertrophy), with rainbow trout as model.

151

152 **2. Materials and methods**

153

154 The experiments were carried out with a certificate for experimenting on live animals
155 (N°B6410006) and in accordance with the EU Directive for the protection of animals used for
156 scientific purposes (N°2010/63/EU) and the French Decree for animal experimentation
157 (N°2013-118).

158

159 2.1. Experiment 1 on rainbow trout embryos

160

161 Diploid eggs were obtained in November from the INRA rainbow trout strain
162 maintained in the INRA experimental fish farm of Lees Athas (agreement N°A64-104-1) and
163 randomly distributed into 32 small mesh racks (10 x 10 cm, density = 500 eggs per rack)

164 themselves randomly disposed in 2 troughs in a flow-through system supplied with spring
165 water (temperature = $8.0 \pm 0.3^{\circ}\text{C}$). As shown on the schematic diagram of the trial (figure 1),
166 two racks were used to finely characterize somitogenesis in the rainbow trout strain used. For
167 this, 10 embryos (5 x 2 racks) were sampled each day at 9 am and 3 pm, from day 9 to day 24
168 post fertilization (dpf), i.e. from 72 to 192 degree days post-fertilization ($^{\circ}\text{dpf}$), based on
169 results indicating that somite formation can begin at 70 $^{\circ}\text{dpf}$ (Vernier, 1969) and last up to
170 175 $^{\circ}\text{dpf}$ (Ballard, 1973) in rainbow trout. These embryos were fixed in a mixture of 2.5%
171 glutaraldehyde, 2.5% paraformaldehyde, 0.5% sucrose in 0.08M cacodylate buffer, pH 7.4
172 (Galloway et al., 1998) as we found in a preliminary experiment that this fixative kept the
173 transparency of trout embryos and thus allowed to count the number of somite formed with
174 accuracy. These embryos were then dehydrated and kept in methanol at $+4^{\circ}\text{C}$ until
175 quantification of somitogenesis. The other 30 racks were used to characterize the effect of
176 immersion of embryos in arginine or L-NAME baths on molecular and cellular markers of
177 myogenesis and muscle growth. For this, the racks containing the embryos were carefully
178 retrieved from the incubation troughs and immersed during 4 hours in aerated small tanks
179 containing water alone as a control (“C” treatment), or water with diluted L-arginine (2mM/L,
180 “A” treatment) or diluted L-NAME (1mM/L, “N” treatment). These treatments were
181 performed in triplicate on half of the racks at 9 dpf (i.e. 72 $^{\circ}\text{dpf}$) and on the other racks at 13
182 dpf (i.e. 104 $^{\circ}\text{dpf}$). These two time-windows were chosen, based on literature data (Ballard,
183 1973; Vernier, 1969), in order to act before the formation of the first somite or during
184 somitogenesis. As we aimed to modify embryonic myogenesis without altering survival, L-
185 NAME bath concentration (1mM/L) was chosen based on a previous study showing that
186 salmonid embryos and alevins react to this L-NAME concentration, as shown by their
187 cardiovascular response, and that incubation in 1mM/L L-NAME affects neither egg
188 hatchability nor appearance and behaviour of exposed alevins (Eddy et al., 1999). Arg bath

189 concentration (2mM/L) was chosen based on results showing that incubation of cultured
190 mouse myofibres in 2mM/L Arg stimulated the activation of the satellite cells residing
191 alongside them (Better et al., 2008). In order to test if a balneation in 2mM Arg for 4 hours
192 was efficient to increase Arg concentration in eggs, 80 eggs were sampled at the end of the
193 balneation held at 13 dpf in the A and C racks. These eggs were frozen in liquid nitrogen and
194 kept at -80°C until chemical analyses. For *in situ* hybridization (ISH) analyses, 10 embryos
195 per rack having undergone the A, N or C treatment at 9 dpf were sampled at 10, 12, 14, 18
196 and 22 dpf, and 10 embryos per rack having undergone the A, N or C treatment bath at 13 dpf
197 were sampled at 14, 18 and 22 dpf. These embryos were fixed for 24h with 4% PFA in PBS
198 pH 7.4, rinsed in PBS, dehydrated in methanol baths and kept in methanol at -20°C until
199 further analyses. Samples were subsequently taken at the end of hatching (48dpf, i.e.
200 384°dpf). 27 hatched fry were retrieved from each rack, euthanized by overdose of
201 benzocaine (60 mg/L), and individually weighed; 20 of them were frozen in liquid nitrogen
202 and stored at -80 °C for mRNA extraction and generation of molecular probes for *in situ*
203 hybridization analyses; the remaining 7 fry were fixed in Serra's liquid (absolute ethanol 6V;
204 37% formalin 3V; glacial acetic acid 1V), dehydrated and preserved in butanol until
205 histological analyses. In order to test if the embryonic treatments with C, A, or N bath at 9 or
206 13 dph had consequences on post hatch body growth, the fry from the triplicates of each
207 embryonic treatment were pooled at the end of hatching. They were then distributed in new
208 triplicates groups of 100 fry in independent circular tanks (50 L) in a flow-through system
209 supplied with spring water (temperature = $8.0 \pm 0.3^{\circ}\text{C}$) and fed with a commercial feed (Bio
210 optimal start, Biomar) from first feeding onwards (73 dpf, i.e. 584°dpf). The fry were hand-
211 fed 6 to 8 times per day (from 9 am to 5 pm) to apparent satiation, with pellets increasing in
212 size over time (200-500 μm for 2 weeks, 500-800 μm for 3 weeks, and 0.8-1.1 mm
213 thereafter). Fry were group (tank) weighed every 3 weeks to follow growth.

214

215 *2.2. Experiment 2 on first-feeding rainbow trout fry*

216

217 Diploid rainbow trout eggs were obtained in April from a commercial fish farm
218 (Viviers de France) and incubated in the INRA experimental fish farm of Lees Athas in spring
219 water (temperature of $8.0 \pm 0.1^\circ\text{C}$) until hatching (366°dpf). Hatched fry were maintained
220 under these conditions until 495°dpf and then transferred to the INRA experimental fish farm
221 of Donzacq (agreement N°A40-228-1) and distributed into 6 independent circular tanks (50
222 L) in a flow-through system supplied with spring water (temperature of $17 \pm 1^\circ\text{C}$) under
223 natural photoperiod (May-June). At 546°dpf (day 0 of the feeding trial), the emerging fry,
224 still having small yolk reserves, were fed with two diets formulated with plant protein sources
225 (Table 1) for 20 days (triplicate tanks per diet; 100 fry per tank). The control diet (C),
226 formulated to cover the nutritional requirements of rainbow trout (NRC, 2011), was
227 manufactured without oil using a twin-screw extruder (BC 45, Clextal, France) and ground in
228 fractions of 200-500 μ and 500-800 μ m. Both fractions were then divided in two parts. The
229 first part was coated with fish oil to obtain diet C, while the second part was coated with N
230 (ω)-nitro-L-arginine methyl ester (L-NAME, 0.1g/100g diet) mixed with fish oil to obtain diet
231 N. The fry were hand-fed 6 to 8 times per day (from 9 am to 5 pm) to apparent satiation, with
232 diet particle fractions of 200-500 μ m during the first 15 days and 500-800 μ m later. The
233 amount of feed distributed was recorded weekly, and mortality daily. On day 20 of the
234 feeding trial, overnight unfed fry (16h food deprived) were sampled at 9 am (n=9 per diet),
235 just before distribution of an ad libitum single meal to the remaining fry. To study the post-
236 prandial expression of myogenic and muscle growth-related genes, fry were sampled at 1 h, 2
237 h, 3 h, 5 h, 7 h and 9 h after the meal. In order to limit handling stress in successive
238 samplings, the required number of fry (n=9 per diet x 7 sampling time) was withdrawn from

239 one of the three tanks at each sampling time. These fry were euthanized by an overdose of
240 benzocaine (60 mg/L), weighed and measured, frozen in liquid nitrogen, and stored at -80 °C
241 until molecular analysis. For analysis of muscle cellularity, 9 fry per diet (3 per tank) were
242 additionally sampled, euthanized by an overdose of benzocaine, weighed and measured, fixed
243 in Serra's liquid (absolute ethanol 6V; 37% formalin 3V; glacial acetic acid 1V), and
244 dehydrated until further histological analysis.

245

246 2.3. Analysis

247

248 *2.3.1. Somitogenesis and egg free Arg content*

249

250 The embryos kept in ethanol were dechorionated with fine forceps and the number of
251 somites formed counted from 9 to 24 dpf using a binocular microscope. Free Arg content was
252 quantified using K-Lage kit (Megazyme, Ireland) on frozen eggs sampled after the 13-dpf
253 bath.

254

255 *2.3.2. Molecular analysis*

256

257 *RNA extraction and cDNA synthesis*

258 Total RNA was extracted from frozen samples using Trizol reagent (Invitrogen)
259 according to the manufacturer's instructions, and stored in Nuclease-free water at - 20°C.
260 Samples were subjected to electrophoresis on 1% agarose gels to confirm the integrity of the
261 28S and 18S rRNA bands, and RNA purity was assessed by the 260/280 nm absorbance ratio.
262 Thereafter, 2 µg of total RNA was reverse transcribed using 200 U SuperScript™ III Reverse
263 Transcriptase (Invitrogen) and 500 ng of random primers (Promega) in a total volume of

264 20 μL (25°C for 10 min, 55°C for 1 h, 70°C for 15 min). Myf5 (Myogenic factor 5), myod1
265 (myoblast determination protein 1), myog (myogenin), pcna (proliferative cell nuclear
266 antigen), fmhc (fast myosin heavy chain), inos (inducible nitric oxide synthase) and ctsd
267 (cathepsin D) cDNA fragments were obtained by RT-PCR using previously published primers
268 (Alami-Durante et al., 2010, 2014; Chauvigné et al., 2003; McNeill and Perry 2005; Salem et
269 al., 2006). Primers for neuronal nitric oxide synthase (nNOS; F: gccatccttcgagatcagg; R:
270 cttgagactttccggtctcg) were designed for experiment 2 using GeneBank sequence DQ640498
271 and primer 3 software (University of Massachusetts). All primers were synthesised by
272 Eurogentec (Belgium) and amplicons confirmed by sequencing (Eurofins Genomics,
273 Ebersberg, Germany). For real-time RT-PCR assays, the Roche Lightcycler 480 system was
274 used (Roche Diagnostics, Neuilly-sur-Seine, France). The assays were performed using a
275 reaction mix of 6 μL per sample, each of which contained 2 μL of diluted (dilution=35)
276 cDNA template, 0.12 μL of each primer ($10 \mu\text{mol L}^{-1}$), 3 μL iQTM SYBR ® Green Supermix
277 (Bio-Rad) and 0.76 μL DNase/RNase-free water (5 Prime GmbH, Hamburg, Germany). The
278 PCR protocol was initiated at 95°C for 10 min for initial denaturation of the cDNA and hot-
279 start DNA polymerase activation and continued with 40 cycles of a two-step amplification
280 program (15 s at 95°C; 30 s at 60°C). Melting curves were systematically monitored
281 (temperature gradient at $0.5^\circ\text{C } 10 \text{ s}^{-1}$ from 55 to 94°C) at the end of the last amplification
282 cycle to confirm the specificity of the amplification reaction. Each real-time RT-PCR run
283 included replicate samples (duplicate of reverse transcription and PCR amplification) and
284 negative controls (reverse transcriptase- and cDNA template-free samples). Standard curves,
285 consisting of five serial dilutions in triplicate of a pool of cDNAs, were obtained for each
286 cDNA template by plotting CT values against the \log_{10} of the different dilutions. Real-time
287 PCR efficiency (E) was calculated from standard curves according to Pfaffl (2001).

