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Jérôme Roy[1], Laurence Larroquet[1], Anne Surget[1], Anthony Lanuque[1], Franck Sandres[1], Frederic Terrier[1], Geneviève Corraze[1], Jetty Chung-Yung Lee[2], Sandrine Skiba-Cassy[1].

[1]INRAE, Université de Pau et des Pays de l'Adour, E2S UPPA, UMR1419 Nutrition Metabolism and Aquaculture, Aquapôle, F-64310 Saint-Pée-sur-Nivelle, France.

[2]The University of Hong Kong, School of Biological Sciences, Hong Kong Special Administrative Region.

Corresponding author:

Jérôme Roy

Phone: +33 5 59 51 59 65

Email: jerome.roy@inrae.fr

ABSTRACT

Characterization and modulation of cerebral function by *ω*-3 long chain polyunsaturated fatty acids (ω-3 LC-PUFAs), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) enrichment in plant based-diet were studied in rainbow trout (*Oncorhynchus mikyss*). We hypothesized that ω-3 LC-PUFAs are involved in the regulation of cerebral function in fish. During nine weeks, we examined the growth performance of rainbow trout for three experimental plant based-diets containing distinct levels of EPA and DHA. Using RT-qPCR, we assessed mRNA genes related to feeding behavior regulated by the central nervous system of humans, rodents and fish. These include markers of neuropeptides, indicators of cellular specification, animal stress, oxidant status, cytokines and genes regulating animal behaviour. ω-3 LC-PUFAs enrichment decreased daily food intake and induced a simultaneous mRNA expression increase in orexigenic transcript *npy* peptide and a decrease in anorexigen transcript *pomcA* peptide in the hypothalamus. Overall transcript genes related to proinflammatory cytokines, inflammation, antioxidant status, cortisol pathway, serotoninergic pathways and dopaminergic pathways were down-regulated in the juveniles fed the high *ω-3 LC-PUFAs* diet. However, the mRNA expression of transcripts related to cell specification were down regulated, namely *tmem119* markers of microglial cell in forebrain and midbrain, *gfap* markers of astrocyte in the midbrain, and *rbfox3* markers of neurons in the midbrain and hindbrain in juveniles fed high *ω*-3 experimental diet. In conclusion, this study revealed that a diet rich in *ω-3* LC-PUFAs affected a relatively high proportion of the brain function in juvenile rainbow trout through mechanisms comparable to those characterized previously in mammals.

KEYWORDS :

Rainbow trout; *ω*-3 long chain polyunsaturated fatty acid; cerebral function; feed intake

I. INTRODUCTION

All vertebrate species have absolute dietary requirements for certain ω-3 long chain polyunsaturated fatty acids (ω-3 LC-PUFAs), which are termed essential fatty acids since they cannot be synthesized de novo and are required for normal body function [1]. In farmed fish, to cope with the boom and to fit with the environmental and ecological impacts, including social and economic sustainability of the aquaculture, traditional ingredients of aquafeed [2] *i.e.* fishmeal (FM) and fish oil (FO), need to be replaced by renewable and alternative sources like terrestrial plants products. These new sustainable aquafeeds contain high levels of raw terrestrial plant materials that are totally devoid of essential ω-3 LC-PUFAs, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [3]. They are present in FO and FM while it is totally absent in feeds of terrestrial agriculture alternatives that contain mainly alpha-linolenic acid (ALA) as source of ω-3 LC-PUFAs.

To date, studies that investigated the impact of total substitution of FM and FO by alternative terrestrial products devoid of EPA and DHA focused their scope on fish performance [4], metabolism, functional genomic, flesh quality or reproductive performance [5]. However, in farmed fish, qualitative (feed type) and quantitative (consumption) feeding is essential for adequate growth, survival and reproduction.

Geurden et al. indicated that rainbow trout (Oncorhynchus mykiss) has the capacity to discriminate a standard diet formulated with fish oil from a diet containing high levels of ALA (linseed oil), linoleic acid (sunflower oil), or oleic acid (rapeseed oil) [6]. Recently, we demonstrated that DHA and EPA steered the feeding behavior of juvenile rainbow trout [7]. Under voluntary feed intake, we observed that the presence of high DHA and EPA in the diet influences the feeding behavior of rainbow trout in terms of feed preference and showed a greater consumption rate with an overall decrease in wastage of the feed.

Moreover, appetite regulation in fish is a complex process in which the central nervous system plays fundamental role to elicit change in food intake through implication of cerebral mechanisms. Thus, it is interesting to assess whether the brain system of fish, could differ from those of mammals in the ability to sense changes in dietary levels of ω-3 LC-PUFAs. With this in mind, we aimed to characterize the central molecular mechanisms regulating feeding behavior in brain areas of a marine fish species.

The choice of the rainbow trout (a carnivorous fish) as the model species for this study, have both academic and commercial reasons. More specifically, studying the physiology of feeding in rainbow trout is important for aquaculture. After twenty years of research [8], the total replacement of marine products by plant products devoid of ω-3 LC-PUFAs is not attained due to the drastic modification of growth performance and survival rate [4], that are illustrated by lower feed intake and efficiency [9], poor reproductive performance, and first spawning and offspring survival [5]. Secondly, even if it is thought that these alterations are mainly related to the absence of FM and especially FO [10], the physiological explanation is poorly understood and the absence of certain ω-3 LC-PUFAs had never been considered. Finally, for academic reason, understanding the impact of lipid nutrition in vegetal diet for carnivorous species has never been well studied in others mammals species than fish, and will lead to the emergence of new information in the modulation of central molecular mechanisms.

To achieve these objectives, we investigated during nine weeks, the impact of plant-based-diet enriched with DHA and EPA on the growth performance and feeding behavior of the juvenile rainbow trout. The study also examined the central molecular mechanisms regulating feeding behavior by determining the patterns of expression of a set of genes related to brain function and feed intake regulation in humans, rodents and fish [11].

These selected genes include neuropeptides, markers of cell specification, animal stress and oxidant status, cytokines and inflammation, and indicators of animal behavior status (anxiety, depression, reward system).

2. MATERIALS AND METHODS

2.1. Animal handling

The experiment was conducted following the Guidelines of the National Legislation on Animal Care of the French Ministry of Research (Decree No 2013-118, 1 February 2013) and in accordance with EU legal frameworks relating to the protection of animals used for scientific purposes (*i.e.* Directive 2010/63/EU). The scientists in charge of the experiments received training and personal authorization. The experiment was conducted at the experimental facilities at Donzacq (permit number A40-228.1, Landes, France) delivered by French veterinary services, and approved by the ethical committee (C2EA-73) of INRA "Comité d'éthique Aquitain poissons oiseaux" (N° agreement INRA 19869, 28 June, 2019).

2.2. Experimental diets

Diets were manufactured at INRA experimental facilities at Donzacq using a twinscrew extruder (Clextral). Pellets were produced with 3 mm diameter and 3 mm length. Composition and proximate analysis of the three diets are shown in Table 1. All diets were formulated based on the same feed ingredient composition, differing only in their oil derivation. An experimental plant-based diet which is completely devoid of FM and FO was used as a reference diet. In order to avoid exceeding anti-nutrient threshold levels, we used a blend of wheat gluten, extruded peas and whole wheat, corn gluten meal, rapeseed meal and white lupin as protein sources (approximately 41.74% of total diet). Synthetic L-lysine, L-methionine, dicalciumphosphate and soy-lecithin were added to all diets to correct the deficiency in essential amino acids, phosphorous and phospholipids. Mineral and vitamin premix were added to each diet. Diets were isoenergetic (*c.* 24.5 kJg^{-1} of dry diet) and were formulated to cover the nutritional requirements of the rainbow trout [12]. In order to maintain a constant ratio between groups of fatty acids (saturated, monounsaturated, *ω-3 LC-*

PUFAs, ω-6 LC-PUFAs and *ω-9 LC-PUFAs*), the three experimental diets differed mainly by the level of DHA and EPA to the benefit of alpha-linolenic acid (ALA), the *ω-3 LC-PUFAs* precursor of these two fatty acids. To do this, the dietary content of EPA and DHA was increased by adding Omegavie® marine oils, DHA and EPA (Polaris functional lipids, Quimper, France). The three experimental diets contained 23.7% crude lipids (± 0.05% of total diet) with 0.0% of *ω*-3 DHA/EPA (% of total fatty acids) for low, 15.7% for medium (7.6% of EPA and 8.1% of DHA) and 33.5% for high *ω*-3 diets (15.8% of EPA and 17.7% of DHA). Fatty acid composition of the diets are shown in Table 2.

Nutrient compositions of the diets were analyzed by drying the samples to constant weight at 105°C for 24 h. Crude protein was determined by the Kjeldahl method after acid digestion and the concentration was estimated by multiplying the nitrogen content using the 6.25 factor. Crude lipids were quantified by Soxhlet method using petroleum diethyl ether for the extraction. Gross energy was determined in an adiabatic bomb calorimeter (IKA, Heitersheim Gribheimer, Germany). Starch content was evaluated by an enzymatic method (Megazyme). Ash content was determined by combustion in muffle furnace (550°C for 8 h). Total lipid was extracted and measured gravimetrically according to the Folch method [13] using dichloromethane instead of chloroform. Fatty acid methyl esters were prepared by acid-catalyzed transmethylation of total lipids using boron trifluoride (BF_3) in methanol (14%) according to Shantha and Ackman method [14] and analyzed in a Varian 3900 gas chromatograph (Varian, les Ulis, France).

2.3. Fish and experimental design

Female rainbow trout used in this experiment originated from the same parental stock (INRA Fish Farm of Lees-Athas, Permit number A64.104.1, vallée d'Aspe, France). Throughout the experiment, rainbow trout were reared at 18ºC in the INRA experimental

facilities at Donzacq, France, under natural photoperiod. Juveniles trout (60.70 ± 0.4 g) (Table 3) were randomly distributed into 9 tanks (25 fish/tank). The fish were reared in triplicates and fed low, medium or high *ω-3 LC-PUFAs* diet. All fish were fed by hand twice a day with an interval of 8 hours, to the point of apparent satiation. Dietary treatments were applied during 9 weeks. Throughout the trial, dead fish (if any) were removed daily and weighed. During the trial, water dissolved oxygen was 9 mg L^{-1}, ammonia < 0.01 mg L^{-1}, nitrite < 0.04 mg L^{-1} and nitrate approximately 17 ppm. Water flow was $0.3s^{-1}$ per tank (130 L volume) that renews 6 times per hour.

At the end of 9 weeks of feeding, the fish were first anaesthetized in 30 mg L^{-1} benzocaine bath and then sacrificed in 60 mg l^{-1} benzocaine bath for 6 h after the last meal. Six brain tissues per condition (two fish per tank) were dissected into five areas (forebrain, midbrain, hindbrain, hypothalamus, and cerebellum), immediately frozen in liquid nitrogen, and stored at −80°C for further analysis.

2.4. Body weight gain and feed efficiency

Total biomass was recorded at the beginning and at the end of the feeding trial. Variables related to growth were initial (IBW) and final body weight (FBW), body weight gain (BWG = FBW - IBW), and feed efficiency (FBW/food intake, FI). Variables related to FI were expressed in relative terms (% of body weight, BW per day). Variables related to zootechnic parameters are presented in Figure 1.

2.5. Gene expression measurement by real-time quantitative PCR

Total RNA was extracted from the brain divided in five areas (forebrain, midbrain, hindbrain, hypothalamus, and cerebellum) using the TRIzol reagent method (Invitrogen, Carlsbad, CA) with Precellys 24 (Bertin technologies, Montigny le Bretonneux, France)

following Trizol manufacturer's instructions. Total RNA (2 µg) was used for cDNA synthesis. The Super-Script III RNAse H-Reverse transcriptase kit (Invitrogen) was used with random primers (Promega, Charbonniéres, France) to synthesize cDNA. High throughput real-time quantitative PCR (RT-qPCR) was performed using the Biomark microfluidic system from Fluidigm (Fluidigm, USA) in which each gene combination was quantified using a 96.96 Dynamic Array™ IFCs (BMK-M-96.96, Fluidigm). Pre-amplification of the samples, chip loading and RT-qPCR were performed according to manufacturer's protocol. The results were analyzed using the Fluidigm PCR analysis software v.4.1.3. Firstly, 6.5 ng of each cDNA were initially pre-amplified (10 min 95°C activation and 14 PCR cycles at 15 sec 95°C for 4 min, 60°C) with PreAmp Master Mix (100-5581, Fluidigm) with a pool containing all the primers (200 nM), excluding the 16S rRNA primer sets. Pre-amplified samples were diluted to 1/5 after an exonuclease treatment (M02935, NEB, Biolabs USA). In order to prepare samples for loading into the IFC, a mix was prepared consisting of 440 µL 2X TaqMan Master Mix (Applied Biosystem, 4369016, USA), 44 µL 20× DNA Binding Dye Sample Loading Reagent (100-7609, Fluidigm), 44 µl 20X EvaGreen dye (31000, Biotium USA) plus 132 µL TE buffer. A volume of 6 µL of the mix was dispensed to each well of a 96-well assay plate. Two microliters of pre-amplified and diluted cDNA sample was added to each well and the plate was briefly vortexed and centrifuged.

