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Effects of dietary sunflower oil on growth parameters, fatty acid profiles and expression of genes regulating growth and metabolism in the pejerrey (*Odontesthes bonariensis*) fry

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Abstract

Aquaculture fish diets usually contain an addition of fish oil to improve their nutritional value. The effect of the replacement of dietary fish oil (FO) by sunflower oil (SfO) on growth, fatty acid composition and expression of genes implicated in somatic growth, feed intake and fatty acid metabolism was studied in pejerrey fry. Fry were fed per 45 days with diets containing FO/SfO ratios of 100% FO; 50% FO:50% SfO; 20% FO:80% SfO; and 100% SfO. No differences were detected in growth and in the total per cent of saturated and monounsaturated fatty acids. *Gh*, *ghr-I* and *ghr-II* showed a higher mRNA expression in head and trunk of fry fed with 100% SfO diet. Expression of *igf-II* was higher in trunk of fry fed with 100% SfO diet compared with 100% FO diet. The *Δ6-desaturase* gene expression was upregulated in head and trunk of fry fed with 100% SfO diet. The *nucb2/nesfatin-1* gene expression decreased in the trunk of fry with increasing dietary SfO. We conclude that the replacement of fish oil by sunflower oil in pejerrey fry feed does not affect growth and is a viable strategy to reduce production costs of this fish.

KEYWORDS

Δ6-desaturase, growth hormone, insulin-like growth factor, lipid, nesfatin-1, polyunsaturated fatty acid

1 | INTRODUCTION

Pejerrey, *Odontesthes bonariensis* (Valenciennes, 1835), is an inland water fish from Argentina highly appreciated for the quality of its flesh that has taste, smell, texture and even chemical characteristics similar to those of expensive marine species, converting it in a good candidate for aquaculture (Somoza et al., 2008). Despite this high potential of pejerrey as an aquaculture species, no specific diets were formulated to develop and improve its aquaculture. In fact, pejerrey farming presents several difficulties related to low growth rates, high mortality in the first life stages and early sexual maturation (Grosman & González Castelain, 1995; Miranda & Somoza, 2001). These factors made its aquaculture expensive, raising the need for improvement, to reduce production costs. In this sense, one approach might lay on the replacement of expensive fish oil (commonly employed to increase the nutritional value of the commercial fish feed) by cheaper vegetable oils such as sunflower oil (Glencross & Turchini, 2010). In addition, it will contribute to develop a sustainable pejerrey aquaculture as this practice would avoid the usage of wild fish (such as sardines and anchovies) to produce aqua feeds for fish farming.

Replacement of fish oil by vegetable oil in fish diet has been studied in several species, such as the *Atlantic salmon* (Bransden, Carter, & Nichols, 2003), sea bass (Mourente, Good, & Bell, 2005) and rainbow trout (Turchini & Francis, 2009), among others.

Particularly, the use of Sunflower oil was widely studied (Bransden et al., 2003; Wijekoon, Parrish, & Mansour, 2014; Yildiz & Sener, 1997) due to its low production cost and high availability. A general conclusion of all of these works is that the success of oil replacement depends on the ability of fish to convert polyunsaturated fatty acids (PUFAs) with low number of unsaturations, present in vegetable oils, into long-chain PUFAs (>20C), also referred to as highly unsaturated fatty acids (HUFAs), that are present in fish oil and are important for growth and development. In this regard, the ability of fish to synthesize long-chain PUFAs depends on the enzyme Δ6-desaturase. This key enzyme regulates the first step in the pathway which produces arachidonic acid (AA), docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), converting linolenic acid (LNA) and linoleic acid (LA) obtained from food to 18:4n−3 and 18:3n−6, respectively (Vagner & Santigosa, 2011), and participates in the synthesis of DHA through the process named Sprecher's shunt (Sprecher, 2000). Previous studies of our group showed that pejerrey expressed mRNA from a *Δ6-desaturase*-like gene (article in preparation). Moreover, studies of fatty acid composition performed in wild pejerrey larvae (Koppprio, Graeve, Kattner, & Lara, 2015) and also in larvae from a related species, the Mexican silverside (*Chirostoma estor*) (Palacios, Racotta, Aparicio, Arjona, & Martínez-Palacios, 2007), suggest that these two fish have the ability to elongate and desaturate dietary PUFAs to long-chain PUFAs through both Δ6 and Δ4 desaturation pathways (Fonseca-Madrigal et al., 2014).

Nesfatin-1 is a peptide orphan ligand with hormone-like actions, produced by the N-terminal cleavage of its precursor nucleobindin-2 (NUCB2), encoded in the *nucb2* gene (Oh-I et al., 2006). Nesfatin-1 reduces feed intake after central or peripheral administration in mammals (Gonzalez, Mohan, & Unniappan, 2012) and fish (Kerbel & Unniappan, 2012), which supports an anorexigenic role of this hormone. In a previous work (Bertucci, Blanco, Canosa, & Unniappan, 2017), we found that nucb2/nesfatin-1 mRNA expression is modified by fatty acids in vitro. Therefore, we aimed to study whether dietary fatty acids can alter its gene expression at central and or peripheral level in vivo. These data together with other results from this experiment could help to elucidate the role of nesfatin-1 in fish metabolism and food intake regulation.

Macronutrient composition of diets is an important factor regulating not only weight gain and metabolism, but also growth performance by the regulation of genes involved in somatic growth, commonly referred to as GH-IGF axis genes (Reinecke, 2010). Growth hormone (GH) is secreted from the pituitary and has been involved in different physiological functions in fish, mainly associated with somatic growth (Reinecke et al., 2005) and stress resistance (Deane & Woo, 2009; Yousefian & Shirzad, 2011), among others. This hormone exerts its action through the receptors GHR-I and GHR-II that show high expression in liver, still in early stages of development (Ozaki et al., 2006; Rhee et al., 2012). In response to this binding, the liver expresses and releases the insulin-like growth factor I and II (IGF-I and IGF-II), which act in an endocrine manner promoting the somatic growth on different tissues (Wood, Duan, & Bern, 2005). Also, IGF-I can be expressed and act in an autocrine–paracrine manner in some tissues such as brain, gastrointestinal tract, pancreas, kidney, gonads, muscle, bone, and skin (Reinecke, 2010).

In summary, dietary fish oil replacement could decrease pejerrey aquaculture production costs. This might not be possible if this produce negative consequences on fish growth rate, survival and flesh properties including fatty acid composition. Therefore, the aims of this research were to determine whether sun flower oil can replace fish oil in pejerrey fry feed, and to elucidate how this change might affect somatic growth parameters, survival rate and fatty acid composition of fish as well as the expression of genes implicated in somatic growth (*gh-igf* axis), feed intake control (*nucb2/nesfatin-1*) and fatty acid metabolism (*Δ6-desaturase*).

