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High broodstock *fads2* expression combined with nutritional programing through broodstock diet improves the use of low fishmeal and low fish oil diets in gilthead seabream (*Sparus aurata*) progeny

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ABSTRACT

One of the factors that limits the replacement of fish meal (FM) and fish oil (FO) by plant ingredients in diets for marine fish, is their lack of long chain-polyunsaturated fatty acids (LC-PUFA). LC-PUFA are essential fatty acids for these fish species, which lack sufficient fatty acyl desaturase 2 (Fads2) activity to synthesize them. Nutritional programing or the use of broodstock with a higher Fads2 activity could improve marine fish ability to synthesize LC-PUFA and their ability to use low FM and FO diets. The aim of this study was to determine the effect of gilthead seabream broodstock with inherently high or inherently low *fads2* gene expression and nutritional programing with broodstock diets rich in FO or rapeseed oil (RO) on the progeny growth performance, liver morphology, biochemical composition and expression of selected genes. Sea bream juveniles (2.31± 0.01 g initial body weight, mean ± SD) obtained from broodstock with either high (H) or low (L) *fads2* expression and fed a broodstock diet based on FO or RO were randomly distributed into 12 × 250 L tanks and nutritionally challenged for 45 days with a diet containing only 7.5% FM and no FO. The highest growth was found in juveniles from broodstock with a high *fads2* expression and fed the RO diet, whereas the lowest growth was obtained in those from broodstock with a low *fads2* expression and fed the RO diet. Juveniles from broodstock with high *fads2* expression showed significantly higher *fads2* expression in liver and increased PUFA contents in liver and muscle. Replacement of FO by RO in broodstock diets led to a significantly increased hepatic 18:3n-6/18:2n-6 ratio and reduction in the viscerosomatic index of the progeny juveniles, the hepatocyte size and the *ghr-1/ghr-2* expression in muscle. Overall, the results showed significant trans-generational effects of both the broodstock *fads2* expression and the type of lipid in the broodstock diet on the metabolism and performance of the juvenile progeny challenged with a diet low in FM and FO.

1. Introduction

Besides being a well-balanced source of minerals and highly digestible proteins, fish food are the main source of n-3 long chain polyunsaturated fatty acid (n-3 LC-PUFA) for people. Therefore, fish demand by consumers is continuously increasing. Due to the stagnant production of fisheries, aquaculture is taking over the responsibility to provide safe and sustainable fish food to satisfy market demands (FAO, 2020). However, further development of aquaculture is restricted by the limited availability and increasing prices of fishmeal (FM) and fish oil (FO),

Abbreviations: ALA, α-linolenic acid; *cox2*, cycloxigenase-2 gene; *cpt-1*, carnitin palmitoil transferase 1 gene; DHA, docosahexaenoic acid; *elovl6*, elongase 6 gene; EPA, eicosapentaenoic acid; Fads2, Fatty acyl desaturase 2; *fads2*, Fatty acyl desaturase 2 gene; FAMES, fatty acid methyl esters; FCR, feed conversion ratio; FI, feed intake; FM, fishmeal; FO, fish oil; gh, growth hormone; Ghr, Growth hormone receptor; HSI, hepatosomatic index; LC-PUFA, long-chain polyunsaturated fatty acid; Lpl, lipoprotein lipase; LNA, linoleic acid; LO, linseed oil; OA, oleic acid; PA, palmitic acid; RO, rapeseed oil; *rpl-27*, ribosomal protein l27 gene; SGR, specific growth rate; *tnf-α*, tumor necrosis factor-alpha gene; VSI, viscerosomatic index..