For the assays, 5 µL of each Assay (5 µM each primer in primer-mix (2X assay loading reagent, 100-7611, Fluidigm) with Tris EDTA) were dispensed to each Detector Inlet of the 96.96 IFC. Following priming of the IFC in the IFC Controller HX, 5 µL of the cDNA sample + reagent mix and 5 µL of Assay were dispensed to each sample inlet of the 96.96 IFC. After loading the assays and samples into the IFC, it was transferred to the BioMark, and PCR was performed using the following thermal protocol: thermal mix of 50 °C, 2 min; 70 °C, 30 min; 25°C, 10 min; hot start at 50 °C, 2 min; 95°C, 10 min PCR Cycle of 35 cycles of (95 °C,

15 s; 60 °C, 60 s), and melting analysis (60°C, 30s; 95°C, 1°C/3s). Results were analyzed using the Fluidigm real-time PCR analysis software v.4.1.3. Transcripts were normalized using Elongation Factor 1α gene (ef1α) as reference gene transcript. Primer sequences used to amplify all paralogs genes and accession numbers of the primers are presented in Table 3.

2.6. Statistical analysis

Comparisons among groups were carried out using a one-way ANOVA followed by a student-Newman-Keul's test. All data are expressed as mean ± S.E. treatment (diet) effects and interactions were considered statistically significant at $P<0.05$. Statistical analyses were performed using R software (v3.5.2)/R Commander package. Analyses were carried out on untransformed data since criteria for normality and homogeneity of variances were fulfilled (Shapiro-Wilk's and Levene's test, respectively).

3. RESULTS

Growth performance

Fish growth performance is presented in Figure 1. At the end of the nine weeks, the mean body weight tripled for all diet groups. This represented a daily growth coefficient of 2.25 ± 0.05 % (for all groups) of their initial body weight (data not shown). The values for body weight gain (Figure 1A) and final body weight (Figure 1B) were unaffected by the three diets. Feed efficiency was not affected between the three diets (Figure 1C) while daily feed intake (Figure 1D) was lower for high ω-3 diet compared to low ω-3 diet (1.31% BW/day for low ω-3 diet *vs*. 1.25% BW/day for high ω-3 diet).

Changes in mRNA gene transcript expressions related to feed intake

Changes in the mRNA expression of neuropeptides genes related to homeostatic control of food intake are presented in Figure 2. In the hypothalamus, the expression of orexigen *npy* peptide differed between the paralogs genes. No differences were observed between the three experimental diets for *npyA,* whereas expression of *npyB* significantly increased in the high ω-3 diet compared to medium and low ω-3 diets (P=0.036). For the orexigenic peptide *agrp*, no difference expression was found for the two paralogs *agrp1* and *agrp2* between the diets. The expression of anorexigenic *pomc* peptide differed also between paralogs genes. No difference was observed between the three diets for *pomcB,* while expression *pomcA* was reduced in high ω-3 diet compared to low ω-3 diet (P=0.029). For the expression anorexigenic peptide *cartpt*, no difference was observed between the three diets.

Changes in mRNA gene transcript expressions related to cytokines and inflammation markers

Changes in the mRNA expression of transcript genes related to cytokines and inflammation markers are shown in Table 4. It appears that most of the pro-inflammatory markers were inhibited by medium and high ω-3 diets, specifically in the forebrain and midbrain. In forebrain, the expression of pro-inflammatory cytokines *IL-1β1* and *IL-1β2*, *IL-6,*

IL-8, IL-11 and *tnf-α* (for the three paralogs) decreased in the high *ω*-3 diet, and *IL-1β1, IL-1β3, IL-6, IL-8, IL-11* and *tnf-α1* in the medium *ω*-3 diet compared to low *ω*-3 diet. However, anti-inflammatory cytokines *IL-4* and *IL-10* expression decreased in the medium and high *ω*-3 diets compared to low *ω*-3 diet where no change was found for *IL-10.* Interestingly, *tnf-α1* was reduced by medium and high *ω*-3 diets compared to low *ω*-3 diets *ω*-3 diets while *tnf-α2 and tnf-α3* were reduced by high *ω*-3 diets compared to low *ω*-3 diet. No alterations were found for *nfkpb1, ikk-α* and *ikk-β* between all three diets. The significance of diet interaction was further confirmed for *IL-1β, IL-1β3, IL8, tnf-α1 and tnf-α2* expressions in the forebrain of the trout.

In the midbrain, the mRNA expression of pro-inflammatory cytokines *IL-1β* (for the three paralogs)*, IL-11* and *tnf-α* (for the paralogs *tnf-α1* and *tnf-α2*) decreased in the high *ω*-3 diet and in the medium *ω*-3 diets for *IL-1β2, IL-1β3, IL-11, tnf-α1 and tnf-α2* compared to low *ω*-3 diet. Expression of *IL-6* and *IL-8* was reduced by medium *ω*-3 diet compared to low *ω*-3 diet. No change was found for *tnf-α3* expression by the diets. About anti-inflammatory cytokines *IL-4* and *IL-10,* the expression decreased in the medium *ω*-3 diet compared to low *ω*-3 diet while no change was observed for high *ω*-3 diet. Both high and medium *ω*-3 diets reduced *nfkpb1, ikk-α* and *ikk-β* compared to low *ω*-3 diets. The significance of diet interaction was further confirmed for *tnf-α1 ikk-α* and *ikk-β* expressions in the midbrain of the trout.

In the hypothalamus, only one significant change occurred, with a decrease in expression of *tnf-α1* in the high *ω*-3 diet compared to low and medium *ω*-3 diet. This modification was confirmed by a significant diet interaction. Inflammatory gene expressions were not modified by all diets in the hindbrain and cerebellum of the trout brain.

Changes in mRNA gene transcript expressions related to cell type markers

Changes in the mRNA expression of transcript genes related to cells markers are shown in Figure 3. The mRNA expression of *gfap,* the astrocyte marker, decreased in the midbrain for the high ω-3 diet compared to low ω-3 diet. No significant changes were found in other brain regions assessed (forebrain, hindbrain, hypothalamus and cerebellum).

The mRNA expression of *tmem119,* the marker of microglial cell, decreased in the forebrain and midbrain by the high ω-3 diet, and also by medium ω-3 diet in the midbrain compared to low ω-3 diet. No significant change was observed in the hindbrain, hypothalamus and cerebellum by the diets.

The expression of *rbfox3,* the marker of neurons, decreased in the midbrain by the medium and high ω-3 diets compared to low ω-3 diet, and in the hindbrain by the high ω-3 diet compared to low ω-3 diet only. No significant change occurred in forebrain, hypothalamus and cerebellum by the diets.

The expression of *pdgfra-1*, the marker of oligodendrocyte was not significantly changed by the diets in the five brain regions assessed.

Changes in mRNA gene transcript expressions related to antioxidant status

Changes in the mRNA expression of transcript genes related to antioxidant are shown in Table 5. It appears SOD transcriptions were affected by the diets in the forebrain and midbrain. Expression of *sod-3* decreased by medium ω-3 diet compared to low ω-3 diet only in the forebrain. However, there was no significance in the diet interaction. Other expressions including *sod-1, sod-2, cat* and *gsr* were not affected by the diets.

A significant reduction in *sod-1* and *sod-2* was observed in the midbrain by high ω-3 diet compared to low ω-3 diet only. These alterations were confirmed by a statistical significance in diet interaction. However, expressions of *sod-3, cat* and *gsr* were not affected by the diet.

Surprisingly, the diets did not significantly change the antioxidant genes in the forebrain, hindbrain, hypothalamus and cerebellum of the trout.

Changes in mRNA gene transcript expressions related to cortisol pathway

Changes in the mRNA expression of transcript genes related to cortisol pathway are shown in Table 6. In the forebrain, *crhr* the transcript gene of cortisol receptor and *mrap-1* one the transcript gene of the two-receptor accessory protein of melanocortin 2 receptor decreased by the the medium and high *ω*-3 diets compared to low *ω*-3 diet. A statistical significance in diet interaction further confirmed the change. Expressions of *crd, mc2r and mrap-2* were not altered by the diets in the forebrain.

In midbrain, the expression of *crf* gene (corticotropin-releasing factor) decreased by the high *ω*-3 diet compared to low *ω*-3 diet only and decreased for *crhr* gene in the medium *ω*-3 diet compared to low *ω*-3 diet only. Both medium and high *ω*-3 diets reduced *mrap-2* expression compared to low *ω*-3 diet. The modification of *crf* and *mrap-2* were confirmed by statistical significance in dietary interaction. Expressions of *crhr, mc2r* and *mrap-1* were not affected by the diets in the midbrain.

In cerebellum, the expression of *crhr* decreased by the high *ω*-3 diet compared to low *ω*-3 diet only. However, the dietary interaction was not significant. The diets did not significantly *change crf, crhr, mc2r, mrap-1 and mrap*-2 expressions in the hindbrain and hypothalamus of the trout.

Changes in mRNA gene transcript expressions related expression to monoamines pathways

Changes in the mRNA expression of transcript genes related to serotonin pathway are shown in Table 7. The expression of *5ht1A-b,* the transcript gene of serotoninergic receptor, and *drd1,* one of the dopaminergic receptor in the forebrain, were reduced by the high *ω*-3

diet compared to low ω-3 diet only. No significant diet interaction was observed. The expression of *tph-1a* (precursor isoenzyme of serotonin) decreased in the high ω-3 diet compared to low and medium ω-3 diets. Moreover, a statistical significance was found in the diet interaction. Expressions of *5ht1A-a, tph-2, sert* (serotonin carrier), *th* (tyrosine hydroxylase enzyme), *drd2* and *dat* (dopamine carrier) were not altered by all the diets in the forebrain.

Dietary interaction was significant in the midbrain of the trout for *5ht1A-a*, *5ht1A-b, tph-2, drd1* and *drd2*. High ω-3 diet decreased *5ht1A-a* while both medium and high ω-3 diets decreased *5ht1A-b* expressions compared to low ω-3 diet. Expression of *tph-2, drd2* and *dat* was reduced by high ω-3 diet compared to low ω-3 diet only whereas *drd1* was decreased by both medium and high ω-3 diets compared to low ω-3 diet. Expressions of *tph-1a, sert, th* and *dat* were not affected by the diets. However, expressions of *dat* reduced in the hindbrain and *drd1* in the cerebellum by high ω-3 diet compared to low ω-3 diet but no significant interaction in the diet was found. Expressions of other makers were not affected by the diets in the hindbrain and cerebellum while none of the diets altered any of the markers in the hypothalamus.

4. DISCUSSION

In this present study, we revealed that dietary *ω*-3 long chain polyunsaturated fatty acids DHA and EPA affected a large part of the brain functions in juvenile trout. In farmed fish, ω-3 LC-PUFAs are known to be essential for fish life cycle, to promote optimal growth and survival [15], health [12], reproduction [16], egg quality and offspring development [17], and are needed to regulate resistance to different stress response [18] and control the vigilance [19]. Moreover, in rainbow trout (*Oncorhynchus mykiss*), a carnivorous species, the concomitant replacement of dietary FM and FO with alternative ingredients, *i.e.* those containing no ω-3 LC-PUFAs like plant based-diet, leads to the reduction of fish growth [4] and a significant lower survival of offspring [5]. Notably, most of the previous studies seem to indicate a reduction in feed intake [20]. As a result, in spite of 20 years of research, the total replacement of marine products in commercial diet by complete plant products has still not been achieved.

In this study, we hypothesized that the abundance of ω-3 LC-PUFAs in FO and FM could strongly influence the integrity and function of the fish brain, particularly the part controlling feed intake. Thus, by suppressing the dietary ω-3 LC-PUFAs, the mechanisms of feed intake regulation could be severely disrupted and impair growth performances. Recently, we demonstrated that ω-3 LC-PUFAs DHA and EPA steered the feeding behavior of juvenile rainbow trout [7]. Under voluntary feed intake, we observed that the presence of high DHA and EPA in the diet influences the feeding behavior of rainbow trout in terms of feed preference, with a greater consumption rate and an overall decrease in wastage for diet containing high levels of EPA and DHA. Moreover, at early stage (larvae), inadequate levels of dietary ω-3 LC-PUFAs resulted in reduced swimming activities, altered feeding rhythms illustrated by delayed response to a visual stimulus [21], behavioral development (schooling),

and increased abnormal behavior (longer escape latency and defect in spatial retention) [22]. Altogether, understanding the impact of dietary ω-3 LC-PUFAs on feeding behavior related to physiological and molecular mechanisms in regulating feed intake of farmed fish will provide extensive information about the perception of the ingredients by the fish especially during the life cycle including feed conversion efficiency and nutrient losses, which are key elements in fish farming. Theses knowledges obtained will provide information for the use of alternative ingredients, to sustain and promote aquaculture while improving fish growth, survival rate, reproduction and health performance.