288

289 *Whole mount in situ hybridization*

290 *In situ* hybridization (ISH) was performed in order to follow early (commitment) and
291 late (terminal differentiation) stages of myogenesis, marked by myf5 and fmhc expressions,
292 respectively. The myf5 and fmhc cDNA fragments obtained by RT-PCR were cloned into the
293 pCR[®]II-TOPO[®]vector (TOPO TA Cloning kit, Invitrogen) according to the manufacturer's
294 protocol. After appropriate linearization of the plasmids, sense and anti-sense RNA probes
295 were generated using SP6 and T7 polymerases in the presence of digoxigenin-11UTP (DIG
296 RNA labelling kit, Roche). Embryos sampled in C, A, and N-tanks were, following
297 rehydration in graded methanol/PBS baths, dechorionated with fine forceps in PBS and
298 processed according to established procedures with minor modifications. Embryos were
299 incubated in PBST containing 50µg/ml proteinase K (Roche) for 15 min, washed in PBST,
300 refixed in a 4% PFA-0.2% glutaraldehyde solution for 20 min, and washed in PBST. Embryos
301 were then prehybridized for 2 h at 65°C in a solution containing 50% formamide, 5x SSC, 0.1
302 mg/ml yeast transfer RNA (Sigma), 0.1 mg/ml heparin and 0.1% Tween20. Dig-riboprobes
303 were denatured for 5 min at 65°C, diluted in hybridization buffer (0.5-1µg/ml) and incubated
304 with the embryos overnight at 65°C. Negative controls with sense probe and without probe
305 were performed. Embryos were then washed in 2x SSC baths to remove aspecific hybrids. To
306 detect hybridized probes embryos were blocked for 90 min in PBST containing 2% sheep
307 serum and 0.2% triton (blocking solution), and then incubated overnight at 4°C in 1:4000
308 diluted alkaline phosphatase conjugated anti digoxigenin (Roche) in blocking solution. The
309 staining was performed overnight in a dark chamber with a solution containing Levamisol and
310 NBT-BCIP (Boehringer Mannheim). Colour development was stopped in H₂O for 10min, 4%
311 PFA for 10 min, and PBS for 30 min.

312

313 *Quantification of mRNA relative abundances*

314 The pre- and post-prandial gene expression data obtained from qPCR were analysed
315 using the Δ CT method (Pfaffl, 2001) with the C-fed fry at time 0h as a control. Data were
316 then normalized using the NORMA-gene algorithm which uses a least squares method to
317 minimize data variability and which does not require the identification and validation of
318 reference genes (Heckmann et al., 2011), as yet validated in trout (Borey et al., 2016).

319

320 *2.3.3. Quantitative histological analyses*

321

322 The tails of the fry kept in butanol were embedded in paraffin and cut transversely into
323 sections (10 μ m thick) that were stained with haematoxylin and orange G (Gabe, 1968).
324 Cellular analyses were performed on a dorsal quadrant of white myotomal muscle, as
325 previously defined (Alami-Durante et al. 1997). Muscle cellularity was quantified on one
326 section per fish, located at the vent level. Measurements were performed with Image-Pro Plus
327 software (Media Cybernetics). In experiment 1, the total cross-sectional area of one dorsal
328 quadrant of white muscle (TCSAWM) was measured and the total number of white muscle
329 fibres (TNWF) present in the quadrant was counted. In experiment 2, the individual area of all
330 the fibres present the dorsal quadrant of white muscle was additionally measured. The white
331 fibre individual equivalent area diameter (diameter of a circle whose area is the same as that
332 of the muscle fibre; will be referred to as “fibre diameter”) was then calculated.

333

334 *2.3.4. Chemical analyses of diets*

335

336 The chemical composition of C-diet (Table 2) was analysed using the following
337 procedures: dry matter after drying at 105°C for 24h, protein content (N x 6.25) by the
338 Kjeldhal method after acid digestion, lipid content by petroleum diethyl ether extraction

339 (Soxtherm), and gross energy after combustion in an adiabatic bomb calorimeter (IKA). The
340 amino acid composition of the C-diet (Table 2) was analysed by HPLC by AgroBio
341 laboratory (Vezin Le Coquet, France).

342

343 *2.3.5. Statistical analyses*

344

345 Statistical analyses were performed with R 3.2.3 Software (R Development Core
346 Team, Vienna, Austria, www.R-project.org) and SigmaStat 3 computing program (SPSS,
347 Chicago, IL, USA). Differences in survival, body weight and muscle cellularity were analysed,
348 after appropriate transformation (arcsin, log, square root) to verify equality of variance and
349 normality, by two-way anova (with “dpf” and “bath” as factors) followed by Newman-Keuls
350 multiple range test to compare means when significant differences were found (exp. 1) or t-
351 test (with “diet” as factor) (exp. 2). Differences in pre- and post-prandial expressions of
352 myogenic and muscle growth-related genes were analysed in exp.2 by two-way anova (with
353 “diet” and “hours after feeding” as factors) after rank-transformation when necessary
354 followed by Newman-Keuls multiple range test to compare means when significant
355 differences were found. Differences were considered significant when $P < 0.05$.

356

357 **3. Results**

358

359 *3.1. Experiment 1 on embryos*

360

361 *3.1.1. Somitogenesis*

362

363 The first five somites were formed at 10 dpf (80°dpf). Somitogenesis then progressed
364 at a rate of about 5.7 somites formed per day, to reach 65 somites formed at 21 dpf (168°dpf)
365 (figure 2).

366

367 *3.1.2. Effect of A-bath on egg free Arg content*

368

369 The trout eggs immersed during 4h in the A-bath at 13dpf had, at the end of the bath, a
370 free Arg content twice higher ($1.10 \pm 0.07 \mu\text{M/g}$) than that of the eggs immersed in the C-bath
371 ($0.52 \pm 0.09 \mu\text{M/g}$) ($P < 0.0001$).

372

373 *3.1.3. Embryonic pattern of expression of myf5 and fmhc genes*

374

375 Myf5 expression began at 9 dpf (72°dpf) in the rostral domain of presomitic
376 mesoderm, on both sides of median axis (figure 3). At 10 dpf (80°dpf), myf5 was expressed in
377 all neoformed somites and presomitic mesoderm. At 14 dpf (112°dpf), i.e. when 22 somites
378 were formed, myf5 expression was weaker in the somites firstly formed at rostral level than in
379 somites lastly formed at caudal level, and the stronger myf5 expression was always noted in
380 the most caudal part of embryos in unsegmented mesoderm. At 18 dpf (144°dpf) i.e. when 50
381 somites were formed, myf5 transcripts accumulated preferentially in the lateral external
382 bordure and in the posterior region of formed somites; myf5 transcripts were always present
383 in quantity in unsegmented caudal mesoderm. At the end of somitogenesis, i.e. at 22dpf
384 (168°dpf), myf5 expression was localized in the posterior part and in the external lateral part
385 of all somites.

386 The first expression of fmhc was detected at 12dpf (96°dpf), i.e. when 16 somites were
387 formed (figure 4) and transcript location was limited to the most rostral trunk area (in about

388 10 somites). Observation of embryos in dorsal view showed that *fmhc* transcripts were
389 localized in the median part of rostral somites, on both sides of central structures. At 14 dpf
390 (112°dpf), i.e. when 28 somites were formed, the expression domain of *fmhc* spread out
391 laterally in the first formed somites and colonized the caudal region of embryos. From 18 dpf
392 (144°dpf) *fmhc* expression was very important and transcripts were localized in almost all
393 somite area.

394 Differences in the pattern of expression of *myf5* and *fmhc* linked to embryonic
395 treatments (C-, A- or N-bath at 9 or 13 dpf) were not identified (figures 3,4).

396

397 *3.1.4. Survival, body weight, white muscle cross-sectional area, and total number of white*
398 *muscle fibres at hatching*

399

400 Survival at hatching was high (>93%) and not affected by bath at 9dpf or 13dpf in
401 2mM/L Arg or 1mM/L L-NAME (Table 3). Fry body weight at hatching was similar in the
402 different batches. Immersion of embryos in 2mM/L Arg or 1mM/L L-NAME at 9 dpf or 13
403 dpf affected neither the total cross-sectional area of white muscle nor the total number of
404 white muscle fibres at hatching (Table 3).

405

406 *3.1.5. Effect of embryonic treatments on post-hatch fry growth*

407

408 Immersion of embryos for 4h in 2mM/L Arg or 1mM/L L-NAME at 9 dpf or 13 dpf
409 had no effect on the subsequent post-hatch weight increase of fry (Figure 5).

410

411 *3.2. Experiment 2 on first feeding fry*

412

413 *3.2.1. Fry survival and growth*

414

415 The quantities of C- and N-diets distributed ad-libitum during the whole feeding trial
416 were similar (14.5g/tank and 14.7g/tank, respectively). After 20 days of feeding, the survival
417 of C-fed ($96.4 \pm 3.6\%$) and N-fed ($93.4 \pm 5.5\%$) fry was high and not different ($P=0.462$). The
418 body weight ($P<0.0001$) and the total length ($P<0.0001$) of the C-fed fry were higher than
419 those of N-fed fry (Figure 6), but the slopes ($P=0.589$) and the intercepts ($P=0.580$) of the
420 length-weight relationships of C- and N-fed fry were similar (Figure 7).

421

422 *3.2.2. Pre- and post-prandial expressions of genes involved in myogenesis, cell proliferation,*
423 *muscle structure, arginine catabolism, and proteolysis*

424

425 In C-fed fry, alterations in gene expressions occurred rapidly after the distribution of
426 the single meal (Figure 8), with decreased transcript levels of pcna and fmhc at 1h. A similar
427 trend, although not significant, was observed for myod1, myog, inos, nnos and ctcd (Figures
428 8,9). The transcript level of all the genes then increased rapidly to reach at 2h values higher
429 than (myod1) or similar to (myog, pcna, fmhc, inos, nnos, ctcd) initial values (0h). Transcript
430 levels then decreased slowly up to 5h (myog) or 7h (myod1) or did not change until 7h (pcna)
431 and finely increased between 7h and 9h (myod1, myog, pcna) to reach values higher than
432 (myod1, pcna) or similar to (myog) initial values (0h). The gene expression of fmhc, inos,
433 nnos and ctcd did not change significantly between 2h and 9h in C-fry.

