Dietary fatty acid profiling in plant protein-rich diets affects the reproductive performance, egg fatty acid profile and hematological parameters in female rainbow trout (*Oncorhynchus mykiss*)

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Abstract

A three month feeding trial was conducted to determine the effects of replacement of dietary fish oil (FO) with blends of vegetable oils (VOs) on reproductive performance, fatty acid dynamics of embryos, as well as hematological health indices of female rainbow trout (*Oncorhynchus mykiss*) brooders (mean body weight, 1.8 ± 0.1 kg). For this purpose, four isoproteic (*ca.* 42%) and isoenergetic (*ca.* 20 MJ kg^{-1}) diets were formulated in which 50% (FO_{50}/VO_{50}), 75% (FO_{25}/VO_{75}) and 100% (VO_{100}) of FO was replaced by mixture of VOs, whereas the control diet (FO_{100}) was prepared with FO as the major source of lipid. Fish fed the FO_{100} diet had lower survival rates at eyed-embryo stage (83.7 ± 1.6 %) and hatching rate (79.9 ± 3.1%) in comparison to the other experimental groups. Fish fed the FO_{25}/VO_{75} and VO_{100} diets had the higher fry weight at 30 days post hatch than other groups. From the eyed-embryo stage to hatching, the proportions of saturated fatty acids increased in embryos of all experimental groups. Broodfish fed the FO_{50}/VO_{50}, FO_{25}/VO_{75} and VO_{100} diets had higher levels of monounsaturated fatty acids in embryos in comparison with fish fed FO_{100} diet. Broodfish fed the VO_{100} diet had relatively higher arachidonic (ARA) content in embryos in comparison to other treatments. The levels of docosahexaenoic acid of embryos gradually decreased during embryogenesis in all treatments and this trend was more evidenced at hatching, whereas the concentrations of eicosapentaenoic acid and ARA extremely increased at hatching day. Regarding serum biochemical parameters, glucose and triglycerides levels were lower in broodfish fed the FO_{100} diet than those from the other groups. The results of the current study revealed that fish fed the FO_{25}/VO_{75} had better reproductive performance than other groups.

Keywords: Reproductive performance, Nutritional programing, Broodstock feeding, Fishmeal, Fish oil, Vegetable oil

Running title: Dietary fatty acid profiling in female rainbow trout

1. Introduction

The biochemical composition of broodstock diets is one of the key determinants of the eggs' biochemical composition that influences the success of reproduction, as well as the offspring survival, since it provides the necessary nutrients to be utilized during the embryonic development and the lecithotrophic larval period (Izquierdo et al., 2001). Regarding nutrients, lipids and especially long chain polyunsaturated fatty acids (LC-PUFAs), namely arachidonic (20:4n-6, ARA), eicosapentaenoic (20:3n-3, EPA) and docosahexaenoic (22:6n-6, DHA), are critical for the optimal reproductive performance and egg quality in fish (Fernández-Palacios et al., 2011). Several studies have evidenced the importance of these LC-PUFAs in broodstock diets in terms of vitellogenesis and gonadal maturation, production of eicosanoids, control of ovulation, female fecundity, egg quality, and viability of the offspring (see reviews in Izquierdo et al., 2001; Glencross, 2009; Tocher, 2010). In addition, the dietary protein level and essential amino acid (EAA) profile have an important role in egg quality, as well as during the embryonic development, since protein is the reservoir of nutrients and energy for many biosynthetic activities during embryogenesis (Fernández-Palacios et al., 2011).

Due to the continuous growth of global aquaculture, as well as the static supply of fish meal (FM) and fish oil (FO) for the aquafeed industry; nowadays, the use of the plant proteins (PP) and vegetal oils (VO) are the most economical and environmentally sustainable approaches for partial or total replacement of marine ingredients in aquafeeds (Gatlin et al., 2007; Tacon and Metian, 2008). Fish meal and FO replacement in broodstock diets has deeply been addressed by the aquafeed industry and academy, whereas most of the recent works have been focused on just considering the early

nutritional programming of the progeny. In this sense, it has been recently reported that broodfish of gilthead seabream (*Sparus aurata*) fed dietary VO improved the acceptance and utilization of the same diets in their offsprings (Izquierdo et al., 2015). Moreover, early nutritional intervention strategy in swim-up fry of rainbow trout (*Oncorhynchus mykiss*) by a short-term exposure to a plant-based diet also improved its acceptance and utilization at later life stages (Geurden et al., 2013). Therefore, replacement of FO and FM with vegetal ingredients through nutritional programming in broodfish is a new approach for a better utilization and acceptance of plant-based diets in their offsprings (Izquierdo et al., 2015; Lazzarotto et al., 2015). In particular, rainbow trout reared entirely on a plant-based diet devoid of n–3 LC-PUFA over a 3-year breeding cycle was able to produce viable ova in which neo-synthesized n–3 LC-PUFA were preferentially accumulated (Lazzarotto et al., 2015). Regardless of the above-mentioned studies, there is scarce information about the impact of FO and FM substitution on spawning and egg quality parameters, as well as on larval performance in fish. On the other hand, using plant-based diets may influence broodstock health by affecting the fatty acid (FA) profile of immune cells (Montero et al., 2015), the EAA profile (Yaghoubi et al., 2017) and/or metabolic disturbances (Torstensen et al., 2011; Sissener et al., 2013). Thus, in addition to broodstock reproduction performance, general health of fish also should be considered, when using dietary alternative protein and lipid sources, an issue that is of special relevance considering the high value and economical cost of broodfish.

The breeding and culture technologies for rainbow trout are well developed in Iran with more than 126,000 MT of this salmonid species being produced in 2014. In fact, Iran is the world-leading producer of this species (FAO, 2016). Due to its

adaptability to salinity changes, this species has been also proposed as a suitable candidate for inland saline aquaculture systems for Iran's central regions, which are nowadays facing high risk of increased salt content (FAO, 2016). Thus, the supply of high quality and quantity of fry and juvenile trouts is of need for supporting the cold-water aquaculture industry in Iran. In this regard, the aim of this research was to determine the effect of different levels of FO replacement by VO sources with different FA ratios in diets containing low levels of FM diet on the reproductive performance, the FA profile of eggs and embryos and the hematological parameters in *O. mykiss* females.

2. Materials and Methods

2.1. Broodstock management

Fish were transferred from a local commercial hatchery to the Artemia and Aquatic Research Institute (Urmia University, Urmia, Iran), where the trial was carried out. Three-year-old *O. mykiss* (n = 108), containing both females (mean body weight (BW) = 1.8 ± 0.1 kg, mean ± SD) and males (BW = 2.0 ± 0.6 Kg, mean ± SD), were randomly divided into four groups of 27 fish (2 females:1 male) in four 25 m^3 circular concrete outdoor tanks supplied by filtered running fresh well water (4.0 l min^{-1}). Average water temperature, dissolved oxygen, hardness ($CaCO_3$) and pH in all treatments were 15.1 ± 0.1 °C, 7.1 ± 0.1 mg l^{-1}, 275.0 ± 0.5 mg l^{-1} and 7.6 ± 0.1, respectively. Each broodstock was fed twice a day at 1.0% of BW with four experimental feeds from August to November 2014 under natural photoperiod (37°40'N, 45°00'E).

2.2. Experimental diets

115 For estimating the effects of PP-rich diets with different FA profiles on the reproductive
116 performance of *O. mykiss*, a three-month feeding trial was conducted using four
117 isonitrogenous (*ca.* 42.3% crude protein), isoenergetic (*ca.* 20 MJ kg^{-1}) and isolipidic (*ca.*
118 19% crude lipids) diets (Table 1). Great portion of the dietary FM was replaced by blends
119 of PPs, including corn gluten (5%), wheat gluten (20%) and soybean meal (15%). Diets
120 were supplemented with L-lysine and DL-methionine in order to balance their AA
121 profiles (NRC, 2011). Regarding their formulation, diets differed in their content in FO
122 and mixture of VO, including FO_{100}; FO_{50}/VO_{50}; FO_{25}/VO_{75} and VO_{100} (Tables 1), as
123 well as in their fatty acid profile (Table 2). Diets were prepared by mixing all ingredients
124 for 30 min, after which, oil and sufficient distilled water were added to form a soft dough,
125 and then mechanically extruded to obtain pellets of the desired size (9.5 mm). Pellets
126 were dried in a convection oven at 25 °C and stored in re-sealable plastic bags at −20 °C
127 until use. Proximate analyses of diets was determined using standard methods (AOAC,
128 2005).