Here, we observed that the daily feed intake was lower in juvenile trout fed high *ω*-3 diet than in trout fed diet devoid of EPA and DHA. This result was in opposite to our previous finding [7] which revealed that juvenile trout preferred diet containing higher level of *ω*-3 LC-PUFAs diet. However, this difference may be due to the experimental approaches implemented in these two studies. In fact, the preference for high EPA and DHA diet was determined in a situation of voluntary feed intake (self-feeding system) where the trout had a choice between two diets, whereas in the present study, they were fed by hand a single diet until visual satiety. Nevertheless, when evaluating the expression of transcript genes related to feed intake mechanisms, our results displayed an inverse correlation between feed intake and neuropeptide expressions. Indeed, in the hypothalamus, which is the main area involved in homeostasis control of food intake [23], and knowing the existence of fatty acid sensing system in the fish [24,25], the expression of orexigenic neuropeptide, *npy* (paralog *npyB*), increased in fish fed with high *ω*-3 diet, whereas the anorexigenic neuropeptide, *pomc* (paralog *pomcA*), decreased. Such observation was not found in the fish with decreased feed intake. If the fish decreased their feed intake, the expression of orexigen neuropeptide could potentially decrease in their hypothalamus while anorexigen neuropeptide expression could increase, as reported in others studies in farmed fish model [24]. Compared to our previous

study [7], the fish were fed by hand twice a day with an interval of 8 hours until apparent satiety. However, it is acknowledged that this method is not perfect for feed intake evaluation since, the fish do not have the choice to consume the feed freely. Also, compared to the observation obtained in voluntary feed intake using self-feeder approaches (with two feeding periods per day for 2.5 hours) [7], the fish in this study had limited feed to eat due to shorter feeding period (less than five minutes). Further, it is possible that *ω*-3 diet improved satiation in short feeding period accompanied by faster hunger pangs as revealed by the neuropeptide expression in the hypothalamus, 6 hours after the last meal.

Other studies also indicated *ω-3 LC-PUFAs* altered feed intake behavior by *ω-3 LC-PUFAs* diet in farmed fish. Conde-Siera et al., revealed that Senegalese sole (*Solea senegalensis*) treated with *ω-3 LC-PUFAs* (intraperitoneal injection) tended to have a lower food intake compared with the control group (saline solution) [24]. Although the animal model differs, the decrease of food intake by high *ω*-3 diet in short feeding period in this study is consistent with reports in rodents where ω-3 LC-PUFAs, including DHA and EPA, but not saturated fatty acids, inhibited food intake upon acute intravenous administration [26,27]. Some explanations could be attributed by ω-3 LC-PUFAs to inhibit feeding by targeting fatty acids receptor in the brain regions and the lingual system, that are important for controlling food intake. Indeed, GPR120, a fatty acids receptor, mainly activated by LC-PUFAs, seemed to be present in NPY expressing neurons in mouse model of diet-induced obesity [27]. In mice, this receptor is suggested to be activated preferentially by ω-3 LC-PUFAs (DHA and EPA) and exert anti-inflammatory, and anxiolytic and anti-depressive behaviors, which could explain the anorectic effect [28] in this study.

Inflammatory pathway are known to be affected by ω-3 LC-PUFAs related to strong anti-inflammatory properties [29,30]. In this study, trout fed with medium or high *ω*-3 LC-

PUFAs diets had lower gene expressions related to cytokines and inflammation in particular the forebrain and midbrain.

Our findings are also consistent with previous findings for the role of *ω*-3 LC-PUFAs in neuroinflammation. In humans, high dietary *ω*-3 LC-PUFAs intakes were associated with a lower risk of neurological disorders that have an inflammatory component, including Alzheimer's disease, Parkinson's disease and depression [31]. These observations has led to the hypothesis that ω-3 LC-PUFAs are potent anti-inflammatory compounds in the brain [30]. In addition, *ω*-3 LC-PUFAs supplementation improved behavior and neurophysiological response caused by neuroinflammation, which could perturbed the feeding behavior. Short-term exposure to dietary *ω*-3 LC-PUFAs reduced spatial memory deficits and anxiolytic behavior induced by intravenous *IL-1β* administration in rats [32]. Furthermore, ageing-associated microglia activation, and the associated production of *IL-1β* and alterations in hippocampus areas (equivalent to forebrain in fish), could be attenuated by dietary *ω*-3 LC-PUFAs supplementation in rats [33,34]. Moreover, dietary *ω*-3 LC-PUFAs supplementation attenuated pro-inflammatory cytokines expression and astrogliosis and restored behavioral spatial memory deficits in the hippocampus areas of mice [35].

Additionally, DHA supplementation blocked macrophage- and microglia-induced activation of *nfkpb* in the brain of rodents with neuroinflammation [36,37]. Low dietary consumption of *ω*-3 LC-PUFAs led to microglia activation and the production of pro-inflammatory cytokines in the hippocampus of mice at weaning [38]. All these studies supports our finding in this study for the diminution of mRNA expression gene transcripts related to cells markers of microglial but also astrocyte cells for fish fed with high *ω*-3 diet. These results confirmed that *ω*-3 LC-PUFAs exerted anti-inflammatory properties in fish associated with reduced microglia and astrocyte production. In parallel, fish fed with high *ω*-3 diet showed reduction of transcripts related to antioxidant enzyme genes, in the midbrain of

the trout. Some studies also suggested that low brain ω-3 LC-PUFAs levels impaired behavior and increased pathology in a mouse model of Alzheimer's disease by increasing oxidative stress [39]. Although our study model is not diseased or under oxidative stress, it is anticipated the lessening of inflammation and reduced antioxidant enzyme genes by the ω-3 diet indeed benefitted the fish in maintaining a healthy eating, growth and behavior during the farming period.

In this study, metabolic homeostasis and adaptation to stress by the trout was investigated by determining transcript genes related to cortisol pathway. It is long known that abnormal regulation of the cortisol in fish affects growth and reproduction [40]. The neuroendocrine hypothalamus–pituitary–adrenal (HPA) axis mediates the endocrine component of the stress response and is controlled, in large part by *crhr* (cortisol receptor). It also mediates other behavioral and autonomic responses to stress via its expression and signaling, in many other regions of the brain. High ω-3 LC-PUFAs diet decreased the corticotropin-releasing factor (*crf*) in midbrain and cerebellum, *crhr* in forebrain and midbrain, and melanocortin 2 receptor one in forebrain and one in midbrain of the trout. Similar findings were made in mammals. In rats subjected to chronic low dietary ω-3 LC-PUFAs, an exaggerated axis response to acute stress [41] was observed, whereas ω-3 LC-PUFAs supplementation have prevented chronic stress-induced increase in plasma corticosterone levels [42,43]. Psychogenic stressors are processed in the forebrain of the fish and respond to potential environmental or physiological stress. It is an adaptive mechanism in acute stress for the fish to strive for homeostasis while chronic stress can be poorly responded, create anxiety and cognitive impairment, and subsequent behavioral change [44]. Altogether, high ω-3 LC-PUFAs diet in the control of cortisol pathway suggests that fish to adapt to the diet and feeding regime during the experimental period.

We also observed changes in the expression of gene transcripts of monoamines related to serotonin and dopaminergic neurotransmitters pathways which were mainly reduced by high ω-3 LC-PUFAs diet. Monoamine neurotransmitters have been shown to affect feeding in both mammals and fish. In fish, tyrosine forms dopamine, under the action of tyrosine hydroxylase. It takes control in physical movements, learning and eating behaviors [45]. Additionally, serotonin supply in the forebrain area regulates memory and learning processes. The reduced expressions of certain serotonin precursors and dopamine carrier genes in the forebrain and midbrain may be for homeostatic and adaptation control, and is postulated to be attributed to the behavioral and autonomic responses in the cortisol pathways leading to reduced feed intake by the trout. Our results are consistent with previous studies, which observed that long-term dietary ω-3 LC-PUFAs deprivation also impaired brain monoamine pathways in rats [46] and piglets [47], with increased basal synaptic 5-hydroxytryptamine levels and decreased stimulated serotonin release; this effect was reversed by early ω-3 LC-PUFAs supplementation [48]. In addition, tyrosine hydroxylase expression was increased in the brain of adolescent rats that were chronically subjected to a low ω-3 LC-PUFAs supplementation, whereas it was decreased in the brains of adults rodents receiving dietary ω-3 PUFAs supplementation [49].

Consistent with our results, researchers observed increased serotonergic activity and expression of POMC and decreased the expression of NPY [50,51]. Particularly, the activation of hypothalamic serotonin receptors (theoretically located in POMC neurons) had an inhibitory effect on food intake in rainbow trout [51–53]. On the other side, central administration of dopamine induced no effect on POMC and NPY gene expression [54] but decreased transcript of AgRP. In the same way, oral treatments of dopamine precursor induced an increase of NPY expression, and of cortisol pathways (CRF transcript) in sea bass hypothalamus whereas feed intake was inhibited by this treatment [55]. Other study

confirmed that monoamines pathways were implicated in feeding and/or searching behavior regulation [45].

In conclusion, we have identified for the first time in farmed fish, a mechanism linking ω-3 LC-PUFAs (DHA and EPA) consumption to rapid but transient anorectic response characterized by reduced feed intake that consequently affected a relatively high proportion of the brain function in juveniles trout. This central regulation of farmed fish by ω-3 LC-PUFAs diet revealed to be close to mammals species with modulation of central molecular mechanisms including inflammatory and oxidant status, cortisol pathways and genes regulating animal behavior. These knowledges obtained are important in formulating feed ingredients for aquaculture to sustain and promote growth and development, survival rate, reproduction and health performance of the fish.

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DISCLOSURES

The authors declare that there are no conflicts of interests.

CREDIT AUTHOR STATEMENT

J.R.: Conceptualization, Methodology, Software, Data curation, Writing- Original draft preparation. **L.L.:** data curation, analysed lipids content. **A.S.:** data curation, analysed diets composition. **A.L., F.S., F.T.:** Data curation, conducted the study at the experimental facilities. **G.C., J.CYL., S.SC.:** Visualization, Investigation, Writing- Reviewing and Editing

REFERENCES

[1] P.C. Calder, Very long-chain n-3 fatty acids and human health: fact, fiction and the future, Proc. Nutr. Soc. 77 (2018) 52–72. https://doi.org/10.1017/S0029665117003950.
[2] R.L. Naylor, R.W. Hardy, D.P. Bureau, A. Chiu, M. Elliott, A.P. Farrell, I. Forster, D.M. Gatlin, R.J. Goldburg, K. Hua, P.D. Nichols, Feeding aquaculture in an era of finite resources, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 15103–15110. https://doi.org/10.1073/pnas.0905235106.
[3] M.J. Leaver, L.A. Villeneuve, A. Obach, L. Jensen, J.E. Bron, D.R. Tocher, J.B. Taggart, Functional genomics reveals increases in cholesterol biosynthetic genes and highly unsaturated fatty acid biosynthesis after dietary substitution of fish oil with vegetable oils in Atlantic salmon (Salmo salar), BMC Genomics. 9 (2008) 299. https://doi.org/10.1186/1471-2164-9-299.
[4] V. Lazzarotto, F. Médale, L. Larroquet, G. Corraze, Long-term dietary replacement of fishmeal and fish oil in diets for rainbow trout (Oncorhynchus mykiss): Effects on growth, whole body fatty acids and intestinal and hepatic gene expression, PloS One. 13 (2018) e0190730. https://doi.org/10.1371/journal.pone.0190730.
[5] V. Lazzarotto, G. Corraze, A. Leprevost, E. Quillet, M. Dupont-Nivet, F. Médale, Three-year breeding cycle of rainbow trout (Oncorhynchus mykiss) fed a plant-based diet, totally free of marine resources: consequences for reproduction, fatty acid composition and progeny survival, PloS One. 10 (2015) e0117609. https://doi.org/10.1371/journal.pone.0117609.
[6] I. Geurden, A. Cuvier, E. Gondouin, R.E. Olsen, K. Ruohonen, S. Kaushik, T. Boujard, Rainbow trout can discriminate between feeds with different oil sources, Physiol. Behav. 85 (2005) 107–114. https://doi.org/10.1016/j.physbeh.2005.03.010.
[7] J. Roy, Y. Mercier, L. Tonnet, C. Burel, A. Lanuque, A. Surget, L. Larroquet, G. Corraze, F. Terrier, S. Panserat, S. Skiba, Rainbow trout prefer diets rich in omega-3 long chain polyunsaturated fatty acids DHA and EPA, Physiol. Behav. 213 (2020) 112692. https://doi.org/10.1016/j.physbeh.2019.112692.
[8] Expanding the utilization of sustainable plant products in aquafeeds: a review Delbert M Gatlin III Frederic T Barrows Paul Brown Konrad Dabrowski T Gibson Gaylord Ronald W Hardy Eliot Herman Gongshe Hu Åshild Krogdahl Richard Nelson Kenneth Overturf Michael Rust Wendy Sealey Denise Skonberg Edward J Souza David Stone Rich Wilson Eve Wurtele https://doi.org/10.1111/j.1365-2109.2007.01704.x, (n.d.).
[9] B.E. Torstensen, M. Espe, M. Sanden, I. Stubhaug, R. Waagbø, G.-I. Hemre, R. Fontanillas, U. Nordgarden, E.M. Hevrøy, P. Olsvik, M.H.G. Berntssen, Novel production of Atlantic salmon (Salmo salar) protein based on combined replacement of fish meal and fish oil with plant meal and vegetable oil blends, Aquaculture. 285 (2008) 193–200. https://doi.org/10.1016/j.aquaculture.2008.08.025.