434 In N-fed fry (Figures 8,9), the transcript level of pcna did not decrease between 0h and
435 1h, and was lower at 0h, 3h, 9h than at 7h. Myod1 expression was upregulated 1h and 2h after
436 distribution of the single meal, decreased at 3h, and then not significantly changed. Fmhc
437 expression remained stable between 0h and 2h, decreased at 3h, increased at 5h and then did

438 not change. Inos expression decreased between 1h and 3h and then remained stable. Ctsd
439 expression was higher at 1h than at 9h. Myog and nnos expressions did not vary significantly
440 after the single meal.

441 Compared to C-fed fry, the N-fed fry had higher expressions of pcna at 1h, 7h, of
442 myod1 at 1h, of fmhc at 0h, 1h, 2h, 5h, 9h, of inos at 0h, 1h, 5h, of nnos at 0h, 1h, 3h, 5h, 7h,
443 of ctsd at 0h, 1h, 7h, and lower expressions of pcna at 0h, 9h and of Myog at 0h, 2h, 3h, 9h
444 (Figures 8,9).

445

446 *3.2.3. Recruitment and growth dynamics of skeletal white muscle fibres*

447

448 In the dorsal muscle of 20 days fed fry, white muscle fibres presented a main stratified
449 spatial organisation, with numerous small white fibres located at white muscle epi-axial
450 extremity and at white muscle edge under red muscle, and biggest white fibres located in the
451 deep part of myotomes. Small white muscle fibres were also present in the deep part of white
452 muscle, around largest fibres (Figure 10). Twenty days of feeding with the N-diet led to a
453 significant decrease in white muscle total cross-sectional area (30% lower than in C-fed fry)
454 and total number of white muscle fibres (17% lower than C-fed fry) but did not change the
455 mean diameter of white muscle fibres (Table 4). The comparison of the distributions of
456 muscle fibre diameters in C- and N-fed fry showed that feeding the N-diet led to a lowering of
457 muscle fibre number in almost all diameter classes (Figure 11). In the 5-7.5 μm and 10-
458 12.5 μm classes, this decrease failed to be significant ($P=0.097$ and $P=0.072$, respectively) due
459 to high individual variability. The N-induced decrease in white fibre number was significant
460 for the 12.5-15 μm ($P<0.001$), 17.5-20 μm ($P=0.047$), 20-22.5 μm ($P=0.030$) and 22.5-25 μm
461 ($P=0.010$) classes of diameters.

462

463 **4. Discussion**

464

465 *Embryonic myogenesis and Arg or L-NAME baths*

466

467 The rate of somitogenesis observed here (5.7 somites formed/day at 8 °C, i.e. 0.7
468 somite formed/degree-day) is consistent with previous observations in the same species (Bobe
469 et al., 2000). Other data with rainbow trout have indicated that the initial differentiation of
470 fast (white) muscle takes place in proximity to axial structures and that fmhc transcript is
471 detected from 25 somites onwards (Rescan et al., 2001). Our results allowed to precise that
472 fmhc expression began earlier, as soon as 96°dpf (12 dpf at 8 °C), i.e. in 19-somite embryo.
473 Concerning myf5 transcript, we report here for the first time its presence in trout at 72 °dpf (9
474 dpf at 8 °C) at the level of paraxial presomitic mesoderm, i.e. before the beginning of the
475 morphological differentiation of somites. This early expression of myf5 is consistent with its
476 known function in myogenic determination (Buckingham and Rigby, 2014).

477 The free Arg content presently found in control eggs at 13dpf (0.52µM/g) is consistent
478 with previous data on the same species (Rice and Stokes, 1974). Immersion during 4h in a
479 bath containing 2mM/L Arg allowed to double the free Arg content of eggs, confirming that
480 trout eggs might be enriched in some nutrients through delivery in embryonic baths, as yet
481 shown with other water-soluble nutrients (Falahatkar et al., 2006). We enriched trout eggs
482 with Arg in order to stimulate myogenic proliferation, act on the expression of genes
483 regulating myogenic steps and increase the number of muscle fibres, based on results obtained
484 in chicken hatched from eggs injected with Arg, in foetuses from gilts fed diets supplemented
485 with Arg during early gestation, and in growing-finishing pigs from sows fed diets
486 supplemented with Arg during early gestation (Bérard and Bee, 2010; Kalbe et al., 2013; Shi
487 et al., 2018 ; Subramaniyan et al., 2019). In present conditions, in spite of increasing trout egg

488 free Arg content, an embryonic bath in 2mM/L Arg performed at 13dph (when 21 somites
489 were formed) failed to modify significantly the embryonic pattern of expression of myf5 and
490 fmhc, and the number of white muscle formed at hatching. An earlier bath (at 9dph, just
491 before the beginning of somitogenesis) in 2mM/L Arg also did not act on these myogenic
492 markers. Perhaps the concentration of 2mM/L Arg, chosen for its ability to stimulate *in vitro*
493 the activation of mice satellite cells (Better et al., 2008), was not sufficient to induce changes
494 *in vivo* on the myogenic markers studied, or the duration of Arg treatment too short. We used
495 embryonic L-NAME bath in order to determine if treatment of trout eggs with a NOS
496 inhibitor would act on the fate of muscle precursor cells and on muscle cellularity, as it occurs
497 *in vivo* in mice and rats injected intraperitoneally with L-NAME or fed with L-NAME in
498 drinking water and in chick injected *in ovo* by NPLA (another NOS inhibitor) (Anderson et
499 al., 2000; Carrazo et al., 2014; Tatsumi et al., 2006; Wang et al., 2001). We chose to use a low
500 concentration of L-NAME (1mM/L) as we did not want to alter embryo survival and this aim
501 was attained, confirming previous results (Eddy et al., 1999), but this treatment did not alter
502 neither the embryonic pattern of expression of myf5 and fmhc nor the number of white
503 muscle fibres formed at hatching. Perhaps, like for 2mM/L Arg, 1mM/L L-NAME was too
504 low to alter the activation of muscle precursor cells, their differentiation, and the formation of
505 muscle fibres, or bath duration too short. Perhaps also, despite the described occurrence of
506 nNOS transcripts in the tail bud of zebrafish embryos (Yamamoto et al., 2003) and of NOS
507 positive cells in rainbow trout embryos (Gallo and Civinini, 2001), NOS activity was too low
508 in 9 and 13 dpf trout embryos to respond to an external stimulation by the tested doses of Arg
509 and L-NAME. This latter hypothesis led us to try to modify myogenesis by acting on NOS
510 activity later in development. Our second experiment was thus performed on first feeding fry,
511 as the weeks following first feeding constitute in trout a time window during which i) an
512 important recruitment of white muscle fibres occurs and ii) a nutritional control of

513 myogenesis has been demonstrated at molecular and cellular levels (Alami-Durante et al.,
514 2014; Fontagné-Dicharry et al., 2017).

515

516 *Pre- and post-prandial expressions of myogenic and muscle growth-related genes in fry fed*
517 *from first feeding onwards and during 20 days a diet covering nutritional requirements*

518

519 Although the temporal changes in expression of myogenic and muscle growth-related
520 genes that occurred after a long starvation period followed by a period of refeeding of
521 numerous days/weeks have been documented in different species of farmed fish (e.g. Bower
522 et al., 2009; Chauvigné et al., 2003; Cleveland and Weber, 2014; Dhillon et al., 2009; Fuentes
523 et al., 2012; Garcia de la serrana et al., 2012; Hagen et al., 2009; Johansen and Overturf,
524 2006; Paula et al., 2017; Rescan et al., 2007, 2017, Zhu et al., 2014), these data only concern
525 juveniles and less data are available for shorter time intervals. To our knowledge, the effect of
526 the distribution of a single meal on the kinetics of transcription of myogenic genes was only
527 studied in juveniles of species of interest to aquaculture (Valente et al., 2012; Zhu et al., 2016)
528 or in model fish (Seilliez et al., 2013) previously submitted to several days of fasting. Our
529 study is the first one undertaken under farming conditions with just an overnight fast and
530 dealing with fry. We found that the expressions of pcna (necessary for cell proliferation) and
531 fmhc (a myosin chain expressed in white muscle) were downregulated 1h after refeeding, and
532 the expressions of the myogenic factors myod1 (involved in activation of muscle precursor
533 cells, MPC) and myog (involved in early differentiation and fusion of MPC), of nos (the
534 constitutive nos isoform providing low levels of nitric oxide under physiological conditions)
535 and ctssd (involved in lysosomal proteolysis) followed, although not significantly, the same
536 trend. The expression of the isoform of nos providing high level of nitric oxide under stress
537 conditions (inos) was very similar before the single meal and 1h later, suggesting that the

538 down regulation of the expression of other genes at 1h post feeding was not due to stress. It
539 was perhaps due to a transitory down regulation of the transcription of genes involved in
540 myogenesis and muscle growth at the expense of transcription of genes involved in digestion
541 and absorption of nutrients. The rapid upregulation of the transcription of myogenic
542 regulatory factors and myosin heavy chain occurring between 1h and 2h after the single meal
543 was for its part probably triggered by the delivery of absorbed nutrients into muscle cells, and
544 the subsequent progressive downregulation of myogenic regulatory factor transcripts linked to
545 a lowering of nutrient delivery when gut fullness decreased. The kinetics of post-prandial
546 changes in myod1 and myog expressions here found in trout fry after a single meal are not
547 similar to known kinetics in juveniles of other aquaculture fish species after a single meal
548 (Valente et al., 2012; Zhu et al., 2016). This discrepancy might be related to factors intrinsic
549 to the fish (species, developmental stage, size) and to the experimental design
550 (starvation/refeeding protocol, diet composition, rearing temperature, post-prandial sampling
551 times). The post-prandial changes in myod1 and myog expressions here observed are the
552 result of nutrient input after just an overnight fasting, while those previously observed
553 (Valente et al., 2012; Zhu et al., 2016) are based on the effects of feeding after one week of
554 fasting, when fish are in a catabolic state.