2 | MATERIALS AND METHODS

2.1 | Fish management and experimental design

Fertilized eggs of pejerrey were acquired from Estación Hidrobiológica Chascomús (Buenos Aires, Argentina). During all the experiment, fry were maintained in 140-L open flow-through water system tanks (water flow of 5 L/min). The water salinity was 15 g/L, the oxygen concentration 8 ppm, the photoperiod was 12L:12D, and the water temperature was maintained at 18°C. After hatching, fry were fed with nauplii of *Artemia* sp. four times per day during 30 days prior to the assay. One week before the onset of the experiment, a co-feeding schedule with *Artemia* nauplii and a commercial starter feed (crude protein, 430 g/Kg; crude fat, 30 g/Kg; crude fibre, 30 g/Kg; Shullet bebe®, Shulet, Argentina) was established. After this adaptation period, fish were divided into 12 tanks (n = 52 fish/tank). Four experimental groups were established (n = 3 tanks/group), each fed until apparent satiety 3 times per day with diets containing the same composition except from different per cent of fish oil (FO)/sunflower oil (SfO), as described in Table 1. The objective of this experiment was to replace partially or totally the fraction of fish oil added as ingredient of aquafeed formulation and the diets names refer to the replacement of this fraction of fish oil by sunflower oil and no to the overall lipid content of the diet, which is composed by other oil sources such as lecithin, fish meal and Hake fillet. The proximal composition was determined according to Association of Official Analytical Chemists (1990) methods. Crude protein was estimated as 6.25 × total nitrogen (N), determined using the semi-micro-Kjeldahl method. Crude lipid was determined gravimetrically of the sulphuric ether extract of 1 g samples. Moisture was measured gravimetrically after drying in an oven at 105°C for 3 hr, and the ash content by combustion in a muffle at 550°C for 6 hr. Fatty acid composition of each diet was also determined by gas chromatography with FID detector of fatty acid methyl esters (FAME) prepared from aliquots of total lipid extracted by the Folch method (Folch, Lees, & Sloane-Stanley, 1957). Fatty acid profile of diets is shown in Table 1. Feed was weighed every day before the first meal and after the last one to calculate the daily feed consumption. This parameter was calculated as the g of feed consumed per day in each tank divided by

TABLE 1 Ingredients and proximate of the experimental diets

	100% FO	50% SfO	80% SfO	100% SfO
Ingredients (g kg^{-1} Sample)				
CMC	37.0	37.0	37.0	37.0
Agar	15.0	15.0	15.0	15.0
Vitamin and Minerals[a]	52.0	52.0	52.0	52.0
Starch	117.0	117.0	117.0	117.0
NaCl	30.0	30.0	30.0	30.0
Gluten	50.0	50.0	50.0	50.0
Hake Fillet	318.0	318.0	318.0	318.0
Fish meal[b]	318.0	318.0	318.0	318.0
Soy Lecithin	25.0	25.0	25.0	25.0
Fish oil	38.0	19.0	8.0	0.0
Sunflower oil	0.0	19.0	30.0	38.0
PC (g kg^{-1} Sample)				
Humidity	55.40	52.40	73.30	70.40
Crude lipid	159.20	167.00	182.20	157.10
Crude protein	407.70	403.60	404.80	396.60
Ash	203.10	198.40	156.30	189.50
Carbohydrates	174.60	178.60	183.40	186.40
FA (g Kg^{-1} sample)				
14:00	3.50	2.90	3.40	1.80
15:00	0.60	0.50	0.50	0.30
16:00	31.30	28.10	31.70	21.80
17:00	1.20	0.90	0.90	0.50
18:00	5.80	5.90	6.10	5.60
20:00	0.40	0.40	0.30	0.40
24:00:00	–	0.20	0.20	0.20
Total SFA (g Kg^{-1} sample)	42.70	38.80	43.20	30.60
14:01	0.20	–	–	
16:01	9.0	6.9	6.9	4.1
17:01	0.60	0.50	0.50	0.30
18:01	31.60	37.90	43.10	40.10
20:01	4.60	3.50	2.30	1.80
22:01	2.80	2.10	1.30	1.00
24:01:00	0.70	0.60	0.30	0.30
Total MUFA (g Kg^{-1} sample)	49.60	51.50	54.40	47.70
16:02	0.60	0.50	0.40	0.20
18:2n6 cis	21.70	40.50	55.60	54.90
20:2n6	0.40	0.30	0.20	0.80
18:3n3	3.40	3.20	3.20	2.70
20:3n6	0.20	0.40	0.40	0.50
20:3n3	0.20	–	–	–
22:3n3	0.50	0.40	0.30	0.20
18:4n3	2.60	2.00	1.80	1.20
20:4n6	1.40	1.10	0.90	1.20
20:4n3	0.80	0.60	0.40	0.30
22:4n6	0.40	0.40	0.20	0.20
20:5n3	11.80	8.80	6.90	5.60
22:5n3	1.30	0.90	0.70	0.50
22:6n3	20.00	16.00	11.70	10.20
Total PUFA (g Kg^{-1} sample)	65.80	75.30	83.00	78.40
Total UFA (g Kg^{-1} sample)	115.40	127.10	140.40	126.10
Total n-3 Fatty Acids	41.00	32.20	25.20	20.90
Total n-6 Fatty Acids	24.20	42.60	57.30	56.40

[a]"Vitafac Super Acqua." Provided per kg of feed: retinol, 24,000 I.U.; vitamin D_3, 4,800 I.U.; tocopherol, 500 I.U.; vitamin K_3, 16 mg; thiamine, 20 mg; riboflavin, 40 mg; pyrodoxine-HCl, 30 mg; vitamin B_{12}, 0.06 mg; ascorbic acid, 300 mg; niacin, 300 mg; pantothenic acid, 80 mg; folic acid, 12 mg; biotin, 2 mg; zinc, 140 mg; iron, 200 mg; manganese, 100 mg; copper, 10 mg; cobalt, 4 mg; iodine, 3.4 mg; selenium, 6 mg.

[b]620 g of crude protein per kilo, Moliendas del Sur S.A., Mar del Plata, Argentina.

CMC, carboxymethyl cellulose; PC, proximate composition; FA, fatty acids; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; UFA, unsaturated fatty acids.

the number of fry present in each tank. Total number, biomass and standard length (Sl) of fry from each tank were measured day 0 (before start of the assay) and days 15, 30 and 45. We were unable to collect brain, pituitary, gut, liver and muscle due to the small size of the fry. Instead, samples of head (with no gills, head kidney and heart), trunk (from the gills to the anus) and tail (from the anus to the end of the caudal fin) were frozen in liquid nitrogen and stored at −80°C until total RNA extraction.

2.2 | Total RNA extraction and real-time quantitative PCR (RT-qPCR) analysis

Total RNA from fish tissues was extracted using the Ambion® TRIzol® Reagent (Life Technologies, USA) following the protocol provided by the manufacturer. Tissues were initially disrupted using a PRO 200 homogenizer (PRO Scientific Inc, USA), and 1 μg of total RNA was reverse transcribed into cDNA using SuperScript II ReverseTM Transcriptase (Invitrogen, USA). Expression of the genes was measured by real-time quantitative PCR (RT-qPCR) according to Bertucci et al., 2017;. The efficiency of the amplification for all genes studied was 95–100%. Gene expression levels were calculated by the $2^{-\Delta\Delta C_t}$ comparative threshold cycle (C_t) method, where $\Delta C_t = \Delta C_t$ sample − ΔC_t reference (Livak & Schmittgen, 2001). All primer sequences are shown in Table S1.