129

130 *2.3. Spawning and hatchery techniques*

131 At the start of the spawning season (90 days after the onset of the trial), broodstocks were
132 checked every six days and ovulating females were removed from the tanks for artificial
133 spawning. For proper identification of ripe ovulating females, gentle manual pressure was
134 applied onto their abdominal cavity in order to evaluate whether eggs could be stripped. .
135 Then, fish were anesthetized (clove oil, 200 ppm) and sperm and eggs were collected by
136 manual stripping. In each experimental groups, eggs from 10 ovulating females were
137 separately fertilized with the milt of 5 males (2:1, female to male ratio) as described by

Hoitsy et al. (2012). Eggs and milt were gently mixed for 2 min without water, and then eggs were washed gently under running water (10.0 ± 0.5°C). Fertilized eggs from each female were placed in an incubation tray (3,570 ± 140 eggs $tray^{-1}$) in order to monitor their development. In addition, ova (pre-cleavage stage, 2 h post fertilization) from each female (n = 10 eggs from 10 females/incubation trays for each experimental group) were collected, washed with running freshwater and their average weight (mg) (Sartorius, WPS 1790, Germany) and diameter (mm) (Nikon, Japan) measured to the nearest 0.1 mg and 0.1 mm, respectively. Fertilization rate (n = 10 eggs per tray) was estimated at seven days after spawning (day 7, eyed embryo stage) by putting eggs in a solution (acetic acid, distilled water and methanol, 1:1:1 ratio) for 10 min, and viable eggs were identified with an opaque spot on the animal pole. Viable eggs (n = 3) from three trays of each experimental group were sampled at different days: spawning (day 0), day 20 (8 mm embryo with pigmented eyes), day 30 (10-11 mm embryo) and at hatching (13-14 mm yolk-sac fry), and stored at -80 °C until their fatty acid analysis. Survival rate of progeny was measured at two developmental stages: the eyed embryo stage and at hatching. In addition, thirty days after hatching, the weight of yolk-sac fry (n = 10 per replicate; n = 30 per treatment) from each experimental group was determined.

2.4. Fatty acid analyses

For the fatty acid profile determination of experimental diets and eggs, fatty acid methyl esters were prepared by acidic methanolysis of lipid extracts using sulfuric acid in methanol (Christie, 1993). In this regard, the lipid sample (up to 50 mg) was dissolved in 2.5% sulfuric acid in methanol (2 ml) in a test tube. The mixture was left for 1 h in 80°C,

and then the samples were cooled at room temperature. After that, water (1.5 ml) containing sodium chloride (0.9%) was added and the required esters extracted with hexane (2 × 1 ml) using Pasteur pipettes to separate the layers. The solution was centrifuged (4,000 *g*, 50 min, 4°C), and the upper layer, which contained FAME, was separated, and then evaporated under a stream of nitrogen. Finally, the remaining dry FAME dissolved in isooctane (1 ml) and was determined by gas chromatography. The FA composition of diets (n = 1) and eggs (n = 3 eggs per replicate; n = 9 eggs per triplicate) were determined by an auto sampler gas chromatography (GC, Agilent Technologies 7890 N, California, USA) equipped with a flame ionization detector (FID) and a cyanopropyl–phenyl capillary column (DB-225MS, 30 m × 0.250 mm ID × 0.25μm film thickness). The column temperature was programmed as follows: holding at 100 °C for 2 min, raising to 182°C at a rate of 30°C min^{-1}, and again raising to 220 °C at a rate of 2 °C min^{-1}, holding for 5 min, and finally column heating at a rate of 3 °C min^{-1} to 230 °C, then holding at this temperature for 3 min. The injector and detector temperatures were set at 230 and 300 °C respectively. The split ratio was 30:1, and the sample volume injected for each analysis was 1 μl. The total run time was 40 min per sample. Identification of the FAs was performed by comparing their retention time with those of an external commercial standard mixture (GLC-68d, NuChek Prep., Minnesota, USA) run under the same condition (Agh et al., 2014).

2.5. Hematological parameters

At the end of the trial, females close to ovulation were anaesthetized with clove oil (clove oil, 200 ppm) and blood samples were collected from the caudal vein in 10 female fish

using a 1 ml syringe and transferred into heparinized vials for analyzing haematological parameters. For evaluation of serum immunological and biochemical parameters, blood specimens were transferred into vials was allowed to clot at room temperature for 1h, followed by 4 °C for 5 h and was subsequently centrifuged at (3,500 *g* for 5 min) (Kiron et al. 2004). Sera were separated and stored at -80 °C until their posterior analysis. Hematocrit (Hct; %), hemoglobin concentration (Hb; g dl^{-1}), the number of red blood cells (RBCs) and white blood cells (WBCs) counts, as well as differential WBC percentage (lymphocyte, neutrophil and monocyte portions as WBC) were assessed according to methods described by Blaxhall and Daisley (1973). The hemolytic activity of the plasma was determined using rabbit red blood cells as the target cells according to the procedure described by Andani et al. (2012). The levels of lysozyme in plasma were determined using a turbidimetric assay according to Ellis (1990) by measuring the lytic activity of plasma against lyophilized *Micrococcus lysodeikticus* (Sigma, St Louis, MO, USA). Plasma total immunoglobulin (Ig) was measured using the method described by Siwicki et al. (1994). All humoral immune parameters were measured in triplicate by a microplate scanning spectrophotometer (PowerWave HT, BioTek®, USA). Plasma metabolites were analyzed by means of an auto-analyzer (Mindray BS-200, China) using commercial clinical investigation kits (Pars Azmoon Kit, Tehran, Iran). Biochemical measurements were conducted for glucose, total cholesterol, triglyceride, high-density lipoprotein (HDL) and low-density lipoprotein (LDL).

2.6. Statistical analyses

206 Data were analyzed using SPSS ver.19.0 (Chicago, Illinois, USA). All data are presented
207 as mean ± standard error of the mean calculated from three biological replicates. Arcsine
208 transformations were conducted on data expressed as percentage. One way ANOVA was
209 performed at a significance level of 0.05 following confirmation of normality and
210 homogeneity of the variance. Duncan's procedure was used for multiple comparisons
211 when statistical differences were found among groups by the one-way ANOVA. The
212 effects of diet and time and their interactions on the dynamics of FA profiles were
213 analyzed using a two-way ANOVA. The Pearson product moment correlation test was
214 used to determine any correlation among parameters, and in all cases, $P < 0.05$ was
215 considered as significant.
216
217 **3. Results**
218 *3.1 Fatty acid profile of experimental diets*
219 As presented in Table 2, the FO_{100} diet provided the highest levels of saturated fatty acids
220 (SFAs), n–3 PUFA (mainly EPA and DHA) and n-3/n-6 ratio, but lowest levels of
221 monounsaturated fatty acids (MUFAs) and n-6 PUFA. On the other hand, the
222 concentration of MUFAs increased with increasing the inclusion of VO in the diet,
223 whereas the VO_{100} diet provided the highest MUFAs (especially oleic acid), but lowest
224 levels of n-3 PUFA. The content in n-6 PUFA was similar between FO_{25}/VO_{75},
225 FO_{50}/VO_{50} and VO_{100} diets and 14.5 times higher than in the FO_{100} diet.
226
227 *3.2. Reproductive performance*

In general terms, fertilization rates were high in all experimental groups; however, fertilization rates were slightly significantly lower from broodfish fed the VO_{100} diet (81.3 ± 2.3%) in comparison to the other groups whose fertilization rate values ranged from 88.3 to 91.7 % (Table 3, $P < 0.05$). In addition, the progeny from fish fed the FO_{100} diet had the lowest survival at the eyed-embryo stage (83.7 ± 1.6 %) and hatching rates (79.9 ± 3.1%) in comparison to the other experimental groups. Fish fed the FO_{25}/VO_{75} and VO_{100} diets had the higher fry BW at 30 days post hatch than fish fed with FO_{100} and FO_{50}/VO_{50} diets ($P < 0.05$). Other reproductive parameters including the gonadosomatic index, reletive fecundity, egg size in diameter and weight were not significantly affected by experimental diets (Table 3, $P > 0.05$).