555

556 *Effect of L-NAME feeding on fry growth, pre- and post-prandial gene expressions, and*
557 *muscle cellularity*

558

559 The decreased weight of N-fed fry after 3 weeks of feeding with maintenance of a
560 length-weight relation similar in slope and origin to that of C-fed fry indicates that L-NAME-
561 feeding although led to reduced somatic growth of fry, did not modify their growth trajectory.
562 Taking into account the quantity of feed delivered ad-libitum during the whole trial, the mean

563 weight of fry during the trial ((final fry weight + weight of dead fry - initial fry weight)/2) and
564 trial duration, the fry had ingested 100mg L-NAME/kg body weight/day, *i.e.* an L-NAME
565 quantity higher than that (30mg L-NAME/kg body weight/day) decreasing the formation of
566 new myotubes in mouse (Anderson, 2000) and satellite cell activation in rat (Tatsumi et al.,
567 2006), and close to that (90mg L-NAME/kg body weight/day) reducing myonuclear addition
568 in rat (Gordon et al., 2006). Long-term L-NAME feeding of trout fry increased the pre-
569 prandial gene expression level of *nnos* and *inos*. *Inos* expression also increased in different
570 mammalian cell types after chronic treatment with L-NAME (e.g. Kopincova et al., 2012;
571 Miller et al., 1996), and it was suggested that L-NAME induced a feedback-regulated
572 activation of *nos* expression which could, *in vivo*, be or not be associated with changes in NO
573 availability, depending on different factors such as duration of treatment, dose of L-NAME,
574 presence of NOS cofactors, and tissue (Kopincova et al., 2012). In trout fry, long-term L-
575 NAME feeding also increased the pre-prandial gene expression level of *fmhc* and *ctsd*, which
576 could be indicative of increased protein turnover, but it decreased the pre-prandial gene
577 expression level of *pcna* and *myog*, suggesting decreased cell proliferation and early
578 differentiation and fusion of myoblasts. These findings are in line with *in vitro* results
579 showing that L-NAME reduces the number of proliferating satellite cells on mice single fibre
580 cultures (Buono et al., 2012) and reduces the fusion of C₂C₁₂ myoblasts (Long et al., 2006). In
581 trout fry, long-term L-NAME feeding also suppressed the peak of expression of *pcna*, *myog*,
582 and *fmhc* occurring 2 hours after feeding in control fed fry. The L-NAME-induced molecular
583 changes led, at cellular phenotype level, to a lower total number of white muscle fibres and to
584 a lower number of large muscle fibres, indicating that L-NAME feeding had decreased fry
585 muscle growth by altering both fibre recruitment (hyperplasia) and fibre growth
586 (hypertrophy). This suggests, based on the recognised inhibitory role of L-NAME on NOS

587 activity, that NO synthesis, yet recognised as being important for the early myogenesis of
588 mammals and birds, is also important for fish fry myogenesis.

589

590 **5. Conclusion**

591

592 We observed that manipulating the arginine-NO pathway during embryogenesis did
593 not have any significant impact on early myogenic processes in rainbow trout. Feeding trout
594 from first feeding onwards with a L-NAME enriched diet induced alterations in the kinetics of
595 transcription of nos, myogenic and muscle-growth related genes. These observations
596 combined with L-NAME-induced decreased muscle growth, fibre recruitment and fibre
597 hypertrophy support the hypothesis of an involvement of NO in the early myogenesis of fish.

598

599 **Acknowledgments**

600

601 The authors acknowledge the staff of the INRA experimental fish farms at Lees Athas
602 and Donzacq for producing trout embryos and fry, manufacturing diets, conducting the trials
603 and helping during samplings (JP. Fouriot, P. Maunas, F. Sandres, F. Terrier, F. Vallée), and
604 O. Ben Fadhel, M.J. Borthaire and A. Surget for laboratory analyses.

605

606 **Author contributions**

607

608 H.A-D. and S.K. designed the research. M.C. and D.B. realized the molecular and
609 cellular analyses of muscle. C.V. helped during sampling and quantified the free Arg content
610 of eggs. H.A-D. analysed the data, wrote the paper and has primary responsibility for final

611 content. We sincerely regret the demise of one of the co-authors (M.C.). Other authors have
612 read and approved the final manuscript.

613

614 **Declaration of Competing Interest**

615

616 The authors have no competing interests to declare.

617

618 **References**

619

620 Aguiar, D.H., Barros, M.M., Padovani, C.R., Pezzato, L.E., Dal Pai-Silva, M., 2005. Growth
621 characteristics of skeletal muscle tissue in *Oreochromis niloticus* larvae fed on a lysine
622 supplemented diet. J. Fish Biol. 67, 1287-1298.

623

624 Alami-Durante, H., Fauconneau, B., Rouel, M., Escaffre, A.M., Bergot, P., 1997. Growth and
625 multiplication of white skeletal muscle fibres in carp larvae in relation to somatic growth rate.
626 J. Fish Biol. 50, 1285-1302.

627

628 Alami-Durante, H., Olive, N., Rouel, M., 2007. Early thermal history significantly affects the
629 seasonal hyperplastic process occurring in the myotomal white muscle of *Dicentrarchus*
630 *labrax* juveniles. Cell Tissue Res. 327, 553-570.

631

632 Alami-Durante, H., Médale, F., Cluzeaud, M., Kaushik, S.J., 2010. Skeletal muscle growth
633 dynamics and expression of related genes in white and red muscle of rainbow trout fed diets
634 with graded levels of a mixture of plant protein sources as substitutes for fish meal.
635 Aquaculture 303, 50-58.

636

637 Alami-Durante, H., Cluzeaud, M., Duval, C., Maunas, P., Girod-David, V., Médale, F., 2014.
638 Early decrease in dietary protein/energy ratio by fat addition and ontogenetic changes in
639 rainbow trout muscle growth mechanisms: short- and long-term effects. Br. J. Nut. 112, 674-
640 687.

641

642 Anderson, J., 2000. A role for nitric oxide in muscle repair: nitric oxide-mediated satellite cell
643 activation. *Mol. Biol. Cell* 11, 1859-1874.

644

645 Anderson, J., Pilipowicz, O., 2002. Activation of muscle satellite cells in single fiber culture.
646 *Nitric Oxide Biol. Chem.* 7, 36-41.

647

648 Anderson, J., Wozniak, A.C., 2004. Satellite cell activation on fibers: modelling events *in vivo*
649 – an invited review. *Can. J. Physiol. Pharmacol.* 82, 300-310.

650

651 Ballard, W.W., 1973. Normal embryonic stages for salmonid fishes, based on *Salmo*
652 *gairdneri* (Richardson) and *Salvelinus fontinalis* (Mitchill). *J. Exp. Zool.* 184, 7-26.

653

654 Bérard, J., Bee, G., 2010. Effects of dietary L-arginine supplementation to gilts during early
655 gestation on foetal survival, growth and myofibre formation. *Animal* 4, 1680-1687.

656

657 Betters, J.L., Lira, V.A., Soltow, Q.A., Drenning, J.A., Criswell, D.S., 2008. Supplemental
658 nitric oxide augments satellite cell activity on cultured myofibers from aged mice. *Exp.*
659 *Geront.* 43, 1094-1101.

660

661 Bobe, J., Andre, S., Fauconneau, B., 2000. Embryonic muscle development in rainbow trout
662 (*Oncorhynchus mykiss*): a scanning electron microscopy and immunohistological study. *J.*
663 *Exp. Zool.* 286, 379-389.

664

665 Borey, M., Panserat, S., Surget, A., Cluzeaud, M., Plagnes-Juan, E., Herman, A., Lazzarotto,
666 V., Corraze, G., Medale, F., Lauga, B., Burel, C., 2016. Postprandial kinetics of gene

667 expression of proteins involved in the digestive process in rainbow trout (*O. mykiss*) and
668 impact of diet composition. *Fish Physiol. Biochem.* 42, 1187-1202.

669

670 Bower, N.I., Taylor, R.G., Johnston, I.A., 2009. Phasing of muscle gene expression with
671 fasting-induced recovery growth in Atlantic salmon. *Frontiers Zool.* 6, 18.

672

673 Buckingham, M., Rigby, P.W.J., 2014. Gene regulatory networks and transcriptional
674 mechanisms that control myogenesis. *Dev. Cell* 28, 225-238.

675

676 Buono, R., Vantaggiato, C., Pisa, V., Azzoni, E., Bassi, M.T., Brunelli, S., Sciorati, C.,
677 Clementini, E., 2012. Nitric oxide sustains long-term skeletal muscle regeneration by
678 regulating fate of satellite cells via signalling pathways requiring vangl2 and cyclic GMP.
679 *Stem Cells* 30, 197-209.

680

681 Canada, P., Engrola, S., Mira, S., Teodosio, R., Fernandes, J.M.O., Sousa, V., Barriga-Negra,
682 L., Conceicao, L.E.C., Valente, L., 2016. The supplementation of a microdiet with crystalline
683 indispensable aminoacids affects muscle growth and the expression pattern of related genes in
684 Senegalese sole (*Solea senegalensis*) larvae. *Aquaculture* 458, 158-169.

685

686 Canada, P., Engrola, S., Mira, S., Teodosio, R., del Mar Yust, M., Sousa, V., Pedroche, J.,
687 Fernandes, J.M.O., Conceicao, L.E.C., Valente, L., 2018. Larval dietary protein complexity
688 affects the regulation of muscle growth and the expression of DNA methyltransferases in
689 Senegalese sole. *Aquaculture* 491, 28-38.

690

691 Carrazo, D., Assi, E., Moscheni, C., Brunelli, S., De Palma, C., Cervia, D., Perrotta, C.,
692 Clementi, E., 2014. Nitric oxide drives embryonic myogenesis in chicken through the
693 upregulation of myogenic differentiation factors. *Exp. Cell Res.* 320, 269-280.
694

695 Chauvigné, F., Gabillard, J.C., Weil, C., Rescan, P.Y., 2003. Effect of refeeding on IGFI,
696 IGFII, IGF receptors, FGF2, FGF6, and myostatine mRNA expression in rainbow trout
697 myotomal muscle. *Gen. Comp. Endocrinol.* 132, 209:215.
698

699 Cleveland, B.M., Weber, G.M., 2014. Ploidy effects on gene regulating growth mechanisms
700 during fasting and refeeding in juvenile rainbow trout (*Oncorhynchus mykiss*). *Molec. Cell*
701 *Endocrinol.* 382, 139-149.
702

703 Dhillon, R.S., Esbaugh, A.J., Wang, Y.S., Tufts, B.L., 2009. Characterization and expression
704 of a myosin heavy-chain isoform in juvenile walleye, *Sander vitreus*. *J. Fish Biol.* 75, 1048-
705 1062.
706

707 Eddy, F.B., McGovern, L., Acock, N., McGeer, J.C., 1999. Cardiovascular responses of eggs,
708 embryos and alevins of Atlantic salmon and rainbow trout to nitric oxide donors, sodium
709 nitroprusside and isosorbide dinitrate, and inhibitors of nitric oxide synthase, N-nitro-L-
710 arginine methyl ester and aminoguanidine, following short- and long-term exposure. *J. Fish*
711 *Biol.* 55A, 119-127.
712

713 Falahatkar, B., Dabrowski, K., Arslan, M., Rinchar, J., 2006. Effects of ascorbic acid
714 enrichment by immersion of rainbow trout (*Oncorhynchus mykiss*, Walbaum, 1792) eggs and
715 embryos. *Aquac. Res.* 37, 834-841.

716

717 Fernandes, J.I.M., Murakami, A.E., Martins, E.N., Sakamoto, M.I., Garcia, E.R.M., 2009.
718 Effect of arginine on the development of the pectoralis muscle and the diameter and the
719 protein: deoxyribonucleic acid rate of its skeletal myofibers in broilers. Poultry Sci. 88, 1399-
720 1406.

721

722 Fontagné-Dicharry, S., Alami-Durante, H., Aragao, C., Kaushik, S.J., Geurden, I., 2017.
723 Parental and early-feeding effects of dietary methionine in rainbow trout (*Oncorhynchus*
724 *mykiss*). Aquaculture 469, 16-27.