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traditional protein and lipid sources in aquafeeds that are mostly derived from capture fisheries. Therefore, dietary FM and FO need to be replaced by other high quality and nutritious ingredients with a more economically, environmentally and socially sustainable production. Many alternative feedstuffs are used to replace FM and FO in aquafeeds such as plant ingredients, animal byproducts, single cell ingredients or insect meals (Caballero et al., 2002; Rimoldi et al., 2018; Rosales et al., 2017; Wang et al., 2016). However, depending on the type of ingredient and the replacement level, these alternative feedstuffs may lead to malnutritional effects on fish growth, nutrients digestibility, immune system, etc. (Caballero et al., 2004; Castro et al., 2015; Gómez-Requeni et al., 2004; Vergara et al., 1996a; Vergara et al., 1996b).

One of the factors that restricts the replacement of FM and FO by alternative ingredients is their frequent lack of essential fatty acids. FO is rich in n-3 long chain polyunsaturated fatty acids, especially docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), which are important components of biomembranes for normal cell functioning (Gorjão et al., 2009). Therefore, they are necessary for the adequate development of brain and larval tissues (Mourente, 2003), immune system functioning (Zuo et al., 2012), reproductive performance (Fernández-Palacios et al., 2011) and flesh nutritional value (Monge-Ortiz et al., 2018). LC-PUFA can be synthesized from α-linolenic acid (ALA) and linoleic acid (LNA) in vertebrates through a series of desaturation and elongation reactions (Bell and Tocher, 2009), starting from desaturation of oleic acid, ALA or LNA to 18:2n-9, 18:4n-3 or 18:3n-6, respectively, by fatty acid desaturase 2 (Fads2) (Vagner and Santigosa, 2011). Marine fish have a limited capacity to synthetize LC-PUFA, what could be related to the abundance of LC-PUFAs in the marine food webs, originating from phytoplankton (Sargent et al., 2003). Since the plant oils in feeds used to replace FO lack n-3 LC-PUFA but are rich in their precursors (ALA and LNA), enhancing the marine fish capacity to synthetize LC-PUFA would facilitate the replacement of FO by plant oils.

Nutritional programing is a common strategy employed by nature that allows an organism to adapt its metabolism to the environmental conditions. Nutritional programming refers to the metabolic consequences of a nutritional stimulus applied at a critical period during life, such as pre- or postnatal stages (Lucas, 1994). A well-known example can be found in honeybees that may become a fertile queen or a sterile worker through the consumption or not of royal jelly (Kucharski et al., 2008). Specific evidence of the regulation of nutritional programming on lipid metabolism include the long-term reduction of plasma cholesterol, high-density lipoprotein cholesterol and triacylglycerol caused by malnutrition during both pre- and postnatal periods in mouse (Lucas et al., 1996). Similarly, in fish, nutritional stimulus during reproduction by feeding broodstock with different dietary fatty acid profiles markedly affects lipid metabolism and growth of the progeny (Hou and Fuiman, 2019; Izquierdo et al., 2015; Turkmen et al., 2019b). For instance, feeding gilthead sea bream (*Sparus aurata*) broodstock with diets containing partial replacement of FO by linseed oil (LO), low in n-3 LC-PUFA but high in their ALA precursor, up-regulated *fads2* expression and growth in the progeny (Izquierdo et al., 2015). Besides, it also up-regulated other lipid metabolism and health related genes, such as cycloxigenase-2 (*cox2)* and tumor necrosis factor-alpha (*tnf-a)*, lipoprotein lipase (*lpl*), carnitin palmitoil transferase 1 (*cpt1*) or elongase 6 (*elovl6*) (Turkmen et al., 2017a, 2017b; Turkmen et al., 2019a). Moreover, when the 4-month-old progeny were fed a low FM and FO diet, those fish from parents fed partial FO replacement by LO showed improved growth and feed utilization (Izquierdo et al., 2015; Turkmen et al., 2019a). Indeed, broodstock feeding exerts a very long-term effect in the progeny, and the replacement of FO by LO in parental diets, in combination with juvenile feeding with low-FM and low-FO diets, markedly improves 16-month-old offspring growth and feed utilization (Turkmen et al., 2017a, 2017b). These studies demonstrated that it is possible to improve the ability of marine fish to use low FM and low FO diets by nutritional programing though broodstock feeding. Similarly, exposure to a vegetable-based diet in early life of Atlantic salmon (*Salmo salar*) improves growth performance and feed efficiency later in life, when fish are challenged with a low FO and FM diet (Clarkson et al., 2017).