[10] S. Panserat, C. Kolditz, N. Richard, E. Plagnes-Juan, F. Piumi, D. Esquerré, F. Médale, G. Corraze, S. Kaushik, Hepatic gene expression profiles in juvenile rainbow trout (Oncorhynchus mykiss) fed fishmeal or fish oil-free diets, Br. J. Nutr. 100 (2008) 953–967. https://doi.org/10.1017/S0007114508981411.
[11] L. Burri, N. Hoem, S. Banni, K. Berge, Marine omega-3 phospholipids: metabolism and biological activities, Int. J. Mol. Sci. 13 (2012) 15401–15419. https://doi.org/10.3390/ijms131115401.
[12] The National Research Council [NRC] (2011). Nutrient Requirements of Fish and Shrimp. Washington, DC: The National Academies Press. doi: 10.1146/annurev. nutr.17.1.405, (n.d.).
[13] M. J.Floch, G.H.S. Stanley, A simple method for the isolation and purification of total lipids from animal tissues, J. Biol. Chem. 226 (1957) 497–509 (n.d.)., (n.d.).
[14] N.C. Shantha, R.G. Ackman, Nervonic acid versus tricosanoic acid as internal standards in quantitative gas chromatographic analyses of fish oil longer-chain n-3 polyunsaturated fatty acid methyl esters, J. Chromatogr. 533 (1990) 1–10.
[15] T. Watanabe, M.S. Izquierdo, T. Takeuchi, S. Satoh, C. Kitajima, Comparison between eicosapentaenoic and docosahexaenoic acids in terms of essential fatty acid efficacy in larval red seabream., NIPPON SUISAN GAKKAISHI. 55 (1989) 1635–1640. https://doi.org/10.2331/suisan.55.1635.
[16] M.S. Izquierdo, H. Fernández-Palacios, A.G.J. Tacon, Effect of broodstock nutrition on reproductive performance of fish, Aquaculture. 197 (2001) 25–42. https://doi.org/10.1016/S0044-8486(01)00581-6.
[17] Salze G, Tocher DR, Roy WJ, Robertson DA (2005) Egg quality determinants in cod (Gadus morhua L.): egg performance and lipids in eggs from farmed and wild broodstock. Aquaculture Research 36: 1488–1499., (n.d.).
[18] R. Ganga, L. Tort, L. Acerete, D. Montero, M.S. Izquierdo, Modulation of ACTH-induced cortisol release by polyunsaturated fatty acids in interrenal cells from gilthead seabream, Sparus aurata, J. Endocrinol. 190 (2006) 39–45. https://doi.org/10.1677/joe.1.06770.
[19] T. Benítez-Santana, E. Atalah, M.B. Betancor, M.J. Caballero, C.M. Hernández-Cruz, M. Izquierdo, DHA but not EPA, enhances sound induced escape behavior and Mauthner cells activity in Sparus aurata, Physiol. Behav. 124 (2014) 65–71. https://doi.org/10.1016/j.physbeh.2013.10.021.
[20] B.E. Torstensen, M. Espe, I. Stubhaug, Ø. Lie, Dietary plant proteins and vegetable oil blends increase adiposity and plasma lipids in Atlantic salmon (Salmo salar L.), Br. J. Nutr. 106 (2011) 633–647. https://doi.org/10.1017/S0007114511000729.
[21] M.S. Izquierdo, Essential fatty acid requirements of cultured marine fish larvae, Aquac. Nutr. 2 (1996) 183–191. https://doi.org/10.1111/j.1365-2095.1996.tb00058.x.
[22] S.-Y. Lim, J. Hoshiba, N. Salem, An extraordinary degree of structural specificity is required in neural phospholipids for optimal brain function: n-6 docosapentaenoic acid substitution for docosahexaenoic acid leads to a loss in spatial task performance, J. Neurochem. 95 (2005) 848–857. https://doi.org/10.1111/j.1471-4159.2005.03427.x.
[23] J.L. Soengas, J.M. Cerdá-Reverter, M.J. Delgado, Central regulation of food intake in fish: an evolutionary perspective, J. Mol. Endocrinol. 60 (2018) R171–R199. https://doi.org/10.1530/JME-17-0320.
[24] M. Conde-Sieira, K. Bonacic, C. Velasco, L.M.P. Valente, S. Morais, J.L. Soengas, Hypothalamic fatty acid sensing in Senegalese sole (Solea senegalensis): response to long-chain saturated, monounsaturated, and polyunsaturated (n-3) fatty acids, Am. J. Physiol. Regul. Integr. Comp. Physiol. 309 (2015) R1521-1531. https://doi.org/10.1152/ajpregu.00386.2015.

[25] J.L. Soengas, Contribution of glucose- and fatty acid sensing systems to the regulation of food intake in fish. A review, Gen. Comp. Endocrinol. 205 (2014) 36–48. https://doi.org/10.1016/j.ygcen.2014.01.015.
[26] D.R. Schwinkendorf, N.G. Tsatsos, B.A. Gosnell, D.G. Mashek, Effects of central administration of distinct fatty acids on hypothalamic neuropeptide expression and energy metabolism, Int. J. Obes. 2005. 35 (2011) 336–344. https://doi.org/10.1038/ijo.2010.159.
[27] D.E. Cintra, E.R. Ropelle, J.C. Moraes, J.R. Pauli, J. Morari, C.T. de Souza, R. Grimaldi, M. Stahl, J.B. Carvalheira, M.J. Saad, L.A. Velloso, Unsaturated fatty acids revert diet-induced hypothalamic inflammation in obesity, PloS One. 7 (2012) e30571. https://doi.org/10.1371/journal.pone.0030571.
[28] D.-S. Im, Omega-3 fatty acids in anti-inflammation (pro-resolution) and GPCRs, Prog. Lipid Res. 51 (2012) 232–237. https://doi.org/10.1016/j.plipres.2012.02.003.
[29] J.-C. Delpech, C. Madore, C. Joffre, A. Aubert, J.X. Kang, A. Nadjar, S. Layé, Transgenic increase in n-3/n-6 fatty acid ratio protects against cognitive deficits induced by an immune challenge through decrease of neuroinflammation, Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol. 40 (2015) 525–536. https://doi.org/10.1038/npp.2014.196.
[30] S.K. Orr, R.P. Bazinet, The emerging role of docosahexaenoic acid in neuroinflammation, Curr. Opin. Investig. Drugs Lond. Engl. 2000. 9 (2008) 735–743.
[31] S. Layé, Polyunsaturated fatty acids, neuroinflammation and well being, Prostaglandins Leukot. Essent. Fatty Acids. 82 (2010) 295–303. https://doi.org/10.1016/j.plefa.2010.02.006.
[32] C. Song, A.G. Phillips, B.E. Leonard, D.F. Horrobin, Ethyl-eicosapentaenoic acid ingestion prevents corticosterone-mediated memory impairment induced by central administration of interleukin-1beta in rats, Mol. Psychiatry. 9 (2004) 630–638. https://doi.org/10.1038/sj.mp.4001462.
[33] A.M. Lynch, D.J. Loane, A.M. Minogue, R.M. Clarke, D. Kilroy, R.E. Nally, O.J. Roche, F. O'Connell, M.A. Lynch, Eicosapentaenoic acid confers neuroprotection in the amyloid-beta challenged aged hippocampus, Neurobiol. Aging. 28 (2007) 845–855. https://doi.org/10.1016/j.neurobiolaging.2006.04.006.
[34] D.S.D. Martin, P.E. Lonergan, B. Boland, M.P. Fogarty, M. Brady, D.F. Horrobin, V.A. Campbell, M.A. Lynch, Apoptotic changes in the aged brain are triggered by interleukin-1beta-induced activation of p38 and reversed by treatment with eicosapentaenoic acid, J. Biol. Chem. 277 (2002) 34239–34246. https://doi.org/10.1074/jbc.M205289200.
[35] V.F. Labrousse, A. Nadjar, C. Joffre, L. Costes, A. Aubert, S. Grégoire, L. Bretillon, S. Layé, Short-term long chain omega3 diet protects from neuroinflammatory processes and memory impairment in aged mice, PloS One. 7 (2012) e36861. https://doi.org/10.1371/journal.pone.0036861.
[36] V. De Smedt-Peyrusse, F. Sargueil, A. Moranis, H. Harizi, S. Mongrand, S. Layé, Docosahexaenoic acid prevents lipopolysaccharide-induced cytokine production in microglial cells by inhibiting lipopolysaccharide receptor presentation but not its membrane subdomain localization, J. Neurochem. 105 (2008) 296–307. https://doi.org/10.1111/j.1471-4159.2007.05129.x.
[37] J.D. Figueroa, K. Cordero, K. Baldeosingh, A.I. Torrado, R.L. Walker, J.D. Miranda, M.D. Leon, Docosahexaenoic acid pretreatment confers protection and functional improvements after acute spinal cord injury in adult rats, J. Neurotrauma. 29 (2012) 551–566. https://doi.org/10.1089/neu.2011.2141.
[38] C. Madore, A. Nadjar, J.-C. Delpech, A. Sere, A. Aubert, C. Portal, C. Joffre, S. Layé, Nutritional n-3 PUFAs deficiency during perinatal periods alters brain innate immune system