725

726 Fuentes, E.N., Safian, D., Valdes, J.A., Molina, A., 2013. Isolation and selection of suitable
727 reference genes for real-time PCR analyses in the skeletal muscle of the fine flounder in
728 response to nutritional status: assessment and normalization of gene expression of growth-
729 related genes. Fish Physiol. Biochem. 39, 765-777.

730

731 Gabe, M., 1968. Techniques histologiques. 1113 pp. Paris: Masson.

732

733 Gallo, V.P., Civini, A., 2001. Immunohistochemical localization of nNOS in the head kidney
734 of larval and juvenile rainbow trout, *Oncorhynchus mykiss*. Gen. Comp. Endocrinol. 124, 21-
735 29.

736

737 Galloway, T.F., Kjorsvik, E., Kryvi, H., 1998. Effect of temperature on viability and axial
738 muscle development in embryos and yolk sac larvae of the Northeast Arctic cod (*Gadus*
739 *morhua*). Mar. Biol. 132, 559-567.

740

741 Garcia de la serrana, D., Vieira, V.L.A., Andree, K.B., Darias, M., Estevez, A., Gisbert, E.,
742 Johnston, I.A., 2012. Developmental temperature has persistent effects on muscle growth
743 responses in gilthead sea bream. PLoS ONE 7, e51884.
744

745 Gordon, S.E., Westerkamp, C.M., Savage, K.J., Hickner, R.C., George, S.C., Fick, C.A.,
746 McCormick, K.M., 2007. Basal, but not overload-induced, myonuclear addition is attenuated
747 by N^G-nitro-L-arginine methyl ester (L-NAME) administration. Can. J. Physiol. Pharmacol.
748 85, 646-651.
749

750 Hagen, O., Fernandes, J.M.O., Solberg, C., Johnston, I.A., 2009. Expression of growth-related
751 genes in muscle during fasting and refeeding of juvenile Atlantic halibut, *Hippoglossus*
752 *hippoglossus* L. Comp. Biochem. Physiol. Part B 152, 47-53.
753

754 Heckmann, L.H., Sørensen, P.B., Krogh, P.H., Sørensen, J.G., 2011. NORMA-Gene: a simple
755 and robust method for qPCR normalization based on target gene data. BMC Bioinf. 12, 250-
756 257.
757

758 Johansen, K.A., Overturf, K., 2006. Alterations in expression of genes associated with muscle
759 metabolism and growth during nutritional restriction and refeeding in rainbow trout. Comp.
760 Biochem. Physiol. Part B 144, 119-127.
761

762 Kalbe, C., Bérard, J., Porm, M., Rehfeldt, C., Bee G., 2013. Maternal L-arginine
763 supplementation during early gestation affects foetal skeletal myogenesis in pigs. Livest. Sci.
764 157, 322-329.
765

766 Kopincova, J, Puzserova, A., Bernatova, I., 2012. L-NAME in the cardiovascular system –
767 nitric oxide synthase activator? Pharmacol. Rep. 64, 511-520.
768

769 Lall, S.P., Kaushik, S.J., Le Bail, P.Y., Keith, R., Anderson, J.S., Plisetskaya, E., 1994.
770 Quantitative arginine requirement of Atlantic salmon (*Salmo salar*) reared in sea water.
771 Aquaculture 124, 13-25.
772

773 Lee, K.H., Baek, M.Y., Moon, K.Y., Song, W.K., Chung, C.H., Ha, D.B., Kang, M.S., 1994.
774 Nitric oxide as a messenger molecule for myoblast fusion. J Biol. Chem. 269, 14371-14374.
775

776 Li, Y., Wang, Y., Willems, E., Willemsen, H., Franssens, L., Buyse, J., Decuypere, E.,
777 Everaert, N., 2016. In ovo L-arginine supplementation stimulates myoblast differentiation but
778 negatively affects muscle development of broiler chicken after hatching. Anim. Physiol.
779 Nutr.100, 167-177.
780

781 Long, J.H.D., Lira, V.A., Soltow, Q.A., Betters, J.L., Sellman, J.E., Criswell, D.S., 2006.
782 Arginine supplementation induces myoblast fusion via augmentation of nitric oxide
783 production. J. Musc. Res. Cell Motil. 27, 577-584.
784

785 Macqueen, D.J., Robb, D.H.F., Olsen, T., Melstveit, L., Paxton, C.G.M., Johnston, I.A., 2008.
786 Temperature until the 'eyed stage' of embryogenesis programs the growth trajectory and
787 muscle phenotype of adult Atlantic salmon. Biol. Lett. 4, 294-298.
788

789 McNeill, B., Perry, SF., 2005. Nitric oxide and the control of catecholamine secretion in
790 rainbow trout *Oncorhynchus mykiss*. J. Exp. Biol. 208, 2421-2431.

791

792 Miller, M.J.S., Thompson, J.H., Liu, X., Eloby-Childress, S., Sadowska-Krowicka, H., Zhang,
793 X.J., Clark, D.A., 1996. Failure of L-NAME to cause inhibition of nitric oxide synthesis: role
794 of inducible nitric oxide synthase. *Inflamm. Res.* 45, 272-276.

795

796 Miller-Fleming, L., Olin-Sandoval, V., Campbell, K., Ralser, M., 2015. Remaining mysteries
797 of molecular biology: the role of polyamines in the cell. *J. Mol. Biol.* 427, 3389-3406.

798

799 Mommsen, T.P., 2001. Paradigms of growth in fish. *Comp. Biochem. Physiol. Part B* 129,
800 207-219.

801

802 Neu, D., Boscolo, W., Zaminhan, M., Almeida, F., Sary, C., Furuya, W., 2016. Growth
803 performance, biochemical responses, and skeletal muscle development of juvenile Nile
804 tilapia, *Oreochromis niloticus*, fed with increasing levels of arginine. *J. World Aquac. Soc.*
805 47, 248-259.

806

807 NRC, 2011. Nutrient requirements of fish and shrimp: National Academy of Sciences,
808 Washington, DC, USA, 360p.

809

810 Ostaszewska, T., Dabrowski, K., Wegner, A., Krawiec, M., 2008. The effects of feeding on
811 muscle growth dynamics and the proliferation of myogenic progenitor cells during pike perch
812 development (*Sander lucioperca*). *J. World Aquac. Soc.* 39, 184-195.

813

814 Østbye, T.K.K., Ruyter, B., Standal, I.B., Stien, L.H., Bahuaud, D., Dessen, J.E., Latif, M.S.,
815 Fyhn-Terjesen, B., Rørvik, K.A., Mørkøre, T., 2008. Functional amino acids stimulate muscle
816 development and improve fillet texture of Atlantic salmon. *Aquac. Nutr.* 24, 14-26.
817

818 Paula, T.G.d, Thomazini Zanella, B. T. T., de Almeida Fantinatti, B., de Moraes, L.N., da
819 Silva Duran, B.O., de Oliveira, C.B., Simoes Salomao, R.A., da Silva, R.N., Padovani, C.R.,
820 dos Santos, V.B., Mareco, E.A., Carvalho, R.F., Dal-Pai-Silva, M., 2017. Food restriction
821 increase the expression of mTORC1 complex genes in the skeletal muscle of juvenile pacu
822 (*Piaractus mesopotamicus*). *PLoS ONE* 12, e0177679.
823

824 Pfaffl, M.W., 2001. A new mathematical model for relative quantification in real-time RT-
825 PCR. *Nucleic Acid Res.* 29, e45.
826

827 Plisetskaya, E.M., Buchelli-Narvaez, L.I., Hardy, R.W., Dickhoff, W.W., 1991. Effects of
828 injected and dietary arginine on plasma insulin levels and growth of pacific salmon and
829 rainbow trout. *Comp. Biochem. Physiol* 98A, 165-170.
830

831 Rescan, P.Y., Collet, B., Ralliere, C., Cauty, C., Delalande, J.M., Goldspink, G., Fauconneau,
832 B., 2001. Red and white muscle development in the trout (*Oncorhynchus mykiss*) as shown by
833 *in situ* hybridisation of fast and slow myosin heavy chain transcripts. *J. Exp. Biol.* 204, 2097-
834 2101.
835

836 Rescan, P.Y., Montfort, J., Rallièrè, C., Le Cam, A., Esquerré, D., Hugot, K., 2007. Dynamic
837 gene expression in fish muscle during recovery growth induced by a fasting-refeeding
838 schedule. *BMC Genomics* 8, 438.

839

840 Rescan, P.Y., Le Cam, A., Rallièrre, C., Montfort, J., 2017. Global gene expression in muscle
841 from fasted/refed trout reveals up-regulation of genes promoting myofibre hypertrophy but
842 not myofibre production. *BMC Genomics* 18, 447.

843

844 Rice, S.D., Stokes, R.M., 1974. Metabolism of nitrogenous wastes in the eggs and alevins of
845 rainbow trout, *Salmo gairdneri* Richardson. In: Blaxter J.H.S. (eds) *The Early Life History of*
846 *Fish*. pp 325-337. Springer, Berlin, Heidelberg.

847

848 Rowlerson, A., Veggetti, A., 2001. Cellular mechanisms of post-embryonic muscle growth in
849 aquaculture species. In *Fish Physiology*, Vol. 18 (ed. I.A. Johnston), pp. 103-140. London:
850 Academic Press.

851

852 Saavedra, M., Grade, A., Candeias-Mendes, A., Pereira, T.G., Teixeira, B., Yufera, M.,
853 Conceicao, L.E.C., Mendes, R., Pousao-Ferreira, P., 2016. Different dietary protein levels
854 affect meagre (*Argyrosomus regius*) larval survival and muscle cellularity. *Aquaculture* 450,
855 89-94.

856

857 Salem, M., Kenney, P.B., Rexroad, C.E., Yao, J., 2006. Molecular characterization of muscle
858 atrophy and proteolysis associated with spawning in rainbow trout. *Comp. Biochem. Physiol.*
859 *D* 1, 227-237.

860

861 Seiliez, I., Medale, F., Aguirre, P., Larquier, M., Lanneretonne, L., Alami-Durante, H.,
862 Panserat, S., Skiba-Cassy, S., 2013. Postprandial regulation of growth- and metabolism-
863 related factors in zebrafish. *Zebrafish* 10, 237-248.

864

865 Shi, Z., Song, W., Sun, Y., Wang, L., Shi, B., Shan, A., Bi, Z., 2018. Dietary supplementation
866 of L-arginine and chromium picolinate in sows during gestation affects the muscle fibre
867 characteristics but not the performance of their progeny. *J. Sci. Food Agric.* 98, 74-79.

868

869 Stamler, J.S., Meissner, G., 2001. Physiology of nitric oxide in skeletal muscle. *Physiol. Rev.*
870 81, 209-237.

871

872 Stickland, N.C., 1983. Growth and development of muscle fibres in the rainbow trout (*Salmo*
873 *gairdneri*). *J. Anat.* 137, 323-333.