3.3. Dynamics of changes in FA composition during embryogenesis

The results of FA profile of eggs showed that lipids in *O. mykiss* eggs contained proportionally more PUFAs, including n–3 and n–6 PUFAs, than MUFAs or SFAs, although a significant effect of the dietary treatment was observed for all FA analyzed (Tables 4-7; $P < 0.05$).. In particular, embryos of females fed the FO_{50}/VO_{50}, FO_{25}/VO_{50} and VO_{100} diets contained over 40% of PUFAs, more than 30% of MUFAs and *ca.* 20-25% of SFAs, whereas embryos of females fed the FO_{100} diet contained *ca.* 50% of PUFAs, over 25% of MUFAs and *ca.* 20-25% of SFA. However, at hatching, the proportions of PUFA decreased to *ca.* 30%, whereas MUFA increased up to 37% and SFA remained stable (25%) in embryos from FO_{50}/VO_{50}, FO_{25}/VO_{75} and VO_{100} groups (Table 7). The FA profile in newly hatched embryos obtained from females fed the FO_{100} diet was also significantly modified; thus, the proportions of PUFAs decreased from *ca.*

50% to *ca.* 40%, whereas the proportions of the MUFAs increased up to 30%, and SFA remained stable (25%).

During embryogenesis, SFA represented 20–25% of the total FA with no significant variation among different experimental groups ($P > 0.05$). The most abundant SFA were the palmitic (16:0), followed by stearic (18:0) and myristic (14:0) acids (Tables 4-7). From the eyed-embryo stage to hatching, the proportions of SFA, mainly palmitic and stearic acids, increased in embryos of all experimental groups (Table 8). With regard to the MUFAs content, the most abundant MUFA was the oleic acid (18:1; OA), whereas broodfish fed the FO_{50}/VO_{50}, FO_{25}/VO_{75} and VO_{100} diets had higher percentage levels of MUFA in embryos at all sampling times in comparison with fish fed FO_{100} diet (Tables 4-7). At hatching, the proportions of MUFA in newly hatched fry significantly increased in all experimental groups as follows: 20.3, 20.8, 13.9 and 23.5% in broodfish fed FO_{100}, FO_{50}/VO_{50}, FO_{25}/VO_{75} and VO_{100}, respectively (Table 8). Among n–6 PUFA, linoleic (18:2n-6; LA) and ARA contents in the embryos had some fluctuations during embryogenesis; however, broodfish fed the VO_{100} diet had relatively higher ARA content in embryos in all developmental stages in comparison to other treatments (Tables 4-7). At hatching, the LA content in newly hatched fry from the FO_{50}/VO_{50}, FO_{25}/VO_{75} and VO_{100} groups significantly decreased; however, the LA content in eggs of fish fed the FO_{100} diet remained stable (Table 8). Regarding arachidonic acid, at hatching, the ARA content in newly hatched fry significantly increased between 24% (VO_{100}) to 48% (FO_{25}/VO_{75}) ($P < 0.05$; Table 8).

Regarding to (n–3) PUFAs, linolenic acid (18:3n–3; LNA) content in the embryos of broodfish fed the VO_{100} diet gradually decreased to the half at the hatching day.

However, the concentration of LNA did not change in embryos from the FO_{100} group, but its level had some fluctuations during embryogenesis in the FO_{50}/VO_{50} and FO_{25}/VO_{75} groups. In general, the levels of DHA of embryos gradually decreased during embryogenesis in all treatments, and this trend was more evidenced at hatching, especially in FO_{50}/VO_{50} and FO_{25}/VO_{75} groups, where DHA levels decreased 67% with regard to the DHA content of spawned eggs (Table 8). With the exception of the hatching stage, fish fed the FO_{100} diet had higher EPA and DHA content than fish fed other diets; however, the level of EPA significantly increased in the embryos of fish fed FO_{50}/VO_{50} (325.1%), FO_{25}/VO_{75} (324.0%) and VO_{100} (601.4%) diets at hatching day. The levels of LC-PUFAs and n-3/n-6 ratios in embryos from the FO_{100} group were higher than the other groups at spawning, day 20 (8 mm embryo with pigmented eyes) and day 30 (10-11 mm embryo); however, at the hatching day there were no significant differences in their values among experimental groups (Tables 4-7). Moreover, the concentration of LC-PUFAs significantly decreased in all experimental groups at hatching. Fatty acid profile of embryos not only influenced by dietary FA profile but also sampling time had profound effects on dynamics of FAs during embryogenesis (Table 8).

3.4. Hematological parameters

There were not significant differences in complete blood count or humoral immune parameters including serum lysozyme, ACH50 and total Ig between different experimental groups (Table 9; $P > 0.05$). Regarding serum biochemical parameters, glucose and triglycerides levels were lower in broodfish fed the FO_{100} diet than those from the other groups (Table 10; $P < 0.05$). Fish fed the FO_{100} and FO_{50}/VO_{50}, diets had

297 higher serum HDL than fish fed the FO_{25}/VO_{75} and VO_{100} diets ($P < 0.05$). Serum total
298 cholesterol and LDL did not significantly change among fish fed different diets ($P >$
299 0.05).
300
301 **4. Discussion**
302 *4.1. Reproductive performance*
303 In the current study using a mixture of various PP sources supplemented with L-lysine
304 and DL-methionine in order to balance respective EAA profiles in PP-rich diets was a
305 satisfactory strategy in terms reproductive performance and the overall condition of *O.*
306 *mykiss* broodfish fed experimental diets. In this regard, Lazzarotto et al. (2015) reported
307 that in spite of drastic change in biochemical composition of the ova in *O. mykiss* females
308 fed a plant-based diet devoid of FM, this species can achieve a 3-year breeding cycle
309 including two spawnings events, which might be linked to the adaptation of this species
310 to PP-rich diets as added to the ability to synthesize LC-PUFAs from their dietary
311 precursors (Gregory et al., 2016). In this sense, it has been suggested that dietary LNA at
312 1% is sufficient for providing n-3 LC-PUFAs to allow normal growth, egg development
313 and survival of the offsprings in *O. mykiss* (Vassallo-Agius et al., 2001). In our study, the
314 n–3 PUFAs level in diets were between 1.2% in VO_{100} to 3.8% in FO_{100} suggesting all
315 diets provided adequate concentrations of these FA for normal reproduction performance
316 in this species. In addition, the ratios of EFA including ARA/EPA (0.1) and DHA/EPA
317 (3.1–3.6) were not significantly changed by balancing different vegetal lipid sources in
318 experimental diets. Different studies also have illustrated that spawning quality in

319 different fish species directly have affected by the ratios of DHA/ARA/EPA (Bell et al.
320 1997; Bruce et al. 1999).

321 In the current study, fish fed VO_{100} diet had the lowest fertilization rates, which
322 could be related to the lower eggs and/or sperm quality that may be affected by the lower
323 dietary LC-PUFAs or n–3/n–6 ratio values. In fact, high levels of VO blends in the VO_{100}
324 diet not only reduced the concentrations of DHA, EPA and ARA in this diet, but also
325 decreased the dietary ratios of LC-PUFAs/SFAs (0.2) and LC-PUFAs/MUFAs (0.1) in
326 this diet that may affect the biochemical composition of mature ova. The similar may be
327 said about the quality of the sperm, even though its quality from males fed different
328 experimental diets was not evaluated in this study. It has been shown that dietary LC–
329 PUFAs, are essential, not only for the production of good quality ova, but also for the
330 production of good, consistent sperm quality in *O. mykiss* (Vassallo-Agius et al., 2001,
331 Furuita et al., 2000, 2002).