and neuronal plasticity-associated genes, Brain. Behav. Immun. 41 (2014) 22–31. https://doi.org/10.1016/j.bbi.2014.03.021.
[39] F. Calon, G. Cole, Neuroprotective action of omega-3 polyunsaturated fatty acids against neurodegenerative diseases: evidence from animal studies, Prostaglandins Leukot. Essent. Fatty Acids. 77 (2007) 287–293. https://doi.org/10.1016/j.plefa.2007.10.019.
[40] S.E. Wendelaar Bonga, The stress response in fish, Physiol. Rev. 77 (1997) 591–625. https://doi.org/10.1152/physrev.1997.77.3.591.
[41] B. Levant, M.K. Ozias, P.F. Davis, M. Winter, K.L. Russell, S.E. Carlson, G.A. Reed, K.E. McCarson, Decreased brain docosahexaenoic acid content produces neurobiological effects associated with depression: Interactions with reproductive status in female rats, Psychoneuroendocrinology. 33 (2008) 1279–1292. https://doi.org/10.1016/j.psyneuen.2008.06.012.
[42] A.C. Ferraz, A.M. Delattre, R.G. Almendra, M. Sonagli, C. Borges, P. Araujo, M.L. Andersen, S. Tufik, M.M.S. Lima, Chronic ω-3 fatty acids supplementation promotes beneficial effects on anxiety, cognitive and depressive-like behaviors in rats subjected to a restraint stress protocol, Behav. Brain Res. 219 (2011) 116–122. https://doi.org/10.1016/j.bbr.2010.12.028.
[43] T. Larrieu, M.L. Hilal, L.M. Hilal, C. Fourrier, V. De Smedt-Peyrusse, N. Sans, S. N, L. Capuron, S. Layé, Nutritional omega-3 modulates neuronal morphology in the prefrontal cortex along with depression-related behaviour through corticosterone secretion, Transl. Psychiatry. 4 (2014) e437. https://doi.org/10.1038/tp.2014.77.
[44] K.D. Ketchesin, G.S. Stinnett, A.F. Seasholtz, Corticotropin-releasing hormone-binding protein and stress: from invertebrates to humans, Stress Amst. Neth. 20 (2017) 449–464. https://doi.org/10.1080/10253890.2017.1322575.
[45] S. Mandic, H. Volkoff, The effects of fasting and appetite regulators on catecholamine and serotonin synthesis pathways in goldfish (Carassius auratus), Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 223 (2018) 1–9. https://doi.org/10.1016/j.cbpa.2018.04.017.
[46] L. Zimmer, S. Delion-Vancassel, G. Durand, D. Guilloteau, S. Bodard, J.C. Besnard, S. Chalon, Modification of dopamine neurotransmission in the nucleus accumbens of rats deficient in n-3 polyunsaturated fatty acids, J. Lipid Res. 41 (2000) 32–40.
[47] S. de la Presa Owens, S.M. Innis, Docosahexaenoic and arachidonic acid prevent a decrease in dopaminergic and serotoninergic neurotransmitters in frontal cortex caused by a linoleic and alpha-linolenic acid deficient diet in formula-fed piglets, J. Nutr. 129 (1999) 2088–2093. https://doi.org/10.1093/jn/129.11.2088.
[48] E. Kodas, L. Galineau, S. Bodard, S. Vancassel, D. Guilloteau, J.-C. Besnard, S. Chalon, Serotoninergic neurotransmission is affected by n-3 polyunsaturated fatty acids in the rat, J. Neurochem. 89 (2004) 695–702. https://doi.org/10.1111/j.1471-4159.2004.02401.x.
[49] C.O. Bondi, A.Y. Taha, J.L. Tock, N.K.B. Totah, Y. Cheon, G.E. Torres, S.I. Rapoport, B. Moghaddam, Adolescent behavior and dopamine availability are uniquely sensitive to dietary omega-3 fatty acid deficiency, Biol. Psychiatry. 75 (2014) 38–46. https://doi.org/10.1016/j.biopsych.2013.06.007.
[50] C. Ruibal, J.L. Soengas, M. Aldegunde, Brain serotonin and the control of food intake in rainbow trout (Oncorhynchus mykiss): effects of changes in plasma glucose levels, J. Comp. Physiol. A Neuroethol. Sens. Neural. Behav. Physiol. 188 (2002) 479–484. https://doi.org/10.1007/s00359-002-0320-z.
[51] J.J. Pérez-Maceira, C. Otero-Rodiño, M.J. Mancebo, J.L. Soengas, M. Aldegunde, Food intake inhibition in rainbow trout induced by activation of serotonin 5-HT2C receptors is associated with increases in POMC, CART and CRF mRNA abundance in hypothalamus, J. Comp. Physiol. [B]. 186 (2016) 313–321. https://doi.org/10.1007/s00360-016-0961-9.

[52] V.A. Ortega, D.A. Lovejoy, N.J. Bernier, Appetite-suppressing effects and interactions of centrally administered corticotropin-releasing factor, urotensin I and serotonin in rainbow trout (Oncorhynchus mykiss), Front. Neurosci. 7 (2013) 196. https://doi.org/10.3389/fnins.2013.00196.
[53] J.J. Pérez Maceira, M.J. Mancebo, M. Aldegunde, The involvement of 5-HT-like receptors in the regulation of food intake in rainbow trout (Oncorhynchus mykiss), Comp. Biochem. Physiol. Toxicol. Pharmacol. CBP. 161 (2014) 1–6. https://doi.org/10.1016/j.cbpc.2013.12.003.
[54] Y.-H. He, L. Li, X.-F. Liang, S. He, L. Zhao, Y.-P. Zhang, Inhibitory neurotransmitter serotonin and excitatory neurotransmitter dopamine both decrease food intake in Chinese perch (Siniperca chuatsi), Fish Physiol. Biochem. 44 (2018) 175–183. https://doi.org/10.1007/s10695-017-0422-8.
[55] E. Leal, B. Fernández-Durán, M.J. Agulleiro, M. Conde-Siera, J.M. Míguez, J.M. Cerdá-Reverter, Effects of dopaminergic system activation on feeding behavior and growth performance of the sea bass (Dicentrarchus labrax): a self-feeding approach, Horm. Behav. 64 (2013) 113–121. https://doi.org/10.1016/j.yhbeh.2013.05.008.

FIGURE LEGENDS

Figure 1. **Body weight, feed efficiency and daily feed intake of rainbow trout fed with low, medium or high *ω*-3 LC-PUFAs dietary treatment at the end of the trial**.
(A) Representative body weight gain expressed in grams by individual at the end of the trial (9 weeks). (B) Representative final body weight expressed in grams by individual at the end of the trial. (C) Representative feed efficiency expressed in body weight gain / feed intake at the end of the trial. (D) Representative daily feed intake expressed in percentage of body weight / day at the end of the trial. An asterisk indicates a significant difference between the two diets as determined by a *t*-test ($P < 0.05$). Results are expressed in % of distribution as mean ± S.E. ($n = 3$ tanks).

Figure 2. mRNA expression of transcripts related to feed intake mechanisms in hypothalamus of rainbow trout 6h after last meal of low, medium or high *ω*-3 LC-PUFAs dietary treatment.
Relative gene expression measured by RT-PCR of *npyA, npyB, agpr1, agrp2, pomcA, pomcB* and *cartpt* in hypothalamus of rainbow trout fed during nine weeks with different diets rich in *ω*-3 LC-PUFAs DHA EPA. Values are expressed as group mean ± SEM; fold change vs low diet for all genes; Two-way ANOVA, Tukey post hoc; Different letters indicate significant differences ($P < .05$) among diets. Significant diet interaction is noted by an asterisk between diet.

Figure 3. mRNA expression of transcripts related to cell markers in brains areas of rainbow trout 6h after last meal of low, medium or high *ω*-3 LC-PUFAs dietary treatment.

Relative gene expression measured by RT-PCR of *gfap, tmem119, rbfox3 and pdgfr1* in brain areas of rainbow trout fed during nine weeks with different diets rich in ω-3 LC-PUFAs DHA EPA. Values are expressed as group mean ± SEM; fold change vs low diet for all genes; Two-way ANOVA, Tukey post hoc; Different letters indicate significant differences ($P < .05$) among diets. Significant diet interaction is noted by an asterisk between diet.

Table 1. Ingredients and composition of the experimental diets.

Ingredient (%)	DIET		
	LOW ω-3	**MEDIUM ω-3**	**HIGH ω-3**
Extruded whole wheat	20.0	20.0	20.0
Corn gluten	16.0	16.0	16.0
Wheat gluten	13.0	13.0	13.0
Peas meal	10.0	10.0	10.0
Extruded Peas	8.0	8.0	8.0
White lupin seed meal	3.0	3.0	3.0
Rapeseed meal	4.0	4.0	4.0
Soy lecithin	2.0	2.0	2.0
L-Lysine	1.0	1.0	1.0
L-methionine	0.5	0.5	0.5
$CaHPO_4.2H_2O$	0.5	0.5	0.5
Mineral premix[a]	1.5	1.5	1.5
Vitamin premix[b]	1.5	1.5	1.5
Attractant mix[c]	1.5	1.5	1.5
Palm oil	1.5	2.0	1.1
Sunflower oil	3.5	4.15	3.1
Rapeseed oil	3.0	3.9	2.7
Linseed oil	9.5	2.9	1.0
Omegavie® DHA marine oil (min 70%)[d]	0.0	2.6	5.6
Omegavie® EPA marine oil (min 70%)[e]	0.0	1.95	4.0
Composition (% of dry matter)			
Dry matter (in % of diet)	96.73	96.9	96.87
Crude protein	42.62	41.52	41.1
Crude lipid	23.66	23.75	23.7
Starch	14.92	15.21	14.96
Ash	4.37	4.39	4.27
Energy (kJg-1 DM)	24.51	24.62	24.47

[a]Mineral premix: (g or mg kg^{-1} 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).

[b]Vitamin premix : (IU or mg kg^{-1} diet): DL-α tocopherol acetate, 60 IU; sodium menadione bisulphate, 5 mg; retinyl acetate, 15,000 IU; DL-cholecalciferol, 3000 IU; 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).

[c]Attractant mix: glucosamine, 0.5 g; taurine, 0.3 g; betaine, 0.3 g; glycine, 0.2 g; alanine, 0.2 g.

[d]Omegavie® DHA oil (min 70%): concentrated marine oil produced mainly from anchovy and sardine oil. The crude oil is first refined, then purified and concentrated. This oil contains marine DHA Omega 3 fatty acids under Triglycerides form. From Polaris, Quimper, France.

[e]Omegavie® EPA oil (min 75%): concentrated marine oil produced mainly from anchovy and sardine oil. The crude oil is first refined, then purified and concentrated. This oil contains marine EPA Omega 3 fatty acids under Triglycerides form. From Polaris, Quimper, France.

Table 2. Selected fatty acid composition (% of total fatty acids).

	Diet		
	LOW ω-3	**MEDIUM ω-3**	**HIGH ω-3**
C14:0	0.2	0.2	0.1
C15:0	0.0	0.0	0.0
C16:0	9.7	9.4	6.7
C18:0	3.1	2.6	2.2
C20:0	0.3	0.3	0.4
Sum of saturated fatty acids	**13.7**	**13.2**	**9.9**
C16:1 ω-7	0.2	0.2	0.2
C18:1 ω-9	30.3	29.5	21.9
C20:1 ω-9	0.4	0.8	1.2
C22:1 ω-9	0.0	0.1	0.3
Sum of MUFAs	**30.9**	**30.7**	**23.7**
C18:2 ω-6	27.2	25.6	20.3
C18:3 ω-6	0.0	0.0	0.1
C20:2 ω-6	0.0	0.1	0.2
C20:3 ω-6	0.0	0.1	0.2
C20:4 ω-6	0.0	0.6	1.2
Sum of ω-6 LC-PUFAs	**27.4**	**26.5**	**22.0**
C18:3 ω-3	26.8	11.0	5.1
C18:4 ω-3	0.0	0.1	0.1
C20:3 ω-3	0.0	0.0	0.1
C20:4 ω-3	0.0	0.3	0;6
C20:5 ω-3	0.0	7.6	15.8
C22:5 ω-3	0.0	0.5	1.0
C22:6 ω-3	0.0	8.1	17.7
Sum of ω-3 LC-PUFAs	**26.9**	**27.6**	**40.3**
Sum of ω-3 (EPA + DHA)	***0.0***	***15.7***	***33.4***
ω-3 (EPA+DHA)/ALA	***0.0***	***1.4***	***6.6***

Table 3. Nucleotide sequence of the PCR primers used to evaluate mRNA expression of transcripts by RT-qPCR.