874

875 Subramaniyan, S.A., Kang, D.R., Park, J.R., Siddiqui, S.H., Ravichandiran, P., Yoo, D.J., Na,
876 C.S., Shim, K.S., 2019. Effect of in ovo injection of L-arginine in different chicken
877 embryonic developmental stages on post-hatchability, immune response, and Myo-D and
878 Myogenin proteins. *Animal* 9,357.

879

880 Tatsumi, R., Hattori, A., Ikeuchi, Y., Anderson, J., Allen, R.E., 2002a. Release of hepatocyte
881 growth factor from mechanically stretched skeletal muscle satellite cells and role of pH and
882 nitric oxide. *Molec. Biol. Cell* 13, 2909-2918.

883

884 Tatsumi, R., Hattori, A., Allen, R.E., Ikeuchi, Y., Ito, T., 2002b. Mechanical stretch-induced
885 activation of skeletal muscle satellite cells is dependent on nitric oxide production *in vitro*.
886 *Anim. Sci. J.* 73, 235-239.

887

888 Tatsumi, R., Liu, X., Pulido, A., Morales, M., Sakata, T., Dial, S., Hattori, A., Ikeuchi, Y.,
889 Allen, R.E., 2006. Satellite cell activation in stretched skeletal muscle and the role of nitric
890 oxide and hepatocyte growth factor. *Am. J. Physiol. Cell Physiol.* 290, C1487-C1494.
891

892 Ulibarri, J.A., Mozdziak, P.E., Schultz, E., Cook, C., Best, T.M., 1999. Nitric oxide donors,
893 sodium nitroprusside and S-nitroso-N-acetylpenicillamine, stimulate myoblast proliferation *in*
894 *vitro*. *In Vitro Cell. Dev. Biol. –Animal* 35, 215-218.
895

896 Valente, L., Bower, N., Johnston, I.A., 2012. Postprandial expression of growth-related genes
897 in Atlantic salmon (*Salmo salar* L.) juveniles fasted for 1 week and fed a single meal to
898 satiation. *Br. J. Nut.* 108, 2148-2157.
899

900 Vernier J.M., 1969. Table chronologique du développement embryonnaire de la truite arc-en-
901 ciel, *Salmo gairdneri* Rich 1836. *Ann. Embryol. Morphog.* 2, 495-520.
902

903 Wang, M.X., Murrell, D.F., Szabo, C., Warren, R.F., Sarris, M., Murrell, G.A.C., 2001. Nitric
904 oxide in skeletal muscle: inhibition of nitric oxide synthase inhibits walking speed in rats.
905 *Nitric Oxide* 3, 219-232.
906

907 Wozniak, A.C., Anderson, J.E., 2007. Nitric-oxide-dependence of satellite stem cell activation
908 and quiescence on normal skeletal muscle fibers. *Dev. Dyn.* 236, 240-250.
909

910 Xi, S.Q., Mason, P.S., Wilkes, D., Goldspink, G., Fauconneau, B., Stickland, N.C., 2001.
911 Lower environmental temperature delays and prolongs myogenic regulatory factor expression
912 and muscle differentiation in rainbow trout (*Oncorhynchus mykiss*) embryos. Differentiation
913 68, 106-114.

914

915 Yamamoto, T., Yao, Y., Harumi, T., Suzuki, N., 2003. Localization of the nitric oxide/cGMP
916 signalling pathway-related genes and influence of morpholino knock-down of soluble
917 guanylyl cyclase on medaka fish embryogenesis. Zool. Sci. 20, 181-191.

918

919 Yamashiro, D., Neu, D.H., Moro, E.B., Feiden, A., Signor, A., Boscolo, W.R., Bittencourt, F.,
920 2016. Performance and muscular development of Nile tilapia larvae (*Oreochromis niloticus*)
921 fed increasing concentrations of phenylalanine. Agricultural Sciences 7, 900-910.

922

923 Zhang, H., Anderson, J.E., 2014. Satellite cell activation and populations on single muscle-
924 fiber cultures from adult zebrafish (*Danio rerio*). J. Exp. Biol. 217, 1910-1917.

925

926 Zhu, K., Chen, L., Zhao, J., Wang, H., Wang, W., Li, Z., Wang, H., 2014. Molecular
927 characterization and expression patterns of myogenin in compensatory growth of
928 *Megalobrama amblycephala*. Comp. Biochem. Physiol. Part B 170, 10-17.

929

930 Zhu, X., Li, Y.L., Liu, L., Wang, J.H., Li, H.H., Wu, P., Chu, W.Y., Zhang, J.S., 2016.
931 Molecular characterization of Myf5 and comparative expression patterns of myogenic
932 regulatory factors in *Siniperca chuatsi*. Gene Expression Patterns 20, 1-10.

933

934

935 **Figure captions**

936

937 Figure 1.

938 Schematic diagram of experiments 1 and 2 with treatments and sampling stages.

939

940 Figure 2.

941 Somitogenesis in trout embryos.

942

943 Figure 3.

944 Expression of *myf5* in trout embryos treated at 9 dpf or 13 dpf by immersion in C, A or N-
945 bath. Whole mount *in situ* hybridization of embryos at 9, 10, 12, 14, 18 and 22 dpf.

946

947 Figure 4.

948 Expression of *fmhc* in trout embryos treated at 9 dpf or 13 dpf by immersion in C, A or N-
949 bath. Whole mount *in situ* hybridization of embryos at 12, 14, 18 and 22 dpf.

950

951 Figure 5.

952 Growth of the fry hatched from embryos treated at 9 dpf or 13 dpf by immersion in C, A or N-
953 bath. Values are mean \pm SD (n=3).

954

955 Figure 6.

956 Total length and body weight of the C- and N-fed fry after 20 days of feeding.

957 Values are mean \pm SEM (n=63).

958

959 Figure 7.

960 Length-weight relationships of the C- and N-fed fry after 20 days of feeding (n= 63).

961 TL, total length; W, body weight.

962

963 Figure 8.

964 Pre- and post-prandial expressions of myogenic regulatory factors (myod1, myog),
965 proliferative cell nuclear antigen (pcna) and fast myosin heavy chain (fmhc) in C- and N-fed
966 fry after distribution of a single meal.

967 Values are mean \pm SEM (n=9) of NORMA-gene normalized transcript levels. Within C-
968 samples, means with unlike lower-case letter are significantly different (P<0.05). Within N-
969 samples, means with unlike upper-case letter are significantly different (P<0.05). Within time
970 points, symbols indicate diet-induced statistical differences (***P<0.001; **P<0.01;
971 *P<0.05).

972

973 Figure 9.

974 Pre- and post-prandial expressions of inducible nitric oxide synthase (inos), neuronal nitric
975 oxide synthase (nnos) and cathepsin D (ctsd) in C- and N-fed fry after distribution of a single
976 meal.

977 Values are mean \pm SEM (n=9) of NORMA-gene normalized transcript levels. Within C-
978 samples, means with unlike lower-case letter are significantly different (P<0.05). Within N-
979 samples, means with unlike upper-case letter are significantly different (P<0.05). Within time
980 points, diet-induced statistical differences are indicated by symbols (***P<0.001; **P<0.01;
981 *P<0.05).

982

983 Figure 10.

984 Transverse dorsal section of a trout fry fed for 20 days with the C-diet showing the position of
985 white muscle fibres with an area lower than $100\mu\text{m}^2$ (in red), between 100 and $240\mu\text{m}^2$ (in
986 blue) and larger than $240\mu\text{m}^2$ (in green).

987 *WM* white muscle, *RM* red muscle, *m* myosept, *SC* spinal cord, *NC* notochord, *hs* horizontal
988 septum, *s* skin. Scale bar = 100 μm .

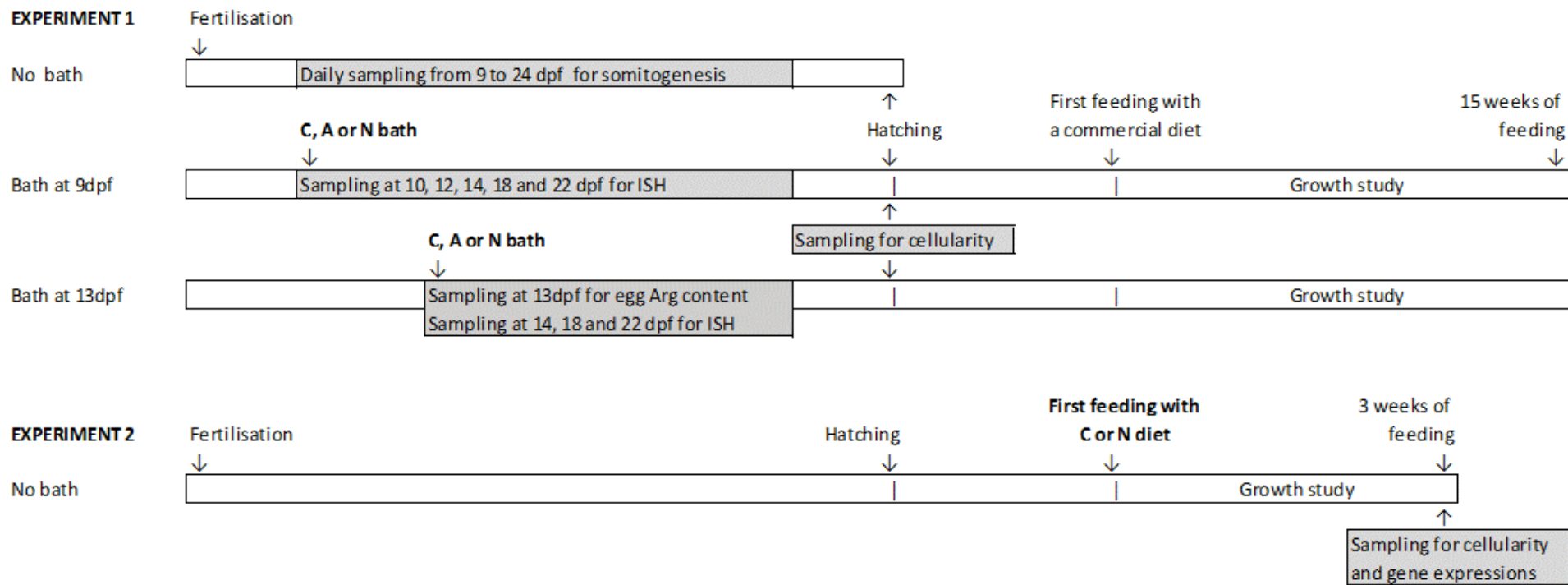
989

990 Figure 11.

991 Distribution of white skeletal muscle fibre diameters in the C- and N-fed fry after 20 days of
992 feeding. Values are mean \pm SEM (n=6). Within each diameter class, means not sharing a
993 common letter are significantly different ($P < 0.05$).