332 What about the discussion of the fertilization rates of the other groups, you only
333 mention the worst results, but no comment on the others. Considering the highest
334 fertlilization rates you may provide some recommendations in terms of FA profile of the
335 diet. Another issue to discuss is the following: different diets and their different FA
336 profile had an impact on ova quality measured as the percentage of fertilization, but it did
337 not affect relative fecundity values. This is something to mention, probably at the
338 beginning of the section before you talk about fertilization rates. In addition, what is the
339 relevance of experimental diets not affecting the fecundity values?

340 The current study showed that fish fed the FO_{100} diet had lower eyed egg and
341 hatchability rates, which might be related to oxidative stress due to high levels of dietary

LC-PUFAs, especially those PUFAs from the n–3 series. Several studies have indicated that hatching rate significantly decreased when broodstock were fed high level of dietary LC-PUFA levels as a consequence of inadequate protection of lipids from oxygen radicals during embryogenesis (Fernández-Palacios et al., 1995; Lavens et al., 1999; Furuita et al., 2000; 2002; Li et al., 2005).

In the present study, fish fed FO_{25}/VO_{75} and VO_{100} diets had higher fry BW than the other groups, which could be a result of enhanced protein utilization due to superior availability of MUFAs in these diets for aerobic metabolism and the production of adenosine triphosphate for energy purposes (Turchini et al., 2011). It has been suggested MUFAs have higher β-oxidation capacity than other FA classes (Karalazos et al., 2014) that may be resulted in growth promotion in fry from the FO_{25}/VO_{75} and VO_{100} groups.

4.2. Dynamics of changes in FA composition during embryogenesis

It is well known that the FA composition of eggs reflects that of dietary lipids (Fernández-Palacios et al., 1995; Bell et al. 1997; Furuita et al., 2000, 2002). In our study, FA composition of eggs partially reflected the FA profile of diets especially in terms of MUFAs and n–3 LC-PUFAs, mainly DHA. As lipids are broken down for metabolism, growth and structural components for new tissues during embryogenesis, the resulting FA profile may be modified into new fatty acids (Tocher, 2003; 2010). In this study, significant quantitative variations were found in the FA composition of *O. mykiss* embryos, as it was also reported by Zengin and Akpinar (2006) in the same species. In particular in all treatments SFA, mainly palmitic acid, increased during embryogenesis especially before the hatching. Similar results have also been reported in *O. mykiss*

365 (Hayes et al., 1974), Atlantic herring (*Clupea harengus*; Tocher, 2003) and Atlantic
366 salmon (Murzina et al., 2012). Palmitic acid plays a key role in metabolism of SFA in
367 fish through *de novo* synthesis by means of the synthetase system (Tocher, 2003;
368 Murzina et al., 2012) and its increase before the hatching may indicate the important role
369 of SFAs as substrates for energy production or lipid biosynthesis (Murzina et al., 2012).
370 In the present study, we observed higher concentrations of MUFAs in the eggs of
371 broodstocks fed diets contained blends of VO that reflected high levels of MUFA in these
372 alternative lipid sources, which in agreement with the results of other studies in different
373 fish species (Nguyen et al., 2010; Zhou et al., 2010; Liang et al., 2014; Lazzarotto et al.,
374 2015). Moreover, the concentrations of MUFA significantly increased before , which
375 might be a result of increasing the activity of desaturases, especially Δ9 FA desaturase
376 (Tocher, 2003) that resulted in increasing OA concentrations in the embryo. In addition,
377 higher proportions of MUFA in embryos of fish fed FO_{25}/VO_{75} and VO_{100} diets may
378 result in higher fry BW as these diets contained 46.5 and 51.4% MUFAs , respectively in
379 comparison with *ca.* 42.0% MUFA levels in other diets.

380 At the spawning day (the first day), the concentrations of LA in the embryos of
381 fish fed the VO_{100} diet was higher than in the other groups as a consequence of high
382 levels of LA (*ca.* 16% of dietary lipid) in diets containing blends of VOs as it has also
383 been reported in other fish species fed diets containing VOs (Nguyen et al., 2010; Zhou et
384 al., 2010; Zakeri et al., 2011; Liang et al., 2014; Lazzarotto et al. 2015). However, levels
385 of LA in the embryos of broodfish fed FO_{50}/VO_{50}, FO_{25}/VO_{75} and VO_{100} diets generally
386 decreased during embryogenesis. These results may be explained by a higher
387 bioconversion of LA into ARA, as well as due to its use as energy source in these groups

(Lazzarotto et al., 2015; Khosravi et al., 2014). Moreover, it seems that the bioconversion of LNA into EPA and DHA was lower than bioconversion of LA to ARA, which was mainly due to the high dietary LA content (Lazzarotto et al., 2015). In addition, the concentrations of EPA and DHA in the embryos significantly decreased with increasing the substitution of dietary FO with blends of VOs. The concentrations of ARA and especially EPA increased at hatching, which may be due to increase in the bioconversion of LA and LNA during this developmental stage and/or as a result of changing in proportions of these FA with regard to other fatty acids, especially DHA. Arachidonic acid and EPA as the main eicosanoids precursors are involved in numerous physiological processes, including stress resistance, metamorphosis, pigmentation success, osmoregulation and immune system development during embryogenesis and early larval stages (Glencross, 2009; Tocher, 2010). Furthermore, the concentrations of DHA drastically decreased in the embryos of all treatments before the hatching, as this fatty acid may catabolized for energy production, as it has also been reported in Eurasian perch (*Perca fluviatilis*; Abi-Ayad et al., 2000) and Caspian Kutum (*Rutilus frisii kutum;* Khosravi et al., 2014).

4.3. Health indices

Dietary n−3 LC-PUFAs especially EPA and DHA are required for erythrocyte production, since they play a major role in the cell membrane functions (Nagasaka et al., 2004). In the present study, there were no differences in the other hematological parameters among fish fed different diets, which in line with the results reported in gilthead seabream (*Sparus aurata*, Montero et al., 2003), largemouth bass (*Micropterus*

411 *salmoides*, Subhadra et al. 2006), European seabass (*Dicentrarchus labrax*, Mourente et
412 al., 2007) and Caspian brown trout (*Salmo trutta caspius*; Kenari et al., 2011) in which
413 dietary FO was replaced with VOs. A close correlation between Hb and Hct values was
414 observed (r = 0.982; P = 0.018), and all values fell within range recorded for trout
415 (Blaxhall and Daisley, 1973; Greene and Selivonchick, 1990). Change in FA profile of
416 immunocytes, especially n−3/n−6 and ARA/EPA ratios can modulate immune
417 competence by alterations in immunocytes membrane FA composition and membrane
418 fluidity, integrity, and permeability, none-specific cellular (*i.e.* phagocytosis) and
419 humoral (*i.e.* lysozyme and alternative complement activity) responses, as well as by
420 eicosanoid production (see reviews by Kiron, 2012; Oliva-Teles, 2012). It has been
421 suggested that normal immune function can be more successfully attained if dietary FO is
422 replaced by a blend of VOs, which provides a more physiologically balanced FA
423 composition in comparison to replacement with a single VO (Mourente et al., 2007). In
424 the current study, replacement of dietary FO with mixture of VOs did not affect humoral
425 immune responses in *O. mykiss*, indicating the adequacy of dietary EFA and their ratios
426 in all diets because of using a blend of VOs. As neutrophils and lymphocytes are the main
427 centers of lysozyme and Ig synthesis and secretion (Magnadóttir et al., 2005), the stable
428 values for these might be correlated with the stable numbers of the above-mentioned
429 immune cells. On the other hand, the same ratios of ARA to EPA, which are the main
430 precursors of prostaglandin E_2 and E_3, respectively (Mourente et al., 2007), as well as the
431 balance EAA profile in experimental diets may have resulted in similar humoral immune
432 responses in rainbow trout. Thus, in the present study replacement of FO with a mixture
433 of VO in plant-protein rich diets did not affect the immune-competence of broodfish.