Transcript	Forward Primer	Reverse Primer	Database	Accession Number
Reference				
eef1a1	TCCTCTTGGTCGTTTCGCTG	ACCCGAGGGACATCCTGTG	GenBank	AF498320
Neuropeptide				
npyA	AAGGCAGAGGTGAGTGCTGT	AGCCTGTGGCTCACTAATCAA	GenBanK	NM_001124266
npyB	CGTATTTGTTAGCGCCTTTC	TCATTCGATTTCTTCACTTCCA	GenBanK	NM_001124266
agrp1	ACCAGCAGTCCTGTCTGGGTAA	AGTAGCAGATGGAGCCGAACA	GenBanK	CR376289
agrp2	CCAGGAGACGGATTTTGCCA	GAGGGGCACAGCTATCCATC	GenBanK	CA343080
pomcA	ACCAGCAGTCCTGTCTGGGTAA	AGTAGCAGATGGAGCCGAACA	GenBanK	NM_001124718.1
pomcB	CCAGGAGACGGATTTTGCCA	GAGGGGCACAGCTATCCATC	GenBanK	NM_001124719.1
cartpt	ACCATGGAGAGCTCCAG	GCGCACTGCTCTCCAA	GenBanK	DQ836925
Cytokines/chemokines				
IL-1β1	AGTGTTGGAGTTGGAGTCGG	CATCAGGACCCAGCACTTGT	GenBanK	NM_001124347
IL-1β2	ACCACTTAGAATGCATGCCCA	CCGACTCCAACTCCAACACT	GenBanK	AJ245925
IL-1β3	AGGACGCCAGAAAGCTCATC	TTCTCAGATTCACCCCGCAC	GenBanK	AJ557021
IL-4	GACAATCTTGGCCTCCGTGA	CCACCTGGTCTTGGCTCTTC	GenBanK	NM_001246341
IL-6	CCCTACTCCCCTCTGTCACA	AACACGCTTCCTCTCACTGG	GenBanK	NM_001124657.1
IL-8	ATTGAGACGGAGAGCAGACG	AATCTCCTGACCGCTCTTGC	GenBanK	NM_001124362.1
IL-10	CGCCTTCTCCACCATCAGAG	CTGTCCATAGCGTGACACCC	GenBanK	NM_001245099
IL-11	ACCACCCTGCTCTCCCTAAA	GGAGAGAGACGCTGAGCATC	GenBanK	AJ535687.1
tnf-α1	AATCTTTCCGCTGACACCGT	CGAAGAAGAGCCCAGTGTGT	GenBanK	NM_001124357
tnf-α2	GCACTATGATGAGAGAGAGGCA	GGCTCAGTTACCATAAAATTGCA	GenBanK	NM_001124374
tnf-α3	GAGTGACGGTGGACTTGGAG	CTGAGACACACAGCGCCAT	GenBanK	HE798544
Inflammation				
nfkpb1	CCAACCTGAAGATTGTGCGG	TAGAAGCGCACCTGGATGTC	GenBanK	XM_021561045.1
ikk-α	GGAGAATGAGGAGAAGGCGG	CTCTGCAGCTCCACGATCTC	GenBanK	FR915835.1
ikk-β	CCAGACGGATCCTAAGCACG	TGCTGTAACCTCTGCCAGTC	GenBanK	FR911637.1
Anti-oxidant				
sod-1	CGTAGTCGTGGCTCAATGGT	CCAAACCAACCCTAGCCACT	GenBanK	NM_001124329.1
sod-2	CTGGGCTTCGACAAGGAGAG	GTAGGCATGCTCCCACACAT	GenBanK	XM_021612540
sod-3	GGCGGCCATTACAACCCTTA	CCGAACAGAGTGGCCTTTGA	GenBanK	XM_021619043.1
cat	GCCTCCAAGATGTGATGCCT	TCTATCGCATTCACGGACGG	GenBanK	XM_021564294
gsr	TCATTGCCACAGGAGGGTCC	CTATGACGCTGCGCTTAGGA	GenBanK	HF969248.1
Stress (cortisol)				
crf	GCAACAGTCTCTTCCCCTCC	CTTGGGGTGCATGACTTTCG	GenBanK	NM_001124286.1
crhr	ACAACCATGCCCTGAGAGTG	AGGTCAAACAGCTGTGGTCT	GenBanK	XM_021613637
mc2r	CGCAGAGCTACGAAACACCT	TGCAGACAAAGCCCTTCAGT	GenBanK	NM_001124680.1
mrap-1	CCCGTTCAGTCAGTCTACCC	CAGAGGGGTTTGGGGATCAA	GenBanK	FR837908.1
mrap-2	GAGGGTCTCAAGGCTCATCG	GTCTTGGTCAGCAGGGTGAG	GenBanK	XM_021611508
Cell markers				
gfap	CAGAGCTTCTCCAACCTGCA	CCCCGTCTCTAGTCTCCACA	GenBanK	XM_021558456
tmem119	GTCTCTGACGGGCCTGATTT	TTCGGTTTGGGCTCCTTACC	GenBanK	XM_021621681
rbfox3	ACGCCACTGTCAACTGTATGA	TCACTCTGCATGCTGTCACA	GenBanK	XM_021556260
pdgfra-1	TCAGTGGAGAGATCAGAGCCA	CTGACAAACTCCACCACTCCA	Sigenae	Scaffold_1131 GSONMG00048607001
pdgfra-2	ATGCTGGAGATGAGTGACGC	CAGCATGTTGTCCATCTCACT	Sigenae	Scaffold_669 GSONMG00023327001
Monoamines				
5ht1A-a	CCCAACACTCCACAGTCCTC	ACCGAGCGTCTTTACCGTTT	GenBanK	XM_021622104
5ht1A-b	GAGGACCAACGGGGACCCGA	AATCGCCGTGCTTGACCGCA	GenBanK	CCAF0100015582
tph-1a	ACACCAGAGCCAGACACATG	TCATCTGAAGCTCCGAGGGA	Sigenae	scaffold_7943 GSONMG00021168001
tph-2	AGCACCTCAAAGACCACGTC	ACTGGTCGAGCTCTGCAATC	GenBanK	MG015698
sert	CCTGCTGCCCTACATGTTGA	GGGGCAGATGTGTTTCCAGA	GenBanK	M_021582096.1
th	ACGCTCTCTCAAGGTGTTCG	AAAGTACTCCAGCCCCTCCA	GenBanK	XM_021564247.1
drd1	CCTCCAGTCCACCACCAATT	CCACTCTCCCACCACCTCTA	GenBanK	NM_001124372.2
drd2	GGAGGAGCTGCAGAAGAAGG	TTTCCAGTGACACATCGGCA	GenBanK	XM_021617454.1
dat	CTACCTCAGCGTCGACTTCC	TAGCACACCAAACCCGACTC	GenBanK	XM_021592557.1

Table 4. Changes in relative mRNA expression of cytokines and inflammation transcripts in brain areas of rainbow trout 6h after last meal of low, medium or high ω-3 LC-PUFAs dietary

treatment. Each value is the mean ± SEM of n=6 fish per treatment. Data are relative to the low diet (results were previously normalized by ef1α mRNA levels, which did not show changes among groups). Superscripts annotated by different letters indicates a significance (*P<0.05*) between the diet groups. Expressions without superscript letter annotations are statistically non-significant between diets. Diet interaction is noted by the P-values in the table.

		FOREBRAIN		MIDBRAIN		HINDBRAIN		HYPOTHALAMUS		CEREBELLUM	
Cytokines	Dietary treatment	effects	*p-values*	effects	*p-values*	effects	*p-values*	effects	*p-values*	effects	*p-values*
IL-1ß1	Low ω-3	1.00 ± 0.03^{a}	**0.0487**	1.00 ± 0.02^{a}	0.119	1.00 ± 0.03	0.771	1.00 ± 0.06	0.503	1.00 ± 0.03	0.117
	Medium ω-3	0.92 ± 0.02^{b}		0.96 ± 0.01^{ab}		1.05 ± 0.05		1.07 ± 0.05		1.01 ± 0.02	
	High ω-3	0.92 ± 0.01^{b}		0.94 ± 0.02^{b}		1.01 ± 0.05		0.99± 0.02		0.99 ± 0.03	
IL-1ß2	Low ω-3	1.00 ± 0.04^{a}	0.080	1.00 ± 0.02^{a}	0.126	1.00 ± 0.02	0.402	1.00 ± 0.06	0.844	1.00 ± 0.03	0.805
	Medium ω-3	0.93 ± 0.03^{ab}		0.94 ± 0.02^{b}		1.01 ± 0.05		1.06 ± 0.05		1.01 ± 0.03	
	High ω-3	0.91 ± 0.01^{b}		0.94 ± 0.03^{b}		0.97 ± 0.05		0.97 ± 0.02		1.01 ± 0.03	
IL-1ß3	Low ω-3	1.00 ± 0.03^{a}	**0.037**	1.00 ± 0.02^{a}	**0.014**	1.00 ± 0.02	0.387	1.00 ± 0.05	0.933	1.00 ± 0.03	0.534
	Medium ω-3	0.94 ± 0.01^{b}		0.94 ± 0.01^{b}		1.04 ± 0.05		1.05 ± 0.06		1.01 ± 0.02	
	High ω-3	0.92 ± 0.01^{b}		0.92 ± 0.02^{b}		0.98 ± 0.05		0.96 ± 0.02		1.00 ± 0.03	
IL-4	Low ω-3	1.00 ± 0.03^{a}	0.007	1.00 ± 0.02^{a}	0.150	1.00 ± 0.01	0.451	1.00 ± 0.06	0.778	1.00 ± 0.02	0.763
	Medium ω-3	0.91 ± 0.02^{b}		0.95 ± 0.01^{b}		1.00 ± 0.05		1.05 ± 0.06		0.98± 0.02	
	High ω-3	0.93 ± 0.01^{b}		0.95 ± 0.03^{ab}		0.96 ± 0.05		0.96 ± 0.02		0.98 ± 0.02	
IL-6	Low ω-3	1.00 ± 0.03^{a}	0.090	1.00 ± 0.02^{a}	0.330	1.00 ± 0.02	0.681	1.00 ± 0.06	0.930	1.00 ± 0.03	0.725
	Medium ω-3	0.92 ± 0.02^{b}		0.96 ± 0.01^{b}		1.04 ± 0.05		1.05 ± 0.06		1.01 ± 0.03	
	High ω-3	0.94 ± 0.01^{b}		0.96 ± 0.03^{ab}		0.99 ± 0.05		0.99 ± 0.02		1.00 ± 0.03	
IL-8	Low ω-3	1.00 ± 0.03^{a}	**0.032**	1.00 ± 0.02a	0.271	1.00 ± 0.01	0.579	1.00 ± 0.06	0.901	1.00 ± 0.03	0.688
	Medium ω-3	0.91 ± 0.02^{b}		0.94 ± 0.01b		1.02 ± 0.06		1.02 ± 0.05		1.02 ± 0.03	
	High ω-3	0.92 ± 0.01^{b}		0.96 ± 0.04^{ab}		0.96 ± 0.06		0.95 ± 0.03		1.01 ± 0.03	
IL-10	Low ω-3	1.00 ± 0.02	0.237	1.00 ± 0.02^{a}	0.156	1.00 ± 0.04	0.549	1.00 ± 0.04	0.743	1.00 ± 0.04	0.757
	Medium ω-3	1.02 ± 0.02		0.95 ± 0.01^{b}		0.96 ± 0.05		1.04 ± 0.03		0.97 ± 0.04	
	High ω-3	0.98 ± 0.01		0.94 ± 0.04^{ab}		0.99 ± 0.03		1.00 ± 0.02		0.97 ± 0.02	
IL-11	Low ω-3	1.00 ± 0.03a	0.093	1.00 ± 0.02a	0.115	1.00 ± 0.01	0.545	1.00 ± 0.07	0.937	1.00 ± 0.03	0.719
	Medium ω-3	0.93 ± 0.02b		0.94 ± 0.02b		0.99 ± 0.05		1.02 ± 0.05		1.01 ± 0.03	
	High ω-3	0.93 ± 0.02b		0.94 ± 0.03b		0.95 ± 0.05		0.95 ± 0.02		1.01 ± 0.03	
tnf-α1	Low ω-3	1.00 ± 0.03a	0.028	1.00 ± 0.02a	**0.038**	1.00 ± 0.02	0.401	1.00 ± 0.06^{a}	**0.015**	1.00 ± 0.03	0.951
	Medium ω-3	0.92 ± 0.02b		0.93 ± 0.02b		0.98 ± 0.04		1.02 ± 0.05^{a}		1.01 ± 0.03	
	High ω-3	0.91 ± 0.01b		0.93 ± 0.02b		0.94 ± 0.05		0.94 ± 0.02^{b}		1.01 ± 0.03	
tnf-α2	Low ω-3	1.00 ± 0.03^{a}	**0.034**	1.00 ± 0.02^{a}	0.060	1.00 ± 0.01	0.456	1.00 ± 0.06	0.992	1.00 ± 0.03	0.569
	Medium ω-3	0.93 ± 0.02^{ab}		0.94 ± 0.01^{b}		1.02 ± 0.05		1.05 ± 0.06		1.00 ± 0.02	
	High ω-3	0.93 ± 0.01^{b}		0.93 ± 0.03^{b}		0.96 ± 0.05		0.97 ± 0.02		1.00 ± 0.03	
tnf-α3	Low ω-3	1.00 ± 0.03^{a}	0.068	1.00 ± 0.02	0.203	1.00 ± 0.01	0.377	1.00 ± 0.05	0.982	1.00 ± 0.03	0.599
	Medium ω-3	0.94 ± 0.02^{ab}		0.96 ± 0.02		1.03 ± 0.05		1.07 ± 0.06		1.01 ± 0.03	
	High ω-3	0.93 ± 0.01^{b}		0.95 ± 0.03		0.97 ± 0.05		0.97 ± 0.02		1.00 ± 0.03	
Inflammation											
nfkpb1	Low ω-3	1.00 ± 0.03	0.472	1.00 ± 0.02^{a}	0.016	1.00 ± 0.02	0.313	1.00 ± 0.04	0.972	1.00 ± 0.04	0.995
	Medium ω-3	0.98 ± 0.02		0.92 ± 0.02^{b}		1.00 ± 0.03		1.03 ± 0.04		0.99 ± 0.03	
	High ω-3	0.96 ± 0.01		0.89 ± 0.03^{b}		1.00 ± 0.03		0.96 ± 0.01		0.99 ± 0.02	
ikk-α	Low ω-3	1.00 ± 0.03	0.300	1.00 ± 0.02^{a}	**0.006**	1.00 ± 0.02	0.390	1.00 ± 0.05	0.982	1.00 ± 0.03	0.850
	Medium ω-3	0.98 ± 0.01		0.94 ± 0.02^{b}		1.02 ± 0.04		1.02 ± 0.04		0.99 ± 0.02	
	High ω-3	0.96 ± 0.01		0.91 ± 0.02^{b}		0.99 ± 0.04		0.95 ± 0.01		0.99 ± 0.02	
ikk-ß	Low ω-3	1.00 ± 0.03	0.377	1.00 ± 0.02^{a}	0.020	1.00 ± 0.02	0.307	1.00 ± 0.05	0.698	1.00 ± 0.02	0.785
	Medium ω-3	0.97 ± 0.02		0.96 ± 0.02^{b}		1.03 ± 0.03		1.07 ± 0.06		0.98 ± 0.03	
	High ω-3	0.96 ± 0.01		0.93 ± 0.02^{b}		1.02 ± 0.04		0.98 ± 0.01		0.97 ± 0.02	

Table 5. Changes in relative mRNA expression of antioxidant transcripts in brain areas of rainbow trout 6 h after last meal of low, medium or high *ω*-3 LC-PUFAs dietary treatment.