994

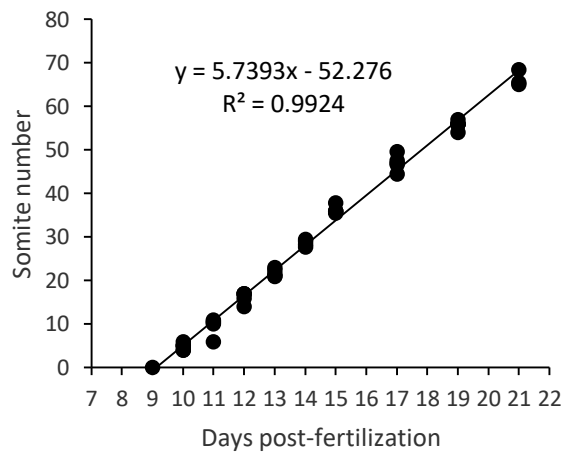
995



996

997 Figure 1.

998



999

1000 Figure

2.

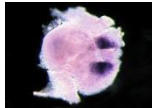
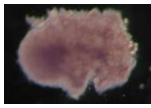
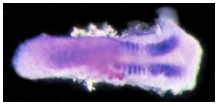
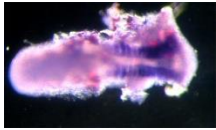

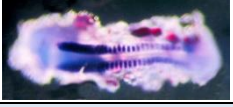

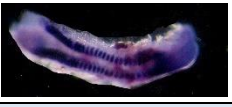
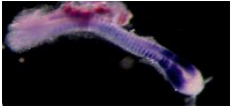
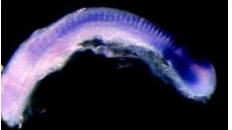

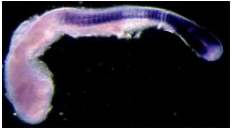

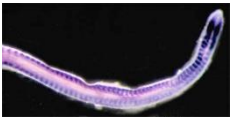




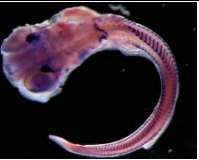



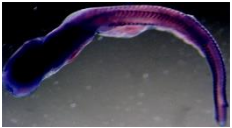

9dpf, before bath		Beginning of expression			Sens probe (control)	
						
9 dpf	C-bath	A-bath	N-bath	no bath	no bath	no bath
10 dpf						
12 dpf						
13 dpf	no bath	no bath	no bath	C-bath	A-bath	N-bath
14 dpf						
18 dpf						
22 dpf						

Figure 3.

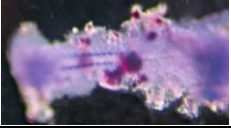
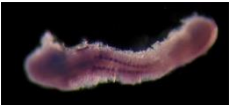
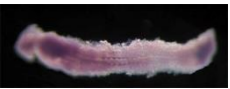



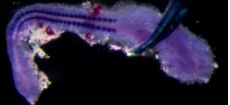

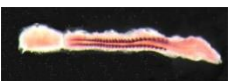
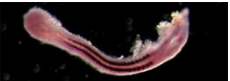
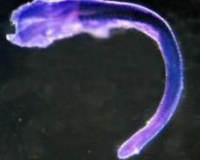



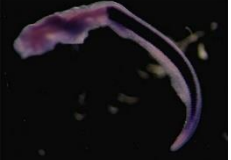

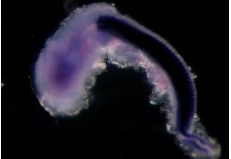

9 dpf	C-bath	A-bath	N-bath	no bath	no bath	no bath
12 dpf				Sens probe (control)		
13 dpf	no bath	no bath	no bath	C-bath	A-bath	N-bath
14 dpf						
18 dpf						
22 dpf						

Figure 4.

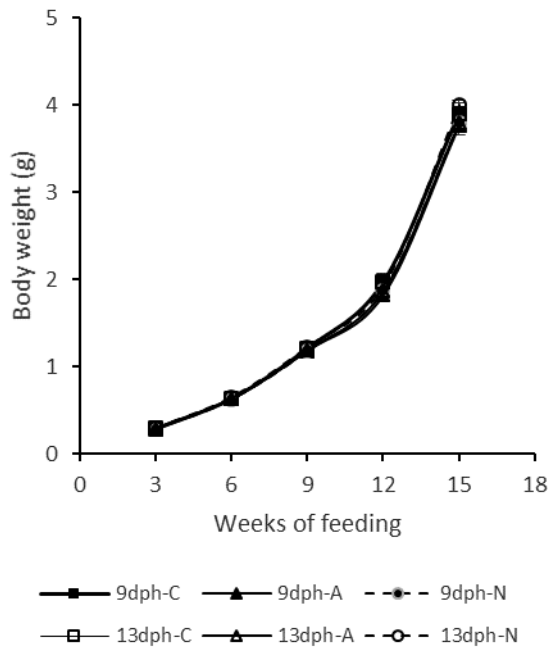


Figure 5.

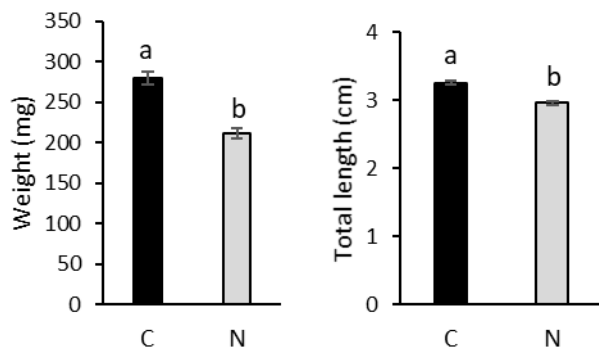


Figure 6.

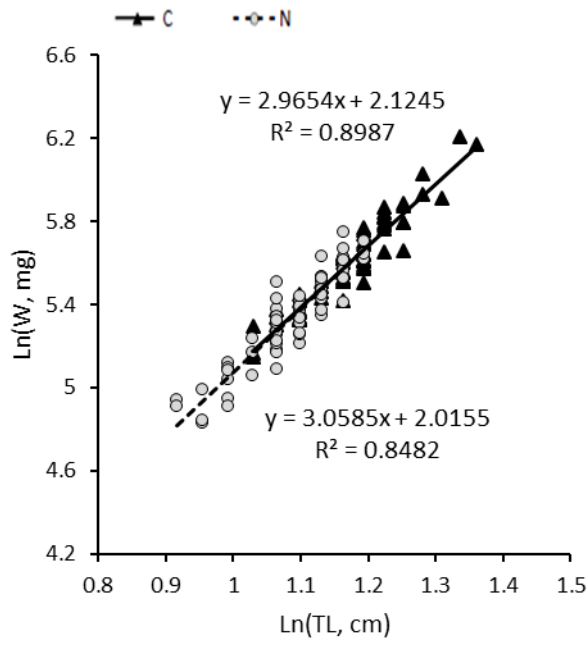


Figure 7.

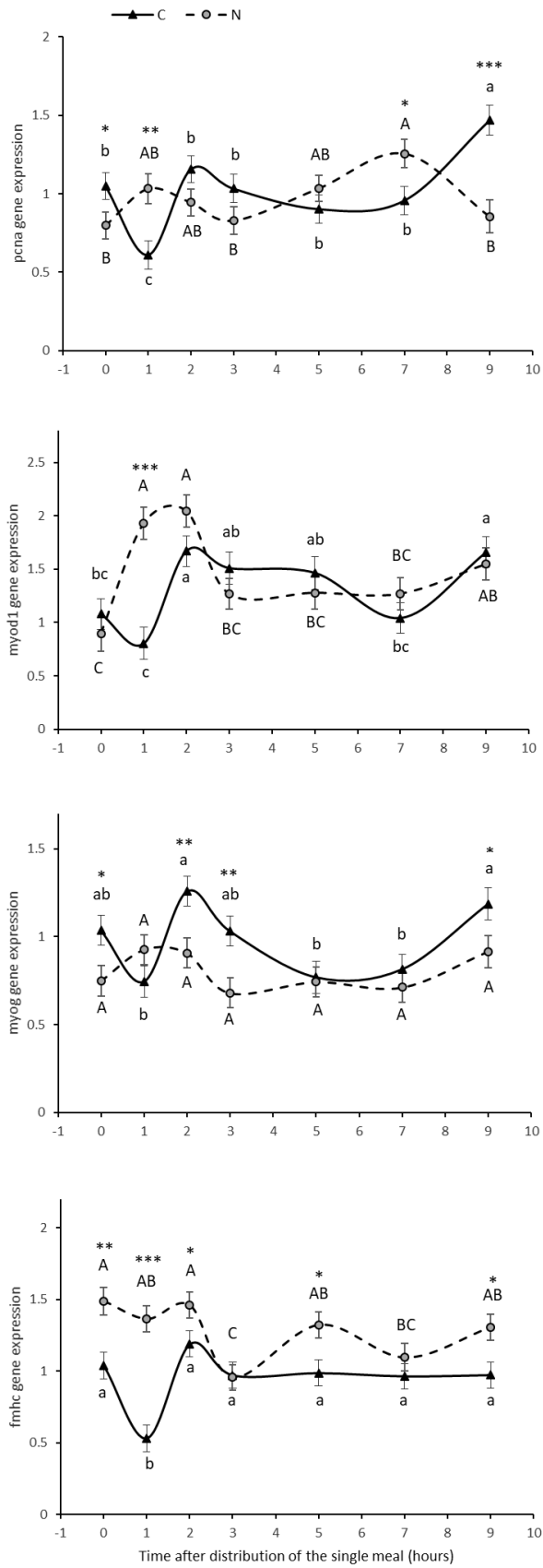


Figure 8.

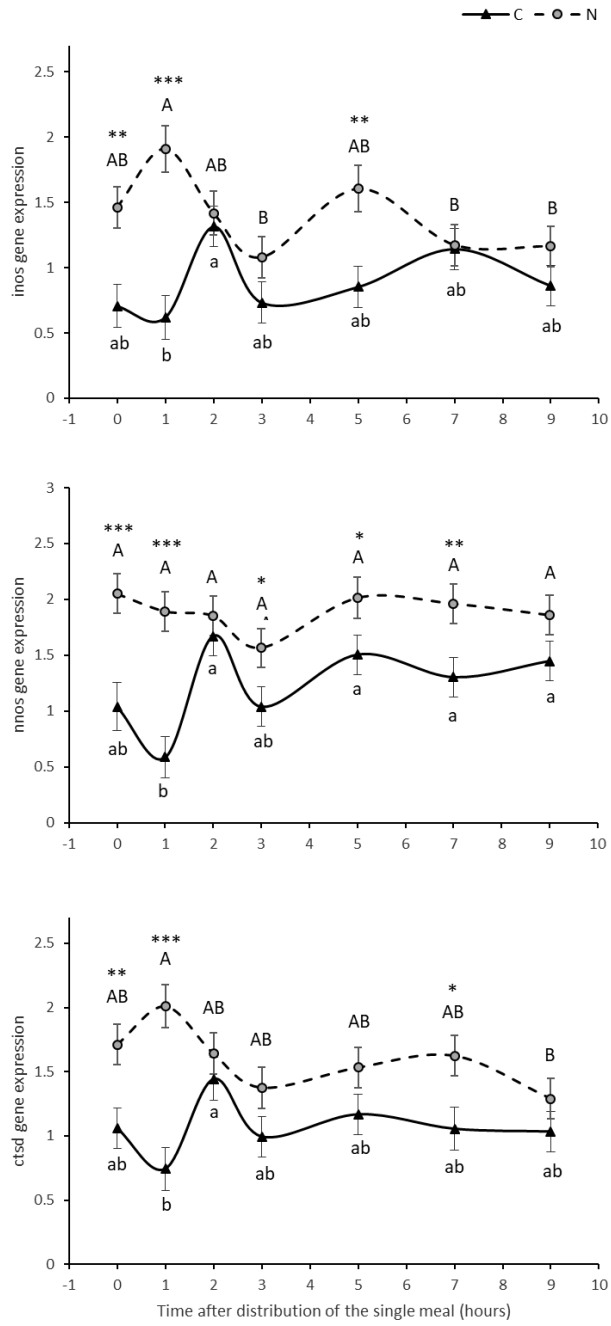


Figure 9.