The replacement of dietary FO with VOs led to an increase in serum glucose as a result of impairment of glucose mobilization or increment of plasma free fatty acids (FFAs) in these groups (Massillon et al., 1997). Moreover, it has been reported that diets rich in n–6 PUFAs induced a higher plasma glucose concentration compared with diets rich in n–3 LC-PUFAs or rich in short-chain n–3 PUFAs as a consequence of stimulating the pentose phosphate pathway enzymes activities (Menoyo et al., 2006; Jordal *et al.*, 2007; Sissener et al., 2013). In this context, it has been reported that dietary VOs led to an increase in plasma glucose in *Salmo trutta caspius* (Kenari et al., 2011). Furthermore, higher plasma triglyceride (TAG) levels in broodfish fed diets containing VOs may indicate higher liver FA synthesis in these groups. It is suggested that VO stimulate liver TAG production and secretion as a result of higher levels of oleic and linoleic acids in these lipid sources (Vegusdal et al., 2005; Ruyter et al., 2006; Kjær et al., 2008). Thus, Caballero et al. (2006) reported that lipogenesis increased in the liver of *S. aurata* fed diets containing VOs like soybean and rapeseed oils. Moreover, replacing FM and FO with high levels (over 70%) of a PP mixture and blends of VOs resulted in increased overall adiposity in *S. salar* post-smolt, liver lipids and plasma TAG contents might be as a consequence of inadequate dietary levels of critical nutrients (*i.e.* methionine, lysine, taurine, EPA, DHA and phosphatidylcholine) (Torstensen et al., 2011). On the other hand, in the present study the replacement of dietary FO with a mixture of VOs led to a lower n–3/n–6 ratios in these diets, which may be induced liver lipid synthesis in these groups. Kjær *et al.,* (2008) reported that low n–3/n–6 ratio in endogenous FA composition of the hepatocytes induce hepatic triglycerides-rich VLDL particle secretion rate in *S. salar*. Similarly, Richard et al. (2006), Kenari et al. (2011), Liland et al. (2013)

and Luo et al. (2014) also reported that dietary VOs led to an increase in plasma TAG in *O. mykiss*, *S. trutta caspius*, *S. salar* and *O. mykiss*, respectively. In the current study, fish fed the FO_{50}/VO_{50} and FO_{25}/VO_{75} diets had higher plasma HDL levels, may because of the higher levels of n–3 LC-PUFAs in these diets due to the down-regulation of the cholesteryl ester transfer protein and lecithin-cholesterol acyltransferase enzymes (Abbey et al., 1990). In this regard, Mozanzadeh et al. (2015; 2016) also reported that plasma HDL levels increased as dietary n–3 LC-PUFA increased in silvery-black porgy (*Sparidentex hasta*).

The results of our study showed that plasma cholesterol and LDL levels were not affected by experimental diets. In contrast, Richard et al., (2006) reported that replacement of dietary FO with blends of VOs led to a decrease in plasma cholesterol and LDL as a result of high levels of OA and LNA. Moreover, VO contains high levels of phytosterols that due to their higher affinity displace cholesterol from micelles in the intestinal lumen and decrease plasma total cholesterol and LDL cholesterol (Richard et al., 2006).

In conclusion, the current study revealed that the replacement of FO with a blend of VOs in PP-rich diets did not have adverse effects on health indices (*i.e.* hemato-immunological and serum biochemical parameters) nor in relative fecundity values in *O. mykiss* females. However, mature ova from broodfish fed the VO_{100} diet had lower fertilizability percentage than other groups because of a drastic reduction in LC-PUFAs in this diet. In addition, high levels of dietary MUFAs in the FO_{25}/VO_{75} and VO_{100} diets promoted fry weight in comparison with other fry obtained from females fed the other diets. The results of this study indicated replacement of 75% of dietary FO with a blend

of VOs, not only improved reproductive performance in terms of fry body weight, but also it did not have detrimental effects on fertilizability, hatchabilty and eyed-eggs survival rates. Moreover, the results of this study showed that dietary FA profile significantly affect dynamics of FA composition of embryos during embryogenesis. Further studies are being conducted to determine early nutritional programming strategy on offsprings performance of this species using more modern "omics" techniques such as transcriptomics, proteomics, metabolomics and lipidomics under a more holistic approach.

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685

Table 1

Ingredient and proximate composition of the basal diet (g kg^{-1})

Dietary ingredients	Basal diet
Fish meal	200
Corn gluten	50
Wheat gluten	200
Blood meal	10
Soybean meal	150
Yeast	20
L-lysine	6.2
DL-methionine	10
Experimental oils	161.9
Starch	20
Wheat middling's	100
Vitamin and mineral premixes	50
Vitamin C	0.6
Vitamin E	0.5
Astaxanthin	0.6
Antioxidant	0.2
Di-calcium phosphate	20
Proximate composition (g kg^{-1})	
Crude protein	422.6
Crude lipid	190.0
Crude carbohydrate	145.2
Crude fiber	22.1
Ash	37.6
Calcium	9.0
Phosphorous	5.0
Energy (kJ g^{-1})	20

Table 2

Oils and fatty acid composition of experimental diets (n = 1)

	Diets			
Lipids Mixture (g kg^{-1} diet)	FO_{100}	FO_{50}/VO_{50}	FO_{25}/VO_{75}	VO_{100}
Fish oil	129.6	64.8	32.4	–
Canola oil	–	32.4	32.4	29.1
Linseed oil	3.2	0.4	1.2	2.4
Corn oil	29.1	16.2	9.8	–
Olive oil	–	27.5	54.2	85.4
Sunflower oil	–	–	2.4	7.7
Coconut oil	–	20.6	29.5	37.3
Fatty acids (mg g^{-1} lipid)				
14:0	29.2	35.7	37.4	39.6
16:0	231.1	150.7	144.9	139.5
18:0	54.8	35.9	36.6	33.3
SFA[a]	322.2	229.3	224.2	212.3
18:1n-9	339.6	379.4	430.1	490.3
MUFA[b]	416.5	427.2	465.0	514.1
18:2n-6	6.1	157.0	157.2	159.5
20:4n-6, ARA[c]	4.1	2.8	1.2	0.1
n-6 PUFA[d]	11.1	160.7	159.0	160.3
18:3n-3	28.2	28.4	27.7	29.2
20:5n-3, EPA[e]	41.9	24.5	16.4	7.3
22:6n-3, DHA[f]	129.4	75.8	57.4	26.4
n-3 PUFA[g]	200.1	132.8	101.8	63.2
LC-PUFA[h]	171.9	107.1	75.4	34.5
n-3 / n-6	18.0	0.8	0.6	0.4
LC-PUFA /SFA	0.5	0.5	0.3	0.2
LC-PUFA / MUFA	0.4	0.3	0.2	0.1
ARA / EPA	0.1	0.1	0.1	0.1
DHA / EPA	3.1	3.1	3.5	3.6

[a] SFA: saturated fatty acids also includes: 20:0 and 22:0.
[b]MUFA: monounsaturated fatty acids also includes: 14:1n−5, 18:1n−7, 20:1n−9, 22:1n−9 and 24:1n−9.
[c] ARA; arachidonic acid.
[d]n-6 PUFA: n-6 polyunsaturated fatty acids also includes:, 20:2n−6 and 20:3n−6.
[e] EPA; eicosapentaenoic acid.
[f] DHA; docosahexaenoic acid.
[g] n-3 PUFA: n-3 polyunsaturated fatty acids also includes: 18:4n−3, 20:3n-3 and 22:5n−3.
[h] LC-PUFA: long chain polyunsaturated fatty acids includes: ARA, 20:2n−6, 20:3n−6, 20:3n-3, EPA, 22:5n−3 and DHA.