Each value is the mean ± SEM of n=6 fish per treatment. Data are relative to the low diet (results were previously normalized by ef1α mRNA levels, which did not show changes among groups). Superscripts annotated by different letters indicates a significance ($P<0.05$) between the diet groups. Expressions without superscript letter annotations are statistically non-significant between diets. Diet interaction is noted by the P-values in the table.

		FOREBRAIN		MIDBRAIN		HINDBRAIN		HYPOTHALAMUS		CEREBELLUM	
Anti-oxidant	**Dietary treatment**	effects	*p-values*	effects	*p-values*	effects	*p-values*	effects	*p-values*	effects	*p-values*
sod-1	***Low ω-3***	1.00 ± 0.04	0.308	1.00 ± 0.02^{a}	**0.025**	1.00 ± 0.02	0.794	1.00 ± 0.04	0.956	1.00 ± 0.04	0.970
	Medium ω-3	0.99 ± 0.02		0.93 ± 0.02^{ab}		0.99 ± 0.04		1.00 ± 0.03		1.00 ± 0.03	
	High ω-3	0.95 ± 0.02		0.91 ± 0.02^{b}		1.00 ± 0.03		0.97 ± 0.01		0.99 ± 0.02	
sod-2	***Low ω-3***	1.00 ± 0.03	0.538	1.00 ± 0.01^{a}	**0.044**	1.00 ± 0.02	0.937	1.00 ± 0.04	0.572	1.00 ± 0.04	0.559
	Medium ω-3	1.02 ± 0.02		0.96 ± 0.02^{ab}		1.02 ± 0.03		1.04 ± 0.05		1.00 ± 0.03	
	High ω-3	0.98 ± 0.01		0.91 ± 0.03^{b}		1.04 ± 0.02		0.99 ± 0.01		0.99 ± 0.02	
sod-3	***Low ω-3***	1.00 ± 0.03^{a}	0.382	1.00 ± 0.02	0.182	1.00 ± 0.02	0.596	1.00 ± 0.06	0.798	1.00 ± 0.03	0.411
	Medium ω-3	0.96 ± 0.02^{b}		0.98 ± 0.01		1.07 ± 0.04		1.09 ± 0.05		1.02 ± 0.03	
	High ω-3	0.97 ± 0.01^{ab}		0.95 ± 0.02		1.03 ± 0.04		1.02 ± 0.02		1.02 ± 0.02	
cat	***Low ω-3***	1.00 ± 0.03	0.366	1.00 ± 0.02^{a}	0.096	1.00 ± 0.01	0.654	1.00 ± 0.05	0.924	1.00 ± 0.03	0.994
	Medium ω-3	0.98 ± 0.01		0.94 ± 0.02^{b}		1.00 ± 0.04		1.02 ± 0.06		1.01 ± 0.03	
	High ω-3	0.96 ± 0.01		0.95 ± 0.02^{b}		1.00 ± 0.03		0.97 ± 0.02		1.01 ± 0.03	
gsr	***Low ω-3***	1.00 ± 0.02	0.683	1.00 ± 0.02^{a}	0.057	1.00 ± 0.01	0.944	1.00 ± 0.03	0.730	1.00 ± 0.03	0.615
	Medium ω-3	1.02 ± 0.02		0.96 ± 0.02^{b}		1.03 ± 0.04		1.00 ± 0.02		1.01 ± 0.02	
	High ω-3	1.00 ± 0.01		0.93 ± 0.02^{b}		1.02 ± 0.02		0.99 ± 0.01		0.98 ± 0.02	

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Table 6. Changes in relative mRNA expression of cortisol pathways transcripts in brain areas of rainbow trout 6 h after last meal of low, medium or high ω-3 LC-PUFAs dietary treatment. Each value is the mean ± SEM of n=6 fish per treatment. Data are relative to the low diet (results were previously normalized by ef1α mRNA levels, which did not show changes among groups). Superscripts annotated by different letters indicates a significance ($P<0.05$) between the diet groups. Expressions without superscript letter annotations are statistically non-significant between diets. Diet interaction is noted by the P-values in the table.

		FOREBRAIN		MIDBRAIN		HINDBRAIN		HYPOTHALAMUS		CEREBELLUM	
Cortisol	Dietary treatment	effects	*p-values*	effects	*p-values*	effects	*p-values*	effects	*p-values*	effects	*p-values*
crf	*Low ω-3*	1.00 ± 0.03	0.808	1.00 ± 0.02^{a}	**0.046**	1.00 ± 0.05	0.808	1.00 ± 0.03	0.980	1.00 ± 0.01	0.667
	Medium ω-3	0.96 ± 0.02		0.94 ± 0.02^{ab}		1.02 ± 0.06		1.00 ± 0.03		1.02 ± 0.05	
	High ω-3	0.94 ± 0.01		0.92 ± 0.02^{b}		0.93 ± 0.01		1.00 ± 0.03		0.98 ± 0.04	
crhr	*Low ω-3*	1.00 ± 0.03^{a}	**0.013**	1.00 ± 0.02^{a}	0.078	1.00 ± 0.06	0.387	1.00 ± 0.03	0.989	1.00 ± 0.01^{a}	0.117
	Medium ω-3	0.91 ± 0.02^{b}		0.93 ± 0.01^{b}		1.03 ± 0.06		1.00 ± 0.03		0.99 ± 0.05^{ab}	
	High ω-3	0.92 ± 0.01^{b}		0.94 ± 0.03^{ab}		0.94 ± 0.02		0.99 ± 0.03		0.94 ± 0.04^{b}	
mc2r	*Low ω-3*	1.00 ± 0.03	0.168	1.00 ± 0.02	0.389	1.00 ± 0.06	0.850	1.00 ± 0.03	0.945	1.00 ± 0.01	0.490
	Medium ω-3	0.94 ± 0.02		0.96 ± 0.01		1.03 ± 0.06		1.01 ± 0.02		1.06 ± 0.04	
	High ω-3	0.96 ± 0.01		0.97 ± 0.03		0.99 ± 0.02		1.00 ± 0.03		1.00 ± 0.05	
mrap-1	*Low ω-3*	1.00 ± 0.03^{a}	**0.033**	1.00 ± 0.02	0.149	1.00 ± 0.02	0.641	1.00 ± 0.02	0.385	1.00 ± 0.02	0.973
	Medium ω-3	0.92 ± 0.02^{b}		0.96 ± 0.01		0.96 ± 0.01		1.00 ± 0.01		1.08 ± 0.06	
	High ω-3	0.92 ± 0.01^{b}		0.96 ± 0.02		0.96 ± 0.02		0.96 ± 0.03		1.00 ± 0.04	
mrap-2	*Low ω-3*	1.00 ± 0.04	0.921	1.00 ± 0.02^{a}	0.008	1.00 ± 0.04	0.279	1.00 ± 0.04	0.685	1.00 ± 0.02	0.428
	Medium ω-3	1.01 ± 0.02		0.92 ± 0.03^{b}		1.01 ± 0.05		1.01 ± 0.04		1.04 ± 0.03	
	High ω-3	0.99 ± 0.03		0.88 ± 0.02^{b}		0.94 ± 0.01		0.97 ± 0.02		1.05 ± 0.02	

Table 7. Changes in relative mRNA expression of monoamines transcripts in brain areas of rainbow trout 6 h after last meal of low, medium or high ω-3 PUFAs dietary treatment.

Each value is the mean ± SEM of n=6 fish per treatment. Data are relative to the low diet (results were previously normalized by ef1α mRNA levels, which did not show changes among groups). Superscripts annotated by different letters indicates a significance ($P<0.05$) between the diet groups. Expressions without superscript letter annotations are statistically non-significant between diets. Diet interaction is noted by the P-values in the table.

		FOREBRAIN		MIDBRAIN		HINDBRAIN		HYPOTHALAMUS		CEREBELLUM	
Monoamines	Dietary treatment	effects	*p-values*	effects	*p-values*	effects	*p-values*	effects	*p-values*	effects	*p-values*
5ht1A-a	*Low ω-3*	1.00 ± 0.03	0.201	1.00 ± 0.02^{a}	**0.012**	1.00 ± 0.05	0.318	1.00 ± 0.04	0.962	1.00 ± 0.01	0.748
	Medium ω-3	0.97 ± 0.02		0.93 ± 0.02^{ab}		1.01 ± 0.05		0.99 ± 0.03		1.00 ± 0.05	
	High ω-3	0.95 ± 0.01		0.91 ± 0.02^{b}		0.92 ± 0.01		0.99 ± 0.03		0.96 ± 0.05	
5ht1A-b	*Low ω-3*	1.00 ± 0.03^{a}	0.097	1.00 ± 0.02^{a}	**0.001**	1.00 ± 0.04	0.270	1.00 ± 0.03	0.822	1.00 ± 0.01	0.634
	Medium ω-3	0.95 ± 0.02^{ab}		0.92 ± 0.02^{b}		1.00 ± 0.04		0.98 ± 0.03		0.99 ± 0.05	
	High ω-3	0.93 ± 0.02^{b}		0.88 ± 0.02^{b}		0.93 ± 0.02		0.98 ± 0.03		0.95 ± 0.05	
tph-1α	*Low ω-3*	1.00 ± 0.04^{a}	**0.019**	1.00 ± 0.01	0.287	1.00 ± 0.05	0.653	1.00 ± 0.03	0.627	1.00 ± 0.03	0.625
	Medium ω-3	1.00 ± 0.03^{a}		0.96 ± 0.01		1.04 ± 0.06		0.98 ± 0.03		1.01 ± 0.04	
	High ω-3	0.87 ± 0.03^{b}		0.95 ± 0.04		0.98 ± 0.02		0.96 ± 0.02		0.96 ± 0.05	
tph-2	*Low ω-3*	1.00 ± 0.03	0.607	1.00 ± 0.02^{a}	0.048	1.00 ± 0.03	0.227	1.00 ± 0.05	0.158	1.00 ± 0.02	0.367
	Medium ω-3	1.00 ± 0.03		0.92 ± 0.03^{ab}		1.05 ± 0.04		1.10 ± 0.04		1.05 ± 0.04	
	High ω-3	1.03 ± 0.02		0.90 ± 0.03^{b}		1.03 ± 0.01		1.06 ± 0.04		1.05 ± 0.03	
sert	*Low ω-3*	1.00 ± 0.02	0.680	1.00 ± 0.02^{a}	0.098	1.00 ± 0.04	0.897	1.00 ± 0.03	0.745	1.00 ± 0.01	0.835
	Medium ω-3	0.99 ± 0.01		0.95 ± 0.02^{ab}		1.02 ± 0.04		1.00 ± 0.03		1.03 ± 0.03	
	High ω-3	0.98 ± 0.01		0.94 ± 0.01^{b}		0.99 ± 0.01		0.98 ± 0.03		1.02 ± 0.03	
th	*Low ω-3*	1.00 ± 0.04	0.359	1.00 ± 0.02	0.221	1.00 ± 0.03	0.383	1.00 ± 0.04	0.425	1.00 ± 0.05	0.061
	Medium ω-3	0.93 ± 0.04		0.96 ± 0.03		1.05 ± 0.02		0.96 ± 0.03		0.94 ± 0.05	
	High ω-3	1.00 ± 0.02		0.93 ± 0.04		1.00 ± 0.03		0.94 ± 0.02		1.04 ± 0.03	
drd1	*Low ω-3*	1.00 ± 0.03^{a}	0.129	1.00 ± 0.03^{a}	**0.019**	1.00 ± 0.05	0.374	1.00 ± 0.03	0.985	1.00 ± 0.02^{a}	0.147
	Medium ω-3	0.97 ± 0.01^{ab}		0.92 ± 0.01^{b}		1.01 ± 0.05		1.00 ± 0.03		0.98 ± 0.05^{ab}	
	High ω-3	0.94 ± 0.00^{b}		0.92 ± 0.00^{b}		0.93 ± 0.01		0.99 ± 0.03		0.95 ± 0.05^{b}	
drd2	*Low ω-3*	1.00 ± 0.03	0.785	1.00 ± 0.03^{a}	**0.014**	1.00 ± 0.03	0.201	1.00 ± 0.04	0.671	1.00 ± 0.02	0.763
	Medium ω-3	0.98 ± 0.02		0.98 ± 0.01^{ab}		1.00 ± 0.05		0.99 ± 0.04		1.03 ± 0.04	
	High ω-3	0.99 ± 0.01		0.95 ± 0.00^{b}		0.93 ± 0.02		0.99 ± 0.02		1.03 ± 0.02	
dat	*Low ω-3*	1.00 ± 0.03	0.701	1.00 ± 0.03^{a}	0.115	1.00 ± 0.05^{a}	0.323	1.00 ± 0.03	0.115	1.00 ± 0.02	0.372
	Medium ω-3	0.98 ± 0.03		0.94 ± 0.03^{ab}		0.92 ± 0.04^{ab}		0.96 ± 0.03		1.03 ± 0.04	
	High ω-3	0.96 ± 0.04		0.89 ± 0.04^{b}		0.89 ± 0.01^{b}		0.94 ± 0.02		1.07 ± 0.04	

Figure 1. Body weight, feed efficiency and daily feed intake of rainbow trout fed with low, medium or high ω-3 PUFAs dietary treatment at the end of the trial.