2.3 | Fry fatty acid composition

Total lipids and FA from six lyophilized individuals (randomly selected) were analysed as described in Guinot et al. (2013). When in doubt, identity of FA was further assessed through GC-MS after splitless injection in an Agilent 6850 Gas Chromatograph system, equipped with a Sapiens-5MS (30 m × 0.25 μm × 0.25 μm) capillary column (Teknokroma, Sant Cugat del Vallés, Barcelona, Spain) coupled to a 5975 series MSD (Agilent Technologies, Santa Clara, CA, USA).

2.4 | Statistical analysis

Data were analysed by one-way ANOVA, followed by post hoc Student–Newman–Keuls (SNK) test at a significance level of $p < .05$. Data that failed to pass homogeneity and normality tests were log-transformed and re-tested. All tests were performed using SigmaPlot version 12.0 and Infostat Version 2008, softwares. The FA profiles obtained were integrated chemometrically in a principal component analysis (PCA) model. All the FAs were introduced in the model as variables, but only those showing communality higher than 0.5 were used for the final output. The score plot obtained after the generation of the two principal components was used to identify patterns of distribution of FA among dietary treatments. Statistical analyses were performed with the SPSS for Windows 15.0 statistical package (SPSS Inc., Chicago, IL, USA).

3 | RESULTS

3.1 | Growth and feed intake

After 45 days of feeding with diets containing different per cent of FO/SfO, pejerrey fry almost triplicated their initial weight in all cases, with no statistically significant differences ($p > .05$) detected among the different groups (Table 2). Additionally, the body weight increase (BWI), the specific growth rate (SGR) and the condition factor (K) were not significantly different ($p > .05$) among groups throughout the duration of the assay (Table 2). We did not find any difference ($p > .05$) in the standard length (Sl) among groups of fish fed with the different experimental diets after 45 days (Table 2). Furthermore, the survival per cent indicates that the four diets tested produced the same rates of mortality, without significant differences ($p > .05$) among groups (Table 2). Statistical analysis of the daily feed consumption (grams of dry matter of feed ingested per fry) and feed efficiency ratio indicates that no significant differences ($p > .05$) are present among groups.

3.2 | Influence of dietary lipid source on *gh-igf* axis, *Δ6-desaturase* and *nucb2/nesfatin-1* gene expression

Quantification of *gh* mRNA expression was performed in the head of fry, because we were unable to take pituitary or complete brain. As shown in Figure 1a, after 45 days of feeding with the experimental diets, no differences ($p > .05$) in *gh* expression were observed among groups fed with 100% FO, 50% SfO and 80% SfO diets. However, *gh* showed a higher expression ($p < .05$) in the head of fry fed with diet containing only SfO compared with the other three treatments. Gene expression of GH receptors was measured in trunk (Figure 1b) instead of liver, because due to the small size of fry, we were unable to collect the livers. We found that *ghr-I* and *ghr-II* mRNA expression increased ($p < .05$) in fish fed with 100% SfO diet, compared to those fed with 100% FO, 50% SfO and 80% SfO diets. No differences ($p > .05$) in the expression of these receptors were found in 100% FO, 50% SfO and 80% SfO treatments. Concerning IGFs, mRNA encoding *igf-I* was detectable in head but no differences ($p > .05$) in expression were observed among experimental groups (Figure 1c). Expression of this gene was undetectable in trunk and tail of pejerrey fry in any of the experimental groups. Gene expression of *igf-II* was not significantly different ($p > .05$) among groups in head and tail (Figure 1d), but a higher expression ($p < .05$) was observed in the trunk of fry that were fed with 100% SfO diet compared with fish fed with the other three diets.

As shown in Figure 2, the *Δ6-desaturase* gene expression was up-regulated ($p < .05$) in the head and trunk (Figure 2a) of pejerrey fry feed with diet containing only SfO as an oil source. No differences ($p > .05$) were observed between treatments in tail (Figure 2a). The *nucb2/nesfatin-1* gene expression in the head of pejerrey fry did not change with the dietary FO replacement (Figure 2b). Nonetheless, a decrease ($p < .05$) in *nucb2/nesfatin-1* gene expression with an increase in the dietary SfO inclusion was observed in the trunk of pejerrey fry (Figure 2b).

3.3 | Lipid and fatty acid composition of pejerrey fry

In Table 3, a list of fatty acids as a per cent of total fatty acids from total lipids of pejerrey fry from each dietary group is provided. The substitution of dietary FO with SfO generates a decrease ($p < .05$) in the per cent of fatty acids of the n-3 group, such as alpha-linolenic acid, EPA and DHA. Also, the dietary fish oil replacement generates an increase ($p < .05$) of the n-6 fatty acids, such as linoleic acid and docosapentaenoic acid. In summary, no differences ($p > .05$) were observed in the total per cent of saturated and monounsaturated fatty acids present in fry feed with the four experimental diets, but an increase ($p < .05$) in the total amount of PUFA of the n-6 group and a decrease ($p < .05$) of PUFA of the n-3 group when the dietary FO is replaced partially or totally by SfO was observed. Table 3 also shows the DHA/EPA ratio. This ratio increases ($p < .05$) when the percent of dietary SfO increases from 0% to 80%, while no differences ($p > .05$) were observed between 80% SfO and 100% SfO.

The PCA results are summarized in Fig. S1. The first two components accounted for 83% of total variance. First component (55% variance) was clearly associated with oil substitution in the diets, with n-6 variables typical from SfO loaded on the negative side of the axis, away from n-3 ones who loaded on the positive side. As for the second component that accounted for 28% of total variance, AA and C18 and C20 saturates loaded heavily on the negative side. This results in a score plot (Fig. S1b) that allows to clearly segregate the dietary groups on the first component, with the fatty acid patterns of fish fed FO separated from those fed intermediate substitution (50% SfO) who in turn are also

TABLE 2 Data on growth performance, food consumption and per cent of survival of fry fed with the experimental diets for 45 days

	100% FO	50% SfO	80% SfO	100% SfO
Initial BW (g)	0.061 ± 0.002	0.065 ± 0.002	0.064 ± 0.002	0.062 ± 0.002
Final BW (g)	0.16 ± 0.02	0.16 ± 0.01	0.18 ± 0.01	0.16 ± 0.01
WG (g)	0.10 ± 0.01	0.09 ± 0.01	0.11 ± 0.01	0.01 ± 0.01
BWI %	158 ± 23	140 ± 7	179 ± 9	157 ± 10
SGR % in weight	2.1 ± 0.2	1.9 ± 0.1	2.3 ± 0.1	2.1 ± 0.1
Initial Sl (mm)	20.0 ± 0.5	20.2 ± 0.5	20.0 ± 0.5	19.7 ± 0.5
Final Sl (mm)	29 ± 1	27 ± 1	29 ± 1	28 ± 1
Initial K	0.57 ± 0.02	0.58 ± 0.02	0.59 ± 0.02	0.61 ± 0.04
Final K	0.60 ± 0.03	0.57 ± 0.04	0.54 ± 0.03	0.55 ± 0.01
DFC (g DM/ larvae/ day)	0.279 ± 0.004	0.279 ± 0.002	0.310 ± 0.020	0.310 ± 0.010
FER (g/g DM)	0.90 ± 0.10	0.86 ± 0.03	1.01 ± 0.06	0.87 ± 0.08
Survival %	77 ± 2	73 ± 4	73 ± 3	75.6 ± 1

Each value is the mean ± SEM of triplicate groups. All data were analysed with one-way ANOVA, and no differences were found between treatments. Weight gain = final weight − initial weight. BWI: body weight increase = ([final weight − initial weight]/initial weight) × 100. SGR: specific growth rate (weight) = (100 × [ln final fish wt. − ln initial fish wt.])/45 days. K: Condition factor = (weight/Sl^3) × 100. DFC: daily food consumption = food consumed per day per tank/number of fry in the tank. FER: feed efficiency ratio = weight gain/food consumption.
DM, dry matter; Sl, standard length; BW, body weight; WG, weight gain.

clearly distinct from the two most substituted dietary groups (80% SfO and 100% SfO).