Table 3

Morphometric and reproductive parameters of *O. mykiss* female fed different experimental diets (mean ± SEM, n = 16). A different superscript in the same row denotes statistically significant differences ($P < 0.05$).

	Diets			
	FO_{100}	FO_{50}/VO_{50}	FO_{25}/VO_{75}	VO_{100}
Growth, somatic and feeding parameters				
BW_i (Kg)	1.8 ± 0.1	1.7 ± 0.1	1.8 ± 0.1	1.8 ± 0.1
BW_f (Kg)	2.3 ± 0.3^{ab}	2.0 ± 0.2^{b}	2.6 ± 0.3^{a}	2.3 ± 0.2^{ab}
SGR (% BW day^{-1})	0.3 ± 0.1^{ab}	0.1 ± 0.1^{b}	0.4 ± 0.1^{a}	0.3 ± 0.1^{ab}
Survival (%)	100	91.7	95.8	100
K (%)	1.3 ± 0.1	1.2 ± 0.3	1.2 ± 0.2	1.1 ± 0.2
FI (kg fish^{-1})	1.1	1.0	1.1	1.0
Reproductive parameters				
Absolute fecundity (×10^3)	4.5 ± 0.5^{b}	4.3 ± 0.3^{b}	5.6 ± 0.3^{a}	4.0 ± 0.5^{b}
Relative fecundity (eggs ×10^3 kg^{-1} BW)	1.7 ± 0.1	2.2 ± 0.5	1.9 ± 0.2	1.5 ± 0.2
Fertilizability (%)	91.7 ± 1.7^{a}	88.3 ± 4.4^{ab}	90.0 ± 1.7^{a}	81.3 ± 2.3^{b}
Eyed eggs survival (% of fertilized eggs)	83.7 ± 1.6^{b}	96.6 ± 5.3^{a}	91.9 ± 2.5^{ab}	94.0 ± 1.1^{ab}
Eggs diameter (mm)	5.3 ± 0.1	5.1 ± 0.1	5.3 ± 0.2	5.2 ± 0.1
Egg weight (mg)	87.0 ± 4.4	82.0 ± 4.5	89.2 ± 3.8	92.9 ± 9.2
Hatchability (% of fertilized eggs)	79.9 ± 3.1^{b}	89.3 ± 5.6^{a}	87.5 ± 1.8^{a}	89.6 ± 5.1^{a}
Larval weight at 30 days post hatch (g)	0.7 ± 0.0^{b}	0.8 ± 0.1^{b}	1.0 ± 0.1^{a}	1.0 ± 0.1^{a}

Table 4

Fatty acid composition (mg g^{-1} lipid) of embryos of *O. mykiss* in different experimental groups at the spawning day (the first day) (mean ± SEM, n = 3). A different superscript in the same row denotes statistically significant differences ($P < 0.05$).

	Diets			
	FO_{100}	FO_{50}/VO_{50}	FO_{25}/VO_{75}	VO_{100}
Fatty acids				
14:0	10.3 ± 0.2^{bc}	13.0 ± 1.5^{b}	18.0 ± 0.9^{a}	7.6 ± 1.1^{c}
16:0	139.5 ± 3.5	137.9 ± 1.6	137.0 ± 2.0	133.9 ± 0.8
18:0	65.0 ± 1.4^{b}	56.7 ± 1.2^{c}	61.8 ± 1.4^{b}	68.9 ± 0.5^{a}
SFA[a]	215.8 ± 4.7	208.6 ± 1.5	216.9 ± 2.4	211.6 ± 8.6
18:1n-9	200.5 ± 4.4^{b}	243.2 ± 7.8^{a}	258.5 ± 3.0^{a}	249.5 ± 2.8^{a}
MUFA[b]	263.9 ± 4.4^{c}	307.0 ± 7.8^{b}	333.6 ± 6.7^{a}	304.7 ± 3.1^{b}
18:2n-6	116.5 ± 1.4^{c}	139.8 ± 10.7^{b}	107.6 ± 1.3^{c}	185.8 ± 2.0^{a}
20:4n-6, ARA[c]	32.9 ± 1.5^{b}	40.7 ± 4.0^{b}	36.9 ± 2.3^{b}	51.6 ± 0.9^{a}
n-6 PUFA[d]	193.0 ± 1.5^{c}	222.7 ± 13.4^{b}	190.3 ± 2.7^{c}	291.8 ± 0.8^{a}
18:3n-3	11.8 ± 0.1^{bc}	13.2 ± 1.4^{b}	10.8 ± 0.2^{c}	16.6 ± 0.3^{a}
20:5n-3, EPA[e]	39.1 ± 0.3^{a}	22.3 ± 2.1^{b}	18.3 ± 0.6^{c}	14.2 ± 0.1^{d}
22:6n-3, DHA[f]	244.7 ± 2.4^{a}	180.3 ± 9.2^{b}	195.2 ± 4.0^{b}	125.7 ± 0.8^{c}
n-3 PUFA[g]	302.0 ± 0.5^{a}	222.1 ± 7.4^{b}	229.3 ± 2.8^{b}	161.6 ± 2.8^{c}
LC-PUFA[h]	365.8 ± 0.5^{a}	291.8 ± 6.4^{b}	301.3 ± 2.2^{b}	251.0 ± 0.1^{c}
n-3 / n-6	1.6 ± 0.0^{a}	1.0 ± 0.1^{c}	1.2 ± 0.0^{b}	0.6 ± 0.6^{d}
LC-PUFA /SFA	1.7 ± 0.0^{a}	1.4 ± 0.1^{b}	1.2 ± 0.1^{c}	1.4 ± 0.1^{c}
LC-PUFA / MUFA	1.4 ± 0.0^{a}	1.0 ± 0.0^{b}	0.9 ± 0.0^{b}	0.8 ± 0.0^{b}
ARA / EPA	0.8 ± 0.0^{c}	1.9 ± 0.3^{b}	2.0 ± 0.0^{b}	3.6 ± 0.0^{a}
DHA / EPA	6.3 ± 0.0^{c}	8.2 ± 0.7^{b}	10.7 ± 0.1^{a}	8.9 ± 0.0^{b}

[a] SFA: saturated fatty acids also includes: 20:0 and 22:0.
[b] MUFA: monounsaturated fatty acids also includes: 14:1n−5, 18:1n−7, 20:1n−9, 22:1n−9 and 24:1n−9.
[c] ARA; arachidonic acid.
[d] n-6 PUFA: n-6 polyunsaturated fatty acids also includes:, 20:2n−6 and 20:3n−6.
[e] EPA; eicosapentaenoic acid.
[f] DHA; docosahexaenoic acid.
[g] n-3 PUFA: n-3 polyunsaturated fatty acids also includes: 18:4n−3, 20:3n-3 and 22:5n−3.
[h] LC-PUFA: long chain polyunsaturated fatty acids includes: ARA, 20:2n−6, 20:3n−6, 20:3n-3, EPA, 22:5n−3 and DHA.

Table 5

Fatty acid composition (mg g^{-1} lipid) of embryos of *O. mykiss* in different experimental groups 20 days after spawning (mean ± SEM, n = 3). A different superscript in the same row denotes statistically significant differences ($P < 0.05$).