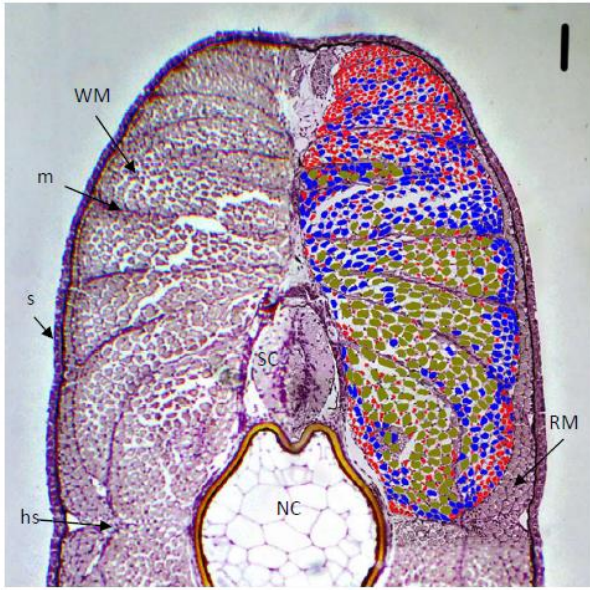


Figure 10.

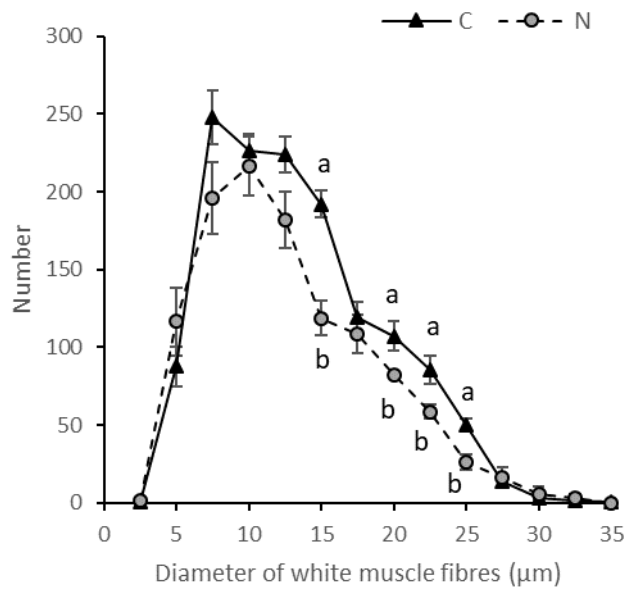


Figure 11.

Table 1.

Ingredient composition of the control (C) and L-NAME (N) diets.

Diets	C	N
<i>Ingredients (g/100g diet)</i>		
Fish soluble protein concentrate ¹	4.0	4.0
Wheat gluten ²	5.0	5.0
Corn gluten meal ³	38.0	38.0
Whole wheat ⁴	29.6	29.6
Soy protein concentrate ⁵	3.0	3.0
L-arginine	0.5	0.5
L-lysine	1.0	1.0
L-histidine	0.1	0.1
L-glutamate	0.8	0.8
Attractant mix ⁶	1.5	1.5
CaHPO ₄ .2H ₂ O (18% P)	3.5	3.5
Soy lecithin ⁷	1.0	1.0
Fish oil ⁸	9.0	9.0
Mineral premix ⁹	1.5	1.5
Vitamin premix ¹⁰	1.5	1.5
L-NAME ¹¹	0	0.1

¹ CPSP-90, Sopropèche (Wimille, France).

² Roquette (Lestrem, France).

³ Inzo (Argentan, France)

⁴ Sud-Ouest Aliment (Pomarez, France).

⁵ Estril 75, Sopropèche (Wimille, France).

⁶ Attractant mix (g/100g feed): Glucosamine 0.5g; taurine 0.3g, betaine 0.3g, glycine 0.2g, alanine 0.2g.

⁷ Louis François (Croissy-Beaubourg, France).

⁸ Sopropèche (Wimille, France).

⁹ Mineral premix (g or mg/kg diet): calcium carbonate (40 % Ca), 2.15 g; magnesium oxide (60 % Mg), 1.24 g; ferric citrate, 0.2 g; potassium iodide (75 % I), 0.4 mg; zinc sulphate (36 % Zn), 0.4 g; copper sulphate (25 % Cu), 0.3 g; manganese sulphate (33 % Mn), 0.3 g; dibasic calcium phosphate (20 % Ca, 18 % P), 5 g; cobalt sulphate, 2 mg; sodium selenite (30 % Se), 3 mg; KCl, 0.9 g; NaCl, 0.4 g (UPAE, INRA, Jouy en Josas, France).

¹⁰ Vitamin premix (μg or mg/kg diet): dl- α -tocopherol acetate, 60 mg; sodium menadione bisulphate, 5 mg; retinyl acetate, 4.5 mg; dl-cholecalciferol, 375 μg ; thiamin, 15 mg; riboflavin, 30 mg; pyridoxine, 15 mg; B₁₂, 0.05 mg; nicotinic acid, 175 mg; folic acid, 500 mg; inositol, 1000 mg; biotin, 2.5 mg; calcium pantothenate, 50 mg; choline chloride, 2000 mg (UPAE, INRA, Jouy en Josas, France).

¹¹ N5751, Sigma (France).

Table 2.

Analysed composition of the control diet (C).

Diet	C
Dry matter (DM, %)	90.6
Crude protein (% DM)	42.1
Total lipid (% DM)	8.3
Gross energy (kJ/g DM)	21.2
<i>Essential amino acids (g/100g dry feed)</i>	
Arginine	1.71
Histidine	0.76
Isoleucine	1.34
Leucine	4.32
Lysine	1.64
Methionine	0.75
Phenylalanine	1.95
Threonine	1.17
Tryptophan	0.22
Valine	1.54
<i>Non-essential amino acids (g/100g dry feed)</i>	
Alanine	2.39
Aspartic acid	2.14
Cysteine	0.58
Glutamic acid	8.37
Glycine	1.38
Proline	2.98

Serine	1.76
Tyrosine	1.34

Table 3

Survival, body weight and muscle cellularity at hatching of the fry hatched from embryos immersed at 9 or 13dpf in C, A or N bath.

	Treatments ¹						<i>P-value</i>		
	9dpf-C	9dpf-A	9dpf-N	13dpf-C	13dpf-A	13dpf-N	dpf	bath	dpf*bath
Survival ²	94.6±0.6	93.2±1.1	94.2±1.5	94.1±1.1	93.9±0.9	94.9±0.2	0.411	0.184	0.569
BW ³	46.3±1.7	48.8±2.1	43.0±1.0	43.6±1.3	46.3±1.8	47.2±1.2	0.917	0.257	0.053
TCSAWM ⁴	52495±4588	59797±4588	71429±4588	63586±5026	56914±4248	59833±4588	0.742	0.331	0.077
TNWF ⁵	768±65	840±65	787±65	923±71	836±60	808±54	0.272	0.768	0.419

¹9dpf-C immersion in C-bath at 9dpf; 9dpf-A immersion in A-bath at 9dpf; 9dpf-N immersion in N-bath at 9dpf; 13dpf-C immersion in C-bath at 13dpf; 13dpf-A immersion in A-bath at 13dpf; 13dpf-N immersion in N-bath at 13dpf.

²Survival (%), values are means ± SD (n=3 tanks). ³BW, body weight (mg), values are means ± SE (n=27 fry). ⁴TCSAWM, total cross-sectional area of white muscle (µm²), values are means ± SE (n=6 fry). ⁵TNWF, total number of white muscle fibres, values are means ± SE (n=6 fry).

Table 4.

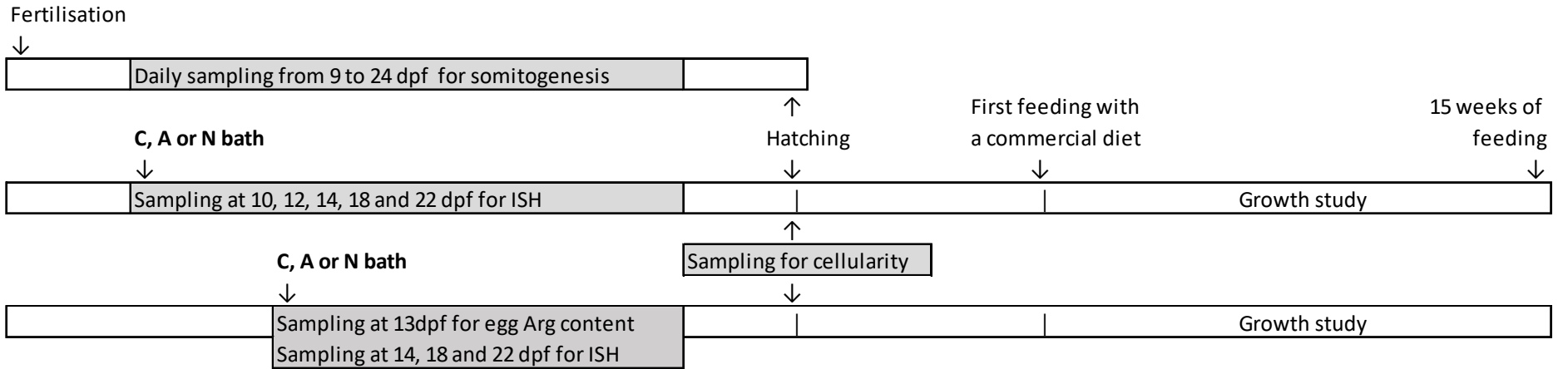
Cellularity of the dorsal skeletal white muscle of fry fed with the C- or N-diet for 20 days.

	Diet		<i>P</i> -value
	C	N	
TCSAWM (μm^2)	419959 \pm 21455 a	293028 \pm 9651 b	<0.001
TNWF	1553 \pm 56 a	1283 \pm 135 b	0.009
d mean (μm)	12.2 \pm 0.2	11.7 \pm 0.5	0.383

TCSAWM, total cross sectional area of white muscle; TNWF, total number of white muscle fibres; d, diameter of white muscle fibres.

Values are means \pm SEM (n=6). Within each row, means not sharing a common superscript letter are significantly different.

EXPERIMENT 1



EXPERIMENT 2