4 | DISCUSSION

In this work, we report a substitution with sunflower oil in diets of pejerrey fry, without compromising somatic growth and weight gain. Additionally, results indicate that replacement of fish oil added as ingredient in the diet might be total, at least in the first stages of pejerrey development. These results are in concordance with studies mentioned above, in which authors reported that fish oil replacement with sunflower oil does not affect growth rate, final body weight, weight gain, total feed consumption, feed efficiency ratio and survival of the species tested (Bransden et al., 2003; Mourente et al., 2005; Turchini & Francis, 2009).

One important aspect observed in the present study is that feed consumption and feed efficiency ratio were not significantly different among fish fed with the four experimental diets tested, indicating that pejerrey might be able to obtain energy and structural precursors from fatty acids with low degree of unsaturation as those present in sunflower oil since early stages of development. Survival rate is an important issue, which influences the production cost of commercial fish culture. It has been demonstrated that a manipulation of dietary n-6/n-3 ratio affects fish pathogen resistance and immune parameters, such as the activity of the alternative complement pathway or the phagocytic activity of head kidney macrophages (Montero et al., 2008). This fact makes it necessary to evaluate whether dietary fish oil replacement affects the survival rate of fish treated in order to discard any negative effect that can generate a decrement in fish production. In our study, we observed that mortality ratios show no significant differences among experimental groups. Indeed, survival per cent in each group was around 70%–75%, which is consistent with previous reports by other authors (Miranda, Berasain, Velasco, Shirojo, & Somoza, 2006) and can be considered as normal in this developmental stage. These results indicate that replacement of FO with SfO at least in early stages of pejerrey development does not compromise fry survival.

The GH-IGF system plays a major role coordinating growth of vertebrates (Reindl & Sheridan, 2012) and could be used as a marker of nutritional status and growth performance (Pérez-Sánchez & Le Bail, 1999). In fish, *gh* gene expression occurs very early in development (Martí-Palanca & Pérez-Sánchez, 1994), and the increase in body weight reported in gilthead sea bream larvae after its oral administration suggests a growth promoting effect of GH during early development (Ben-Atia et al., 1999). *Ghr-I* mRNA expression has been reported in Chilean flounder larvae at 8 days after hatching (dah) in somites and head, and at 9.5 dah in almost the whole larvae (Fuentes et al., 2008). Early mRNA expression of *igf*-I and *igf*-II was also observed in gilthead sea bream (Perrot et al., 1999). In the present work, we found a higher mRNA expression of *gh* in head and of *ghr*-I and *ghr*-II in trunk of fish fed with 100% SfO diet. Moreover, the expression of *igf*-II was higher in the trunk of fish from the same experimental group. Together, all these observations may indicate an action mediated by GH-IGF system in response to a total replacement of dietary fish oil, although this response would not necessarily imply any additional stimulation of somatic growth. Fasting and malnutrition have been previously associated with changes in GH-IGF axis (Pérez-Sánchez, Martí-Palanca, & Kaushik, 1995; Wilkinson, Porter, Woolcott, Longland, & Carragher, 2006). Authors from these works found that, during food deprivation, plasmatic levels of GH increase, while a decrease is observed for

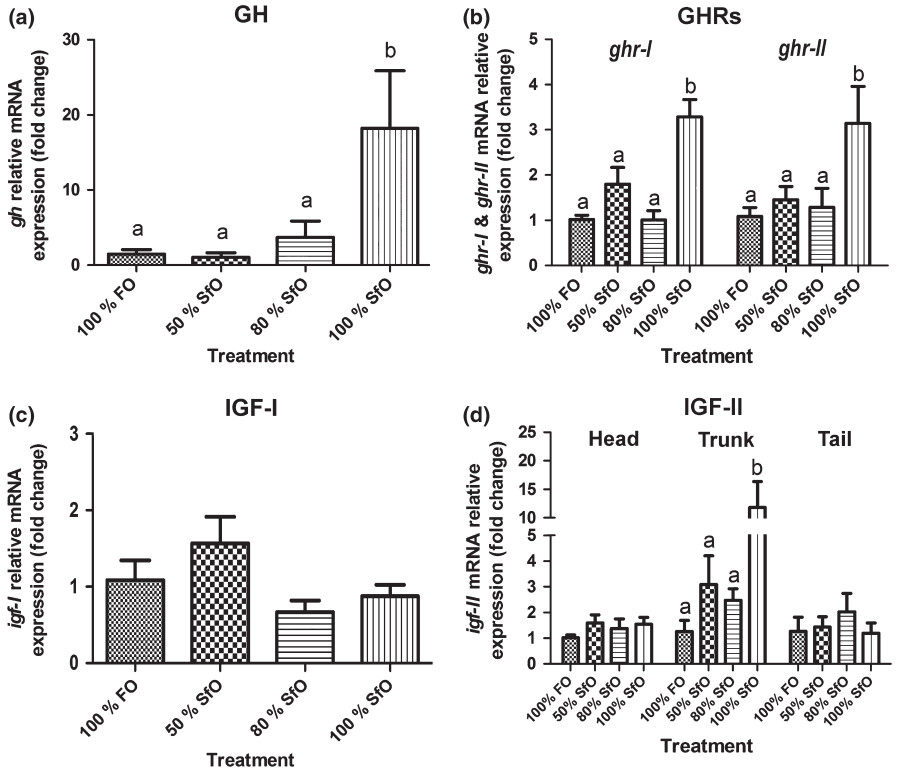

FIGURE 1 (a) *gh* mRNA expression levels in head, (b) *ghr-I* and *ghr-II* expression levels in trunk, (c) *igf-I* expression level in head and (d) *igf-II* expression level in head, trunk and tail of fry of pejerrey after 45 days of treatment. Bars represent mean ± SE (n = 3). Different letters indicate significant differences among treatments (one-way ANOVA, SNK test, $p < 0.05$). In graphs b and d, letters indicate significant differences between treatments for each gene (b) or fry section (d)