	Diets			
	FO_{100}	FO_{50}/VO_{50}	FO_{25}/VO_{75}	VO_{100}
Fatty acids				
14:0	10.5 ± 1.1[c]	12.9 ± 1.0[bc]	18.5 ± 1.0[a]	15.3 ± 0.8[b]
16:0	139.9 ± 5.7	138.7 ± 3.2	136.0 ± 3.6	130.6 ± 2.4
18:0	62.1 ± 1.0	56.6 ± 1.0	61.8 ± 0.7	61.9 ± 1.3
SFA[a]	212.6 ± 5.9	208.3 ± 3.6	216.0 ± 4.6	207.8 ± 3.8
18:1n-9	205.2 ± 5.0[b]	271.7 ± 3.2[a]	260.2 ± 19.4[a]	229.7 ± 9.2[a]
MUFA[b]	272.3 ± 8.3[c]	307.7 ± 4.6[b]	354.4 ± 5.5[a]	339.1 ± 1.6[a]
18:2n-6	118.9 ± 4.7	129.9 ± 8.4	115.8 ± 8.1	136.1 ± 2.4
20:4n-6, ARA[c]	38.1 ± 2.2[b]	39.6 ± 4.0[b]	35.7 ± 1.1[b]	56.7 ± 5.7[a]
n-6 PUFA[d]	197.0 ± 7.6[c]	212.9 ± 10.8[b]	197.7 ± 8.9[c]	245.0 ± 16.3[a]
18:3n-3	13.3 ± 0.2	13.2 ± 0.5	10.7 ± 1.3	14.0 ± 2.0
20:5n-3, EPA[e]	39.8 ± 1.6[a]	19.3 ± 0.4[b]	17.3 ± 0.7[bc]	13.5 ± 1.8[c]
22:6n-3, DHA[f]	241.0 ± 7.7[a]	198.1 ± 4.5[b]	191.0 ± 4.1[b]	141.5 ± 7.9[c]
n-3 PUFA[h]	303.2 ± 10.8[a]	232.2 ± 3.3[b]	221.3 ± 3.6[b]	171.5 ± 10.4[c]
LC-PUFA	367.9 ± 11.4[a]	302.0 ± 3.4[b]	292.4 ± 3.8[b]	266.4 ± 17.7[b]
n-3 / n-6	1.5 ± 0.1[a]	1.1 ± 0.1[b]	1.1 ± 0.1[b]	0.7 ± 0.1[c]
LC-PUFA /SFA	1.7 ± 0.1[a]	1.5 ± 0.0[b]	1.4 ± 0.1[b]	1.3 ± 0.0[b]
LC-PUFA / MUFA	1.4 ± 0.1[a]	1.0 ± 0.0[b]	0.8 ± 0.0[b]	1.1 ± 0.1[b]
ARA / EPA	1.0 ± 0.1[b]	2.1 ± 0.2[b]	2.1 ± 0.1[b]	4.3 ± 0.7[a]
DHA / EPA	6.1 ± 0.3[b]	10.3 ± 0.4[a]	11.1 ± 0.4[a]	10.8 ± 1.2[a]

[a] SFA: saturated fatty acids also includes: 20:0 and 22:0.
[b] MUFA: monounsaturated fatty acids also includes: 14:1n−5, 18:1n−7, 20:1n−9, 22:1n−9 and 24:1n−9.
[c] ARA; arachidonic acid.
[d] n-6 PUFA: n-6 polyunsaturated fatty acids also includes:, 20:2n−6 and 20:3n−6.
[e] EPA; eicosapentaenoic acid.
[f] DHA; docosahexaenoic acid.
[g] n-3 PUFA: n-3 polyunsaturated fatty acids also includes: 18:4n−3, 20:3n-3 and 22:5n−3.
[h] LC-PUFA: long chain polyunsaturated fatty acids includes: ARA, 20:2n−6, 20:3n−6, 20:3n-3, EPA, 22:5n−3 and DHA.

Table 6

Fatty acid composition (mg g^{-1} lipid) of embryos of *O. mykiss* in different experimental groups 30 days after spawning (mean ± SEM, n = 3). A different superscript in the same row denotes statistically significant differences ($P < 0.05$).

	Diets			
	FO_{100}	FO_{50}/VO_{50}	FO_{25}/VO_{75}	VO_{100}
Fatty acids				
14:0	9.0 ± 0.3[b]	13.8 ± 4.1[b]	15.9 ± 3.9[ab]	22.8 ± 2.1[a]
16:0	155.7 ± 0.1[b]	174.2 ± 2.2[a]	140.0 ± 0.3[c]	155.8 ± 0.7[b]
18:0	76.4 ± 4.2	70.5 ± 1.3	65.7 ± 4.1	68.7 ± 0.6
SFA[a]	245.8 ± 4.1[b]	263.6 ± 0.1[a]	227.7 ± 0.6[c]	250.1 ± 3.1[ab]
18:1n-9	197.6 ± 6.5[b]	216 ± 8.9[ab]	243.8 ± 7.8[a]	244.4 ± 1.9[a]
MUFA[b]	265.6 ± 8.4[b]	286.2 ± 8.4[b]	308.0 ± 4.3[a]	314.8 ± 2.0[a]
18:2n-6	108.7 ± 0.8[b]	116.0 ± 3.6[b]	160.9 ± 14.0[a]	117.8 ± 0.5[b]
20:4n-6, ARA[c]	32.2 ± 0.8[b]	33.8 ± 1.4[b]	45.7 ± 1.5[ab]	48.7 ± 2.6[a]
n-6 PUFA[d]	179.3 ± 1.1[c]	186.1 ± 0.1[c]	266.3 ± 13.6[a]	216.2 ± 0.7[b]
18:3n-3	12.3 ± 0.1[b]	12.0 ± 0.4[b]	16.9 ± 1.1[a]	11.8 ± 0.5[b]
20:5n-3, EPA[e]	36.6 ± 1.5[a]	32.7 ± 0.4[b]	15.6 ± 0.4[c]	15.3 ± 0.3[c]
22:6n-3, DHA[d]	222.1 ± 2.5[a]	181.2 ± 10.8[b]	133.3 ± 13.1[c]	138.5 ± 14.2[c]
n-3 PUFA[f]	271.0 ± 3.7[a]	226.0 ± 10.0[b]	165.8 ± 11.6[c]	165.6 ± 8.6[c]
LC-PUFA[h]	329.3 ± 3.4[a]	284.0 ± 14.0[b]	254.3 13.3[b]	252.2 ± 7.2[b]
n-3 / n-6	1.5 ± 0.0[a]	1.2 ± 0.1[b]	0.6 ± 0.1[c]	0.8 ± 0.1[c]
LC-PUFA /SFA	1.3 ± 0.1[a]	1.1 ± 0.1[b]	1.0 ± 0.0[b]	1.0 ± 0.0[b]
LC-PUFA / MUFA	1.2 ± 0.1[a]	1.0 ± 0.1[b]	0.8 ± 0.1[c]	0.8 ± 0.0[c]
ARA / EPA	0.9 ± 0.0[b]	1.0 ± 0.1[bb]	2.9 ± 0.0[a]	2.9 ± 0.2[a]
DHA / EPA	6.1 ± 0.2[b]	5.5 ± 0.4[b]	8.6 ± 1.0[a]	9.0 ± 0.8[a]

[a] SFA: saturated fatty acids also includes: 20:0 and 22:0.
[b] MUFA: monounsaturated fatty acids also includes: 14:1n−5, 18:1n−7, 20:1n−9, 22:1n−9 and 24:1n−9.
[c] ARA; arachidonic acid.
[d] n-6 PUFA: n-6 polyunsaturated fatty acids also includes:, 20:2n−6 and 20:3n−6.
[e] EPA; eicosapentaenoic acid.
[f] DHA; docosahexaenoic acid.
[g] n-3 PUFA: n-3 polyunsaturated fatty acids also includes: 18:4n−3, 20:3n-3 and 22:5n−3.
[h] LC-PUFA: long chain polyunsaturated fatty acids includes: ARA, 20:2n−6, 20:3n−6, 20:3n-3, EPA, 22:5n−3 and DHA.

Table 7

Fatty acid composition (mg g^{-1} lipid) of embryos of *O. mykiss* in different experimental groups at the hatching day (35 days after spawning) (mean ± SEM, n = 3). A different superscript in the same row denotes statistically significant differences ($P < 0.05$).