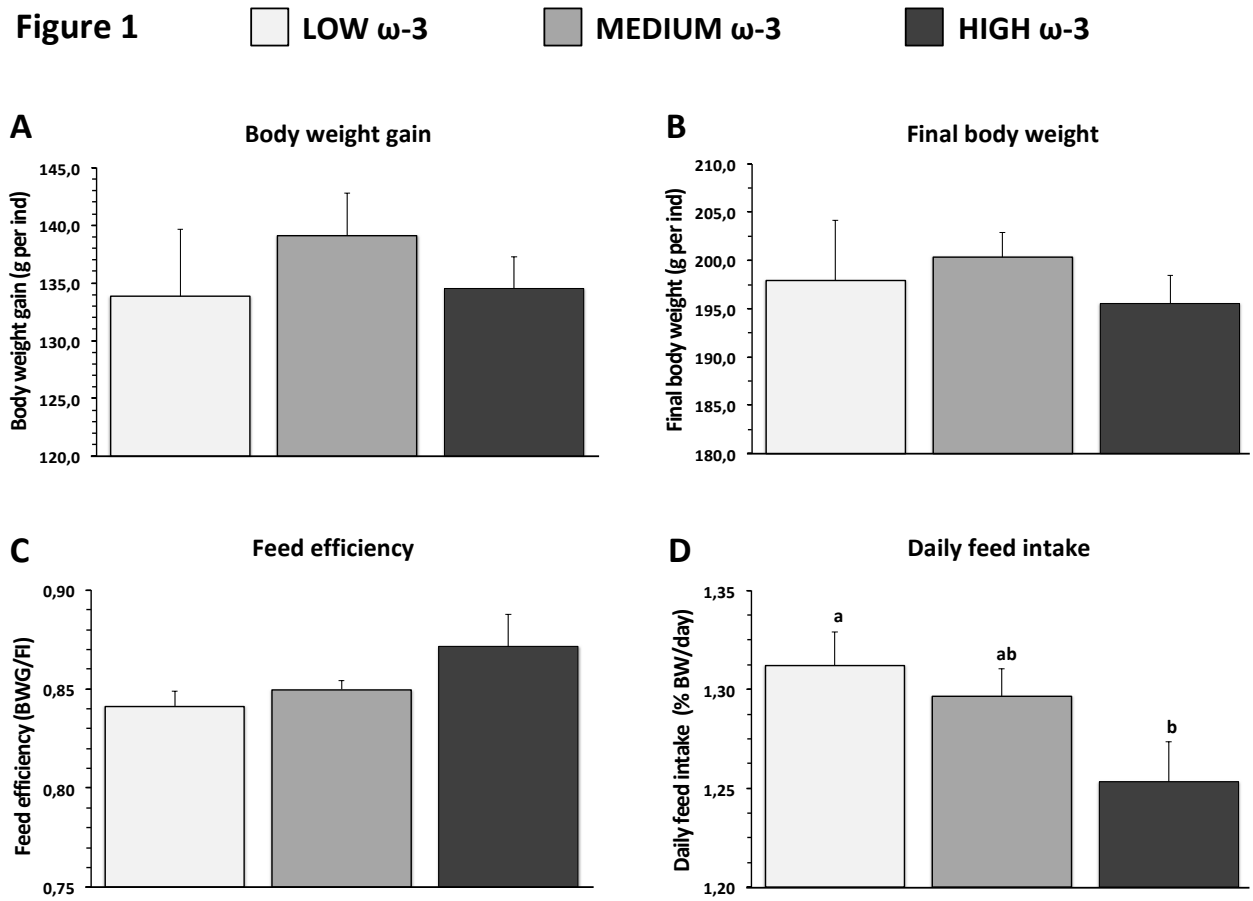


Figure 2. mRNA expression of transcripts related to feed intake mechanisms in hypothalamus of rainbow trout 6h after last meal of low, medium or high ω-3 PUFAs dietary treatment.

Figure 2

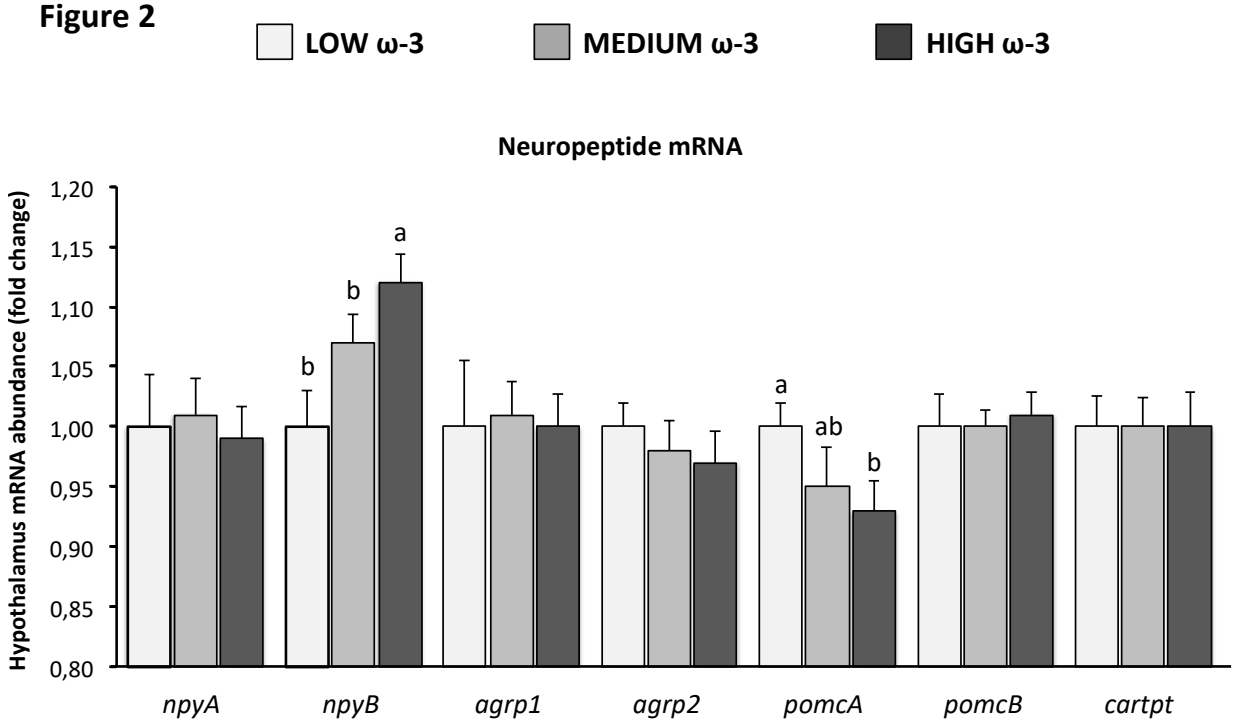
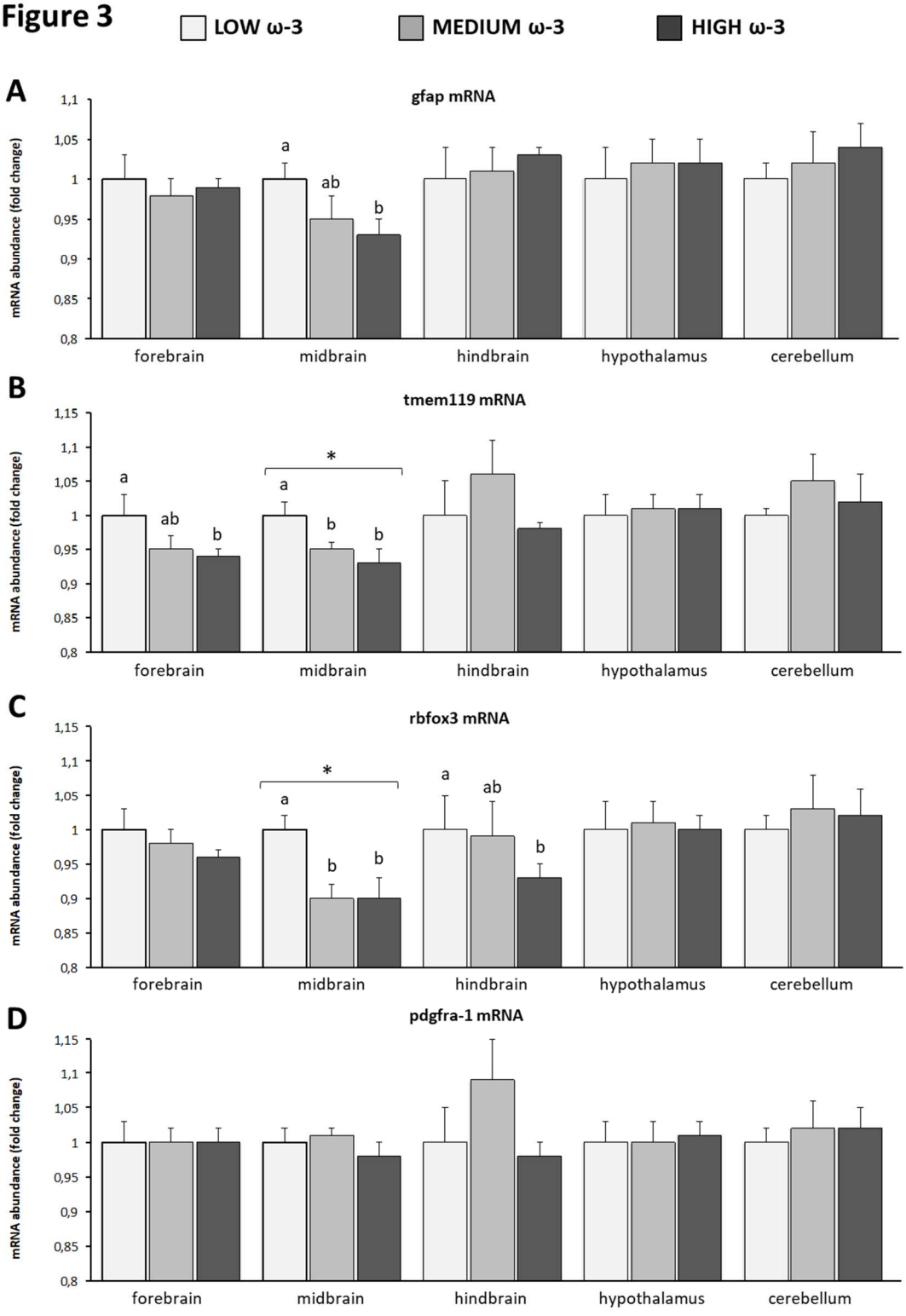


Figure 3. mRNA expression of transcripts related to cell markers in brains areas of rainbow trout 6h after last meal of low, medium or high ω-3 LC-PUFAs dietary treatment.

Figure 3


LOW ω-3
MEDIUM ω-3
HIGH ω-3
A
gfap mRNA
B
tmem119 mRNA
C
rbfox3 mRNA
D
pdgfra-1 mRNA
mRNA abundance (fold change)
forebrain
midbrain
hindbrain
hypothalamus
cerebellum