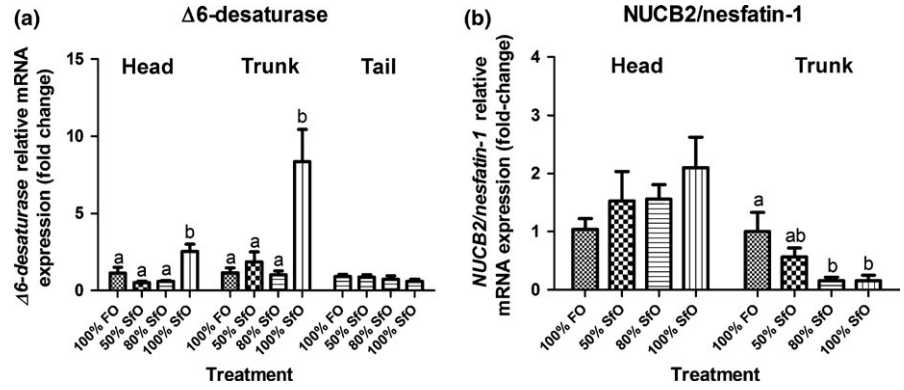

FIGURE 2 Influence of the dietary oil source on the *Δ6-desaturase* (a) and *nucb2*/nesfatin-1 (b) gene expression in the head, trunk and tail of pejerrey fry. Bars represent media ± SE (n = 3). Different letters indicate significant differences among treatments (one-way ANOVA, SNK, $p < .05$)

plasma levels of IGF-I and IGF-II and mRNA levels of *gh* receptors and *igf-I* in liver. Although we found a high expression of *gh* in fry fed with 100% SfO diet, which could be correlated with high plasma levels of GH, but contrary to the results described above, we observed a concomitant increase in the mRNA expression of *ghr-I*, *ghr-II* and *igf-II*. Thus, this could indicate that the change in mRNA expression of *gh-igf* axis in response to 100% SfO diet is not an indication of malnutrition in the pejerrey fry. It would be interesting to further study whether this upregulation of *gh*, *ghr*-I, *ghr-II* and *igf-II* could also have an effect on somatic growth in subsequent stages of pejerrey development. As we did not observe any signal of differential somatic growth between 100% SfO group and the other three treatment groups, we hypothesized that the over expression of *gh*, *ghr-I*, *ghr-II* and *igf-II* might be related with lipid metabolism and energy mobilization (Pérez-Sánchez et al., 2002; Vijayakumar, Novosyadlyy, Wu, Yakar, & LeRoith, 2010). Concerning *igf-I* mRNA expression, we were able to detect it only in the head of pejerrey fry but not in the trunk or tail. Due to the small size of fry tissues, the whole trunk and tail were to be used instead of liver and muscle, and thus, it is unavoidable that samples contain mRNA from other tissues as well and thus a relative lower amount of *igf-I* mRNA which might make it gene expression undetectable. This fact might indicate a higher amount of *igf-II* mRNA present in pejerrey fry compared with *igf-I* at the moment of sample collection. In head, both genes were expressed at the same level in each dietary treatment, indicating a tissue specific action of IGF-I and IGF-II, at least in this stage of pejerrey.

To know how dietary composition could affect fatty acid metabolism, we studied the influence of dietary fish oil replacement on pejerrey *Δ6-desaturase* tissue gene expression. Although Δ4-desaturase activity has been reported in other species of the same family (*Chisrostoma estor*, Fonseca-Madrigal et al., 2014), thus allowing DHA synthesis independently of Sprecher's shunt, *Δ6-desaturase*

TABLE 3 Fatty acid composition (percentage of total fatty acids) of total lipid of pejerrey fry fed with the experimental diets

Fatty acid/Diet	0% SfO	50% SfO	80% SfO	100% SfO
14:0	1.21 ± 0.03^{a}	1.10 ± 0.03^{a}	0.908 ± 0.04^{b}	0.85 ± 0.03^{b}
14:1	0.12 ± 0.01^{a}	0.120 ± 0.004^{a}	0.110 ± 0.002^{a}	0.09 ± 0.004^{b}
15:0	0.37 ± 0.02^{a}	0.34 ± 0.01^{ab}	0.301 ± 0.007^{b}	0.29 ± 0.02^{b}
16:0	20.7 ± 0.4^{a}	20.1 ± 0.8^{a}	17.3 ± 0.3^{b}	17.3 ± 0.7^{b}
16:1n-9	0.67 ± 0.02	0.67 ± 0.01	0.69 ± 0.02	0.66 ± 0.03
16:1n-7	4.37 ± 0.06^{a}	3.1 ± 0.1^{b}	2.73 ± 0.05^{c}	2.33 ± 0.04^{d}
16:2	0.30 ± 0.01	0.32 ± 0.01	0.27 ± 0.05	0.25 ± 0.04
17:0	0.55 ± 0.02^{a}	0.46 ± 0.01^{b}	0.35 ± 0.02^{cd}	0.34 ± 0.02^{d}
16:3	0.61 ± 0.01^{a}	0.470 ± 0.005^{b}	0.41 ± 0.02^{cd}	0.37 ± 0.02^{d}
16:4	0.020 ± 0.004	0.02 ± 0.01	0.07 ± 0.05	0.04 ± 0.02
18:0	5.2 ± 0.2	5.9 ± 0.4	5.0 ± 0.2	5.4 ± 0.3
18:1n-9	19.3 ± 0.3^{a}	19.8 ± 0.5^{a}	23.1 ± 0.5^{b}	22.5 ± 0.2^{b}
18:1n-7	4.26 ± 0.06^{a}	3.39 ± 0.04^{b}	3.15 ± 0.06^{bc}	3.0 ± 0.1^{c}
18:2n-6	11.0 ± 0.2^{a}	16.3 ± 0.6^{b}	20.40 ± 0.06^{cd}	21.8 ± 0.9^{d}
18:3n-6	0.75 ± 0.06^{a}	1.66 ± 0.05^{b}	3.244 ± 0.3^{c}	3.97 ± 0.3^{d}
18:3n-3	1.03 ± 0.03^{a}	0.81 ± 0.06^{b}	0.753 ± 0.008^{b}	0.67 ± 0.04^{b}
18:4n-3	0.57 ± 0.03^{a}	0.40 ± 0.03^{bc}	0.35 ± 0.02^{cd}	0.28 ± 0.02^{d}
20:0	0.17 ± 0.01	0.18 ± 0.02	0.147 ± 0.008	0.150 ± 0.004
20:1n-9	1.78 ± 0.03^{a}	1.23 ± 0.04^{bc}	1.10 ± 0.07^{c}	0.89 ± 0.03^{d}
20:1n-7	0.270 ± 0.004^{a}	0.199 ± 0.002^{ab}	0.164 ± 0.002^{ab}	0.08 ± 0.03^{b}
20:2n-6	0.30 ± 0.01^{a}	0.35 ± 0.01^{b}	0.38 ± 0.02^{b}	0.39 ± 0.02^{b}
21:0	0.21 ± 0.01^{a}	0.41 ± 0.02^{b}	0.89 ± 0.03^{c}	1.03 ± 0.04^{d}
20:3n-6	0.04 ± 0.01^{a}	0^{b}	0.003 ± 0.002^{b}	0 b
20:4n-6	1.23 ± 0.05	1.19 ± 0.06	1.09 ± 0.06	1.2 ± 0.1
20:3n-3	0.16 ± 0.01^{a}	0.11 ± 0.01^{bc}	0.092 ± 0.002^{c}	0.07 ± 0.01^{d}
20:4n-3	0.36 ± 0.01^{a}	0.23 ± 0.03^{bc}	0.19 ± 0.01^{cd}	0.14 ± 0.02^{d}
20:5n-3	2.04 ± 0.09^{a}	1.51 ± 0.07^{b}	0.94 ± 0.04^{cd}	0.88 ± 0.03^{d}
22:0	0.07 ± 0.01^{a}	0.12 ± 0.01^{b}	0.114 ± 0.006^{b}	0.11 ± 0.01^{b}
22:1n-11	0.40 ± 0.05	0.30 ± 0.04	0.29 ± 0.01	0.290 ± 0.004
22:1n-9	0.26 ± 0.02^{ab}	0.22 ± 0.02^{b}	0.16 ± 0.01^{cd}	0.150 ± 0.005^{d}
22:1n-7	0.05 ± 0.01	0.05 ± 0.01	0.058 ± 0.003	0.05 ± 0.001
22:4n-6	0.08 ± 0.01	0.06 ± 0.01	0.086 ± 0.004	0.07 ± 0.01
22:5n-6 22:3n-3	0.330 ± 0.01^{a}	0.310 ± 0.01^{ab}	0.46 ± 0.02^{c}	0.540 ± 0.004^{d}
22:5n-3	1.43 ± 0.03^{a}	1.09 ± 0.08^{b}	0.84 ± 0.03^{cd}	0.76 ± 0.03^{d}
22:6n-3	16.1 ± 0.3^{a}	13.6 ± 0.3^{b}	10.8 ± 0.5^{cd}	10.8 ± 0.5^{d}
24:0	0.57 ± 0.02	0.66 ± 0.09	0.453 ± 0.03	0.50 ± 0.05
24:1	0.02 ± 0.01	0.02 ± 0.01	0.007 ± 0.006	0
Saturated	29.1 ± 0.7	29 ± 1	25.5 ± 0.5	26 ± 1
Monounsaturated	31.5 ± 0.5	29.2 ± 0.7	31.5 ± 0.6	30.1 ± 0.3
PUFA n-6	14.7 ± 0.2^{a}	20.6 ± 0.4^{b}	26.4 ± 0.2^{c}	28.6 ± 0.8^{d}
PUFA n-3	21.8 ± 0.2^{a}	17.8 ± 0.4^{b}	14.0 ± 0.6^{cd}	13.6 ± 0.5^{d}
EPA + DHA	18.1 ± 0.3	15.1 ± 0.3	11.8 ± 0.6	11.7 ± 0.5
DHA/EPA	7.9 ± 0.3^{a}	9.0 ± 0.2^{b}	11.5 ± 0.2^{c}	12.7 ± 0.4^{c}