	Diets			
	FO_{100}	FO_{50}/VO_{50}	FO_{25}/VO_{75}	VO_{100}
Fatty acids				
14:0	28.1 ± 6.5[a]	6.2 ± 1.1[b]	4.6 ± 1.4[b]	5.1 ± 0.7[b]
16:0	158.4 ± 1.5	163.0 ± 6.7	174.0 ± 0.2	168.6 ± 0.5
18:0	67.1 ± 0.5	82.4 ± 0.5	73.5 ± 4.6	78.4 ± 5.9
SFA[a]	256.4 ± 4.2	257.0 ± 4.9	255.1 ± 3.4	255.7 ± 3.5
18:1n-9	250.2 ± 6.0[b]	280.8 ± 16.5[a]	288.0 ± 8.3[a]	295.3 ± 10.5[a]
MUFA[b]	317.5 ± 8.3[b]	371.0 ± 19.3[a]	380.0 ± 8.7[a]	376.3 ± 6.3[a]
18:2n-6	118.3 ± 4.4[a]	93.6 ± 1.4[b]	93.6 ± 4.4[b]	102.8 ± 3.3[b]
20:4n-6, ARA[c]	45.0 ± 4.6[b]	56.4 ± 7.5[ab]	54.5 ± 1.6[ab]	64.4 ± 3.2[a]
n-6 PUFA[d]	216.8 ± 1.8[a]	171.0 ± 1.6[b]	157.8 ± 2.7[b]	178.6 ± 0.2[b]
18:3n-3	11.0 ± 0.5[ab]	14.4 ± 0.2[a]	10.5 ± 0.0[b]	8.8 ± 0.1[c]
20:5n-3, EPA[e]	13.4 ± 1.4[b]	94.8 ± 12.2[a]	77.6 ± 15.5[a]	99.6 ±1.0[a]
22:6n-3, DHA[f]	156.2 ± 8.0[a]	59.5 ± 5.6[b]	64.6 ± 8.2[b]	56.6 ± 0.1[b]
n-3 PUFA[g]	180.6 ± 8.9	167.8 ± 18.5	152.6 ± 7.2	166.6 ± 0.1
LC-PUFA[h]	268.1 ± 15.6	231.6 ± 27.6	214.3 ± 9.0	231.9 ± 9.0
n-3 / n-6	0.8 ± 0.0	1.0 ± 0.1	0.9 ± 0.1	0.9 ± 0.0
LC-PUFA /SFA	1.0 ± 0.1	0.9 ± 0.1	0.8 ± 0.0	0.9 ± 0.0
LC-PUFA / MUFA	0.8 ± 0.1	0.6 ± 0.1	0.6 ± 0.0	0.6 ± 0.0
ARA / EPA	3.4 ± 0.0[a]	0.6 ± 0.0[b]	0.8 ± 0.1[b]	0.6 ± 0.0[b]
DHA / EPA	11.8 ± 0.7[a]	0.6 ± 0.0[b]	1.0 ± 0.1[b]	0.6 ± 0.0[b]

[a] SFA: saturated fatty acids also includes: 20:0 and 22:0.
[b] MUFA: monounsaturated fatty acids also includes: 14:1n−5, 18:1n−7, 20:1n−9, 22:1n−9 and 24:1n−9.
[c] ARA; arachidonic acid.
[d] n-6 PUFA: n-6 polyunsaturated fatty acids also includes:, 20:2n−6 and 20:3n−6.
[e] EPA; eicosapentaenoic acid.
[f] DHA; docosahexaenoic acid.
[g] n-3 PUFA: n-3 polyunsaturated fatty acids also includes: 18:4n−3, 20:3n-3 and 22:5n−3.
[h] LC-PUFA: long chain polyunsaturated fatty acids includes: ARA, 20:2n−6, 20:3n−6, 20:3n-3, EPA, 22:5n−3 and DHA.

Table 8

Dynamics of fatty acid profile (%) of embryos of *O. mykiss* in different experimental groups during embryogenesis.

		Fatty acids*							
Diets	Sampling days	SFA	MUFA	LA	LNA	ARA	EPA	DHA	LC-PUFA
FO_{100}	20	Δ = -1.5 (=)	Δ = +3.2 (=)	Δ = +2.1 (=)	Δ = +12.2 (=)	Δ = +15.8 (↑)	Δ = +1.8 (=)	Δ = -1.5 (=)	Δ = +0.6 (=)
	30	Δ = +13.9 (↑)	Δ = +0.6 (=)	Δ = -7.7 (=)	Δ = +4.2 (=)	Δ = -2.1 (=)	Δ = -6.4 (=)	Δ = -9.2 (↓)	Δ = -10.0 (↓)
	35	Δ = +18.8 (↑)	Δ = +20.3 (↑)	Δ = +1.5 (=)	Δ = -6.8 (=)	Δ = +36.8 (↑)	Δ = -65.7 (↓)	Δ = -36.2 (↓)	Δ = -26.7 (↓)
FO_{50}/VO_{50}	20	Δ = -0.2 (=)	Δ = +0.2 (=)	Δ = -7.1 (=)	Δ = 0.0 (=)	Δ = -2.7 (=)	Δ = -13.5 (↓)	Δ = +9.9 (↑)	Δ = +3.5 (=)
	30	Δ = +26.4 (↑)	Δ = -6.8 (=)	Δ = -17.0 (↓)	Δ = -9.9 (↓)	Δ = -17.0 (↓)	Δ = +46.6 (↑)	Δ = +0.5 (=)	Δ = -2.7 (=)
	35	Δ = +23.2 (↑)	Δ = +20.8 (↑)	Δ = -33.0 (↓)	Δ = +9.0 (↑)	Δ = +38.6 (↑)	Δ = +325.1 (↑)	Δ = -67.0 (↓)	Δ = -20.6 (↓)
FO_{25}/VO_{75}	20	Δ = -0.4 (=)	Δ = +6.2 (=)	Δ = +7.6 (=)	Δ = -0.9 (=)	Δ = -3.3 (=)	Δ = -5.5 (=)	Δ = -2.2 (=)	Δ = -3.0 (=)
	30	Δ = +5.0 (=)	Δ = -7.7 (=)	Δ = +49.5 (↑)	Δ = +56.5 (↑)	Δ = +23.8 (↑)	Δ = -14.8 (↓)	Δ = -31.7 (↓)	Δ = -15.6(↓)
	35	Δ = +17.6 (↑)	Δ = +13.9 (↑)	Δ = -13.0 (↓)	Δ = -2.8 (=)	Δ = +47.7 (↑)	Δ = +324.0 (↑)	Δ = -66.9 (↓)	Δ = -28.9 (↓)
VO_{100}	20	Δ = -1.8 (=)	Δ = +11.3 (↑)	Δ = -26.7 (↓)	Δ = -15.7 (↓)	Δ = +9.9 (↑)	Δ = -4.9 (=)	Δ = +12.6 (↑)	Δ = +6.1 (=)
	30	Δ = +18.2 (↑)	Δ = +3.3 (=)	Δ = -36.6 (↓)	Δ = -28.9 (↓)	Δ = -13.4 (↓)	Δ = +7.7 (↑)	Δ = +10.2 (↑)	Δ = +0.5 (=)
	35	Δ = +20.8 (↑)	Δ = +23.5 (↑)	Δ = -44.8 (↓)	Δ = -47.0 (↓)	Δ = +24.0 (↑)	Δ = +601.4 (↑)	Δ = -55.0 (↓)	Δ = -7.6 (↓)
Two-Way ANOVA									
Diet		0.011	0.001	0.001	0.001	0.033	0.001	0.001	0.001
Time		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Diet × Time		0.001	0.001	0.001	0.001	0.01	0.001	0.001	0.180

*Abbreviations: SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; LA, linoleic acid; LNA, linolenic acid; ARA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; LC-PUFA, long chain-polyunsaturated fatty acid.

Δ is the % difference of the overall mean for each treatment used to calculate the fatty acid differences between spawning and onwards points of development. (↑), (↓) and (=) show increasing, decreasing and no significant differences, respectively, in each FA comparing with the same FA at spawning day.