Different letters (a, b c, d) within a row indicate significant differences in content of the corresponding fatty acid among diets (n = 3, one-way ANOVA, SNK, p < .05). Letters indicate significant differences among values. This is expressed in the legend. Those letters should appear as supper index.
PUFA, polyunsaturated fatty acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

expression seems a better marker of the effects of putative nutritional regulation driven by SfO substitution, as Δ6-desaturases are expected to act over 18:2n-6 and 18:3n-3 in the first steps leading to the synthesis of long-chain PUFA either through direct Δ4 activity or through the above mentioned Sprecher's pathway. We found that mRNAs levels encoding Δ6-desaturase were higher in head and

trunk of fry fed with 100% SfO diet compared with the other groups that were fed with a different mix of SfO and FO. This result is in concordance with previous studies in other fishes reporting a nutritional regulation of *Δ6-desaturase* gene expression by the dietary oil source (Ren, Yu, Xu, & Tang, 2012; Sarker et al., 2011). The observed response of *Δ6-desaturase* gene expression to total FO replacement was tissue specific, as the high expression mentioned above was not found in tail. Therefore, considering that the tail portion of pejerrey fry is composed mainly by muscle, we can consider that changes in *Δ6-desaturase* gene expression in head and trunk were not due to the muscle tissue present there. We attribute those changes to other organs such as brain in head (rich in long-chain PUFA) and liver (lipid metabolism), intestine (absorption) and gonads (rich in long-chain PUFA) in trunk of pejerrey fry. This might indicate a dietary regulation of PUFA and HUFA synthesis in central and peripheral tissues such as those mentioned above. While a higher expression of *Δ6-desaturase* was observed in 100% SfO fed fry, no differences were observed among the other groups despite the decreasing levels of fish oil. This could be explained in two ways. On one hand, a dietary FO content of at least the amount present in 80% SfO diet (Table 1) presumably helped by the Δ4 activity, meets the requirements of PUFAs and HUFAs for pejerrey fry. On the other hand, a decrease in the dietary FO content with the concomitant reduction of dietary PUFAs and HUFAs could promote compensatory changes in *Δ6-desaturase* activity not observed at gene expression level, but presumably taking place at enzyme activity or protein translation levels. Thus, changes in gene expression would be observed only when dietary PUFAs and HUFAs content fall below a minimum level, such as the amount supplied by 80% SfO diet (dietary inclusion of 8 gr/Kg of FO, Table 1).

Although the substitution of dietary FO with oil from plant sources is a topic of increasing interest in aquaculture, only few studies have been carried out to know how that replacement could affect the expression of peptides involved in food intake regulation (Bonacic et al., 2016). In this experiment, we tested how this replacement affects *nucb2/nesfatin-1* mRNA expression in pejerrey fry. While no effects were found in head, a decrement in the *nucb2/nesfatin-1* mRNA expression with an increase in the dietary SfO inclusion was found in trunk. In the work by Bonacic et al. (2016), the authors found that fish oil and plant oil led to differential regulation of genes involved in the control of feed intake, although no apparent pattern of expression was detected for orexigenic and anorexigenic genes. As we observed in a previous work (Bertucci et al., 2017), fatty acids alter the expression of *nucb/nesfatin-1* in goldfish intestine and hepatopancreas organ culture, suggesting an involvement of nesfatin-1 in the fatty acid metabolism. Additionally, in that work we found a downregulation of *nucb2/nesfatin-1* gene expression by oleic acid, which is present in a high proportion in the sunflower oil. These in vitro results are consistent with the in vivo results found in the present paper. Considering these results, we hypothesize that changes found in the *nucb2/nesfatin-1* mRNA expression could be associated not only with feed intake regulation but also with changes in the fatty acid metabolism.

We also studied the fatty acid composition from total lipids of the complete pejerrey fry. Results show that the dietary FO replacement with SfO generates a decrease in the total amount of the n-3 PUFAs and an increase in the n-6 PUFAs. This change is predictable, because the increases in the dietary SfO elevate the amount of n-6 PUFAs, which are typical components of the vegetable oils, and a decrease in the n-3, which are presents in fish oil. Despite this fact, pejerrey fry were able to generate EPA and DHA (n-3 PUFAs) and DPA (n-6 PUFA), which are the ultimate products of each biosynthetic pathway, even when FO is not added in the diet as suggested by the increase of DHA/EPA and DPA/AA ratios. Our results, together with those from Kopprio et al. (2015), suggest that pejerrey has the capability to desaturate and elongate fatty acids, which are necessary for survival and growth. As n-3 PUFAs are a desirable component of fish flesh and the replacement of dietary FO by SfO does not decrease dramatically the total amount of n-3 PUFAs (specifically the amount of DHA and EPA) in whole fry, this strategy could be useful for pejerrey aquaculture to reduce the production costs.

In summary, the present work shows for the first time in pejerrey that the supplemented fraction of dietary fish oil could be totally replaced by sunflower oil since early stages of development without compromising fish growth, BWI and other important aquaculture parameters, such as survival rate. Moreover, we found a high expression of *gh-igf* axis genes in response to complete fish oil replacement. We attribute this to changes in lipid metabolism, although future studies are required to investigate this and whether these changes can generate an increase in somatic growth. Concerning lipid metabolism, we show a tissue specific regulation of *Δ6-desaturase* expression by the dietary fish oil content. This result together with the fatty acid composition analysis of fry support the idea that pejerrey has the enzymes necessary to synthesize PUFAs from precursors as those present in vegetable oils. In conclusion, replacement of fish oil by sunflower oil in pejerrey fry feed does not affect growth and this strategy could be used to reduce production costs of this commercial fish.

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REFERENCES

Association of Official Analytical Chemists (1990). *Official methods of analysis of the Association of Official Analytical Chemists* (15th edn, p. 684). Arlington, VA: Association of Official Analytical Chemists.

Ben-Atia, I., Fine, M., Tandler, a, Funkenstein, B., Maurice, S., Cavari, B., & Gertler, A. (1999). Preparation of recombinant gilthead seabream (*Sparus aurata*) growth hormone and its use for stimulation of larvae growth by oral administration. *General and Comparative Endocrinology*, *113*(1), 155–164.

Bertucci, J. I., Blanco, A. M., Canosa, L. F., & Unniappan, S. (2017). Glucose, Amino Acids and Fatty Acids Directly Regulate Ghrelin and NUCB2/Nesfatin-1 in the Intestine and Hepatopancreas of Goldfish (Carassius auratus) In Vitro. *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology*, *206*, 24–35.

Bonacic, K., Campoverde, C., Gómez-Arbonés, J., Gisbert, E., Estevez, A., & Morais, S. (2016). Dietary fatty acid composition affects food intake and gut–brain satiety signaling in Senegalese sole (Solea senegalensis, Kaup 1858) larvae and post-larvae. *General and Comparative Endocrinology*, *228*, 79–94.

Bransden, M. P., Carter, C. G., & Nichols, P. D. (2003). Replacement of fish oil with sunflower oil in feeds for Atlantic salmon (*Salmo salar* L.): Effect on growth performance, tissue fatty acid composition and disease resistance. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *135*(4), 611–625.

Deane, E. E., & Woo, N. Y. S. (2009). Modulation of fish growth hormone levels by salinity, temperature, pollutants and aquaculture related stress: A review. *Reviews in Fish Biology and Fisheries*,

Folch, J., Lees, M., & Sloane-Stanley, G. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, *226*, 497–509.

Fonseca-Madrigal, J., Navarro, J. C., Hontoria, F., Tocher, D. R., Martinez-Palacios, C. A., & Monroig, O. (2014). Diversification of substrate specificities in teleostei Fads2: Characterization of Δ4 and Δ6-Δ5 desaturases of *Chirostoma estor*. *The Journal of Lipid Research*, *55*(7), 1408–1419.

Fuentes, E., Poblete, E., Reyes, A. E., Vera, M. I., Alvarez, M., & Molina, A. (2008). Dynamic expression pattern of the growth hormone receptor during early development of the Chilean flounder. *Comparative Biochemistry and Physiology B-Biochemistry and Molecular Biology*, *150*(1), 93–102.

Glencross, B., & Turchini, G. (2010). Fish oil replacement in starter, grow-out, and finishing feeds for farmed aquatic animals. In Fish Oil (Ed.), *Replacement and alternative lipid sources in aquaculture feeds* (pp. 373–404). Boca Raton, FL: CRC Press.

Gonzalez, R., Mohan, H., & Unniappan, S. (2012). Nucleobindins: Bioactive precursor proteins encoding putative endocrine factors? *General and Comparative Endocrinology*, *176*(3), 341–346.

Grosman, F., & González Castelain, J. (1995). Experiencias de alimentación y crecimiento con alevinos de pejerrey (*Odontesthes bonariensis*) dirigidas a optimizar la siembra. *Revista de Ictiología Argentina*, *4*(1–2), 5–9.

Guinot, D., Monroig, Ó., Navarro, J. C., Varó, I., Amat, F., & Hontoria, F. (2013). Enrichment of *Artemia* metanauplii in phospholipids and essential fatty acids as a diet for common octopus (*Octopus vulgaris*) paralarvae. *Aquaculture Nutrition*, *19*(5), 837–844.

Kerbel, B., & Unniappan, S. (2012). Nesfatin-1 suppresses energy intake, co-localises ghrelin in the brain and gut, and alters ghrelin, cholecystokinin and orexin mRNA expression in goldfish. *Journal of Neuroendocrinology*, *24*(2), 366–377.

Kopprio, G. A., Graeve, M., Kattner, G., & Lara, R. J. (2015). Fatty acid composition of wild *Odontesthes bonariensis* (Valenciennes 1835) larvae: Implications on lipid metabolism and trophic relationships. *Journal of Applied Ichthyology*, *31*, 752–755.

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C}{}_{T}$ Method. *Methods*, *25*, 402–408.

Martí-Palanca, H., & Pérez-Sánchez, J. (1994). Developmental regulation of growth hormone binding in the gilthead sea bream, *Sparus aurata*. *Growth Regulation*, *4*(1), 14–19.

Miranda, L. a., Berasain, G. E., Velasco, C. a M., Shirojo, Y., & Somoza, G. M. (2006). Natural spawning and intensive culture of pejerrey *Odontesthes bonariensis* juveniles. *Biocell*, *30*(1), 157–162.

Miranda, L. A., & Somoza, G. M. (2001). Biología reproductiva del pejerrey *Odontesthes bonariensis*: Diferenciación sexual y endocrinología de la reproducción. Aspectos básicos y su potencial aplicación en acuicultura. In F. Grosman (Ed.), *Fundamentos biológicos, económicos y sociales para una correcta gestión del recurso pejerrey* (pp. 41–45). Buenos Aires: Editorial Astyanax.

Montero, D., Grasso, V., Izquierdo, M. S., Ganga, R., Real, F., Tort, L., … Acosta, F. (2008). Total substitution of fish oil by vegetable oils in gilthead sea bream (*Sparus aurata*) diets: Effects on hepatic Mx expression and some immune parameters. *Fish and Shellfish Immunology*, *24*(2), 147–155.

Mourente, G., Good, J. E., & Bell, J. G. (2005). Partial substitution of fish oil with rapeseed, linseed and olive oils in diets for European sea bass (*Dicentrarchus labrax* L.): Effects on flesh fatty acid composition, plasma prostaglandins E2 and F2α, immune function and effectiveness of a fish oil fin. *Aquaculture Nutrition*, *11*(1), 25–40.

Oh-I, S., Shimizu, H., Satoh, T., Okada, S., Adachi, S., Inoue, K., … Mori, M. (2006). Identification of nesfatin-1 as a satiety molecule in the hypothalamus. *Nature*, *443*(7112), 709–712.

Ozaki, Y., Fukada, H., Tanaka, H., Kagawa, H., Ohta, H., Adachi, S., … Yamauchi, K. (2006). Expression of growth hormone family and growth hormone receptor during early development in the Japanese eel (*Anguilla japonica*). *Comparative Biochemistry and Physiology. Part B, Biochemistry and Molecular Biology*, *145*(1), 27–34.

Palacios, E., Racotta, I. S., Aparicio, B., Arjona, O., & Martínez-Palacios, C. A. (2007). Lipid classes and fatty acids during embryogenesis of captive and wild silverside (*Chirostoma estor estor*) from Pátzcuaro Lake. *Fish Physiology and Biochemistry*, *33*(1), 81–91.

Pérez-Sánchez, J., Calduch-Giner, J. a, Mingarro, M., de Celis, S. V. R., Gomez-Requeni, P., Saera-Vila, a, & , … , M. M. (2002). Overview of fish growth hormone family. New insights in genomic organization and heterogeneity of growth hormone receptors. *Fish Physiology and Biochemistry*, *27*(3–4), 243–258.

Pérez-Sánchez, J., & Le Bail, P. Y. (1999). Growth hormone axis as marker of nutritional status and growth performance in fish. *Aquaculture*, *177*(1), 117–128.

Pérez-Sánchez, J., Martí-Palanca, H., & Kaushik, S. J. (1995). Ration size and protein-intake affect circulating growth-hormone concentration, hepatic growth-hormone binding and plasma insulin-like growth-factor-I immunoreactivity in a marine teleost, the gilthead sea bream (*Sparus-aurata*). *Journal of Nutrition*, *125*(3), 546–552.

Perrot, V., Moiseeva, E. B., Gozes, Y., Chan, S. J., Ingleton, P., & Funkenstein, B. (1999). Ontogeny of the insulin-like growth factor system (IGF-I, IGF-II, and IGF-1R) in gilthead seabream (*Sparus aurata*): Expression and cellular localization. *General and Comparative Endocrinology*, *116*, 445–460.

Reindl, K. M., & Sheridan, M. A. (2012). Peripheral regulation of the growth hormone-insulin-like growth factor system in fish and other vertebrates. *Comparative Biochemistry and Physiology. Part A, Molecular and Integrative Physiology*, *163*(3–4), 231–245.

Reinecke, M. (2010). Influences of the environment on the endocrine and paracrine fish growth hormone-insulin-like growth factor-I system. *Journal of Fish Biology*, *76*(6), 1233–1254.

Reinecke, M., Björnsson, B. T., Dickhoff, W. W., McCormick, S. D., Navarro, I., Power, D. M., & Gutiérrez, J. (2005). Growth hormone and insulin-like growth factors in fish: Where we are and where to go. *General and Comparative Endocrinology*, *142*(1), 20–24.

Ren, H., Yu, J., Xu, P., & Tang, Y. (2012). Influence of dietary fatty acids on muscle fatty acid composition and expression levels of Δ6 desaturase-like and Elovl5-like elongase in common carp (Cyprinus carpio varJian).

Comparative Biochemistry and Physiology. Part B, Biochemistry and Molecular Biology, *163*(2), 184–192.

Rhee, J.-S., Kim, B.-M., Seo, J. S., Kim, I.-C., Lee, Y.-M., & Lee, J.-S. (2012). Cloning of growth hormone, somatolactin, and their receptor mRNAs, their expression in organs, during development, and on salinity stress in the hermaphroditic fish, *Kryptolebias marmoratus*. *Comparative Biochemistry and Physiology. Part A, Molecular and Integrative Physiology*, *161*(4), 436–442.

Sarker, M. A.-A., Yamamoto, Y., Haga, Y., Sarker, M. S., Miwa, M., Yoshizaki, G., & Satoh, S. (2011). Influences of low salinity and dietary fatty acids on fatty acid composition and fatty acid desaturase and elongase expression in red sea bream *Pagrus major*. *Fisheries Science*, *77*(3), 385–396.

Somoza, G. M., Miranda, L. A., Berasain, G. E., Colautti, D., Remes Lenicov, M., & Strüssmann, C. a. (2008). Historical aspects, current status and prospects of pejerrey aquaculture in South America. *Aquaculture Research*, *39*(7), 784–793.

Sprecher, H. (2000). Metabolism of highly unsaturated n-3 and n-6 fatty acids. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids*, *1486*(2), 219–231.

Turchini, G. M., & Francis, D. S. (2009). Fatty acid metabolism (desaturation, elongation and beta-oxidation) in rainbow trout fed fish oil- or linseed oil-based diets. *The British Journal of Nutrition*, *102*(1), 69–81.

Vagner, M., & Santigosa, E. (2011). Characterization and modulation of gene expression and enzymatic activity of delta-6 desaturase in teleosts: A review. *Aquaculture*, *315*(1–2), 131–143.

Vijayakumar, A., Novosyadlyy, R., Wu, Y., Yakar, S., & LeRoith, D. (2010). Biological effects of growth hormone on carbohydrate and lipid metabolism. *Growth Hormone and IGF Research: Official Journal of the Growth Hormone Research Society and the International IGF Research Society*, *20*(1), 1–7.

Wijekoon, M. P. A., Parrish, C. C., & Mansour, A. (2014). Effect of dietary substitution of fish oil with flaxseed or sunflower oil on muscle fatty acid composition in juvenile steelhead trout (*Oncorhynchus mykiss*) reared at varying temperatures. *Aquaculture*, *433*, 74–81.

Wilkinson, R. J., Porter, M., Woolcott, H., Longland, R., & Carragher, J. F. (2006). Effects of aquaculture related stressors and nutritional restriction on circulating growth factors (GH, IGF-I and IGF-II) in Atlantic salmon and rainbow trout. *Comparative Biochemistry and Physiology. Part A, Molecular and Integrative Physiology*, *145*(2), 214–224.

Wood, A. W., Duan, C., & Bern, H. A. (2005). Insulin-like growth factor signaling in fish. *International Review of Cytology*, *243*, 215–285.

Yildiz, M., & Sener, E. (1997). Effect of dietary supplementation with soybean oil, sunflower oil or fish oil on the growth of seabass (*Dicentrarchus labrax* L. 1758). Feeding Tomorrow's Fish. Proceedings of the Workshop of the CIHEAM Network on Technology of Aquaculture in the Mediterranean TECAM, Jointly Organized by CIHEAM, FAO and IEO, Mazarron Spain, *22*, 225–233.

Yousefian, M., & Shirzad, E. (2011). The review of the effect of growth hormone on immune system, metabolism and osmoregulation of fish. *Australian Journal of Basic and Applied Sciences*, *5*, 467–475.

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