Finally, choosing broodstock with higher *fads2* expression could also be an effective way to produce fish better able to use low FO diets. Recent studies have demonstrated that progeny obtained from broodstock with an inherently high expression of *fads2* shows improved growth and feed utilization, particularly when challenged with a low FM and low FO diet (Turkmen et al., 2019a). However, the potential beneficial effect of combining broodstock with a high *fads2* expression and nutritional programing failed to be demonstrated. More recently, we conducted a study aimed to evaluate the reproductive performance of broodstock with different *fads2* expression in combination with broodstock diets to stimulate nutritional programing (Ferosekhan et al., 2020). In this study, gilthead seabream broodfish inherently having either a high (H) or low (L) *fads2* expression were fed during the spawning season with two diets containing different fatty acid profiles. The results demonstrated that blood *fads2* expression in females, which tended to be higher than in males, is positively related to plasma 17β-estradiol levels, and improved reproductive performance (Ferosekhan et al., 2020). However, the potential effect of these type of broodstock on progeny performance or *fads2* expression had not been yet determined. Thus, the present study, following the previous one, aimed to determine the combined effect of broodstock with different *fads2* expression and nutritional programing through broodstock diets on the progeny performance. For that purpose, juveniles obtained from the previous study (Ferosekhan et al., 2020) were fed a low FM and FO diet for 45 days, and their growth, feed utilization, fatty acid composition of different tissues and expression of selected genes were studied.

2. Material and methods

All the experiments were performed according to the European Union Directive (2010/63/EU) on the protection of animals for scientific purposes, at Fundación Canaria Parque Científico Tecnológico (FCPCT), University of Las Palmas de Gran Canaria (Las Palmas de Gran Canaria, Spain) (OEBA-ULPGC-20/2018).

2.1. Feeding trial and fish performance

Gilthead sea bream juveniles for this trial were obtained from broodstock with high (H) or low (L) *fads2* expression in blood when fed a low n-3-LC-PUFA diet and fed either with a high fish oil diet (FO) or with a high rapeseed oil diet (RO) during the spawning season (Ferosekhan et al., 2020). Twelve broodstock with relatively high *fads2* expression and 12 broodstock with relatively low *fads2* expression were distributed into twelve 1000 L tanks (6H and 6 L) and fed for 1 month with a diet that contained either FO or a mixture of 20% FO and 80% RO. Then eggs from each of the 4 broodstock groups (HFO, HRO, LFO and LRO) were collected in the same day and incubated in different tanks. The offspring obtained were reared under the same culture conditions and commercial diets (Fig. 1). 300 fish (2.31 ± 0.01 g) from each of the 4 groups were randomly distributed into 3 tanks of 200 L (100 fish/tank, a total of 12 tanks). Tanks were provided with 200 L/h seawater at 23.3 ± 0.3 °C and strong aeration, and dissolved oxygen (6.0 ± 0.3 mg/L) was daily determined. Tanks were illuminated by fluorescent lights placed above the tank at an intensity of 100 lx and programmed for a 12 h light photoperiod (from 8 a.m. to 8 p.m.).

In order to challenge the fish with a very low FM and FO diet for evaluating their potential ability to cope with low n-3 LC-PUFA, an experimental diet was formulated to contain 7.5% of FM and 0% FO (Table 1). Dietary FM was replaced by corn gluten, soybean meal, wheat gluten, soya protein concentrates and faba bean meal in the experimental diets. Poultry oil and RO were used to replace the dietary fish oil. The diet was low in ARA, EPA and DHA and high in oleic acid (OA, 18:1n-9), LNA, palmitic acid (PA, 16:0) and ALA (Table 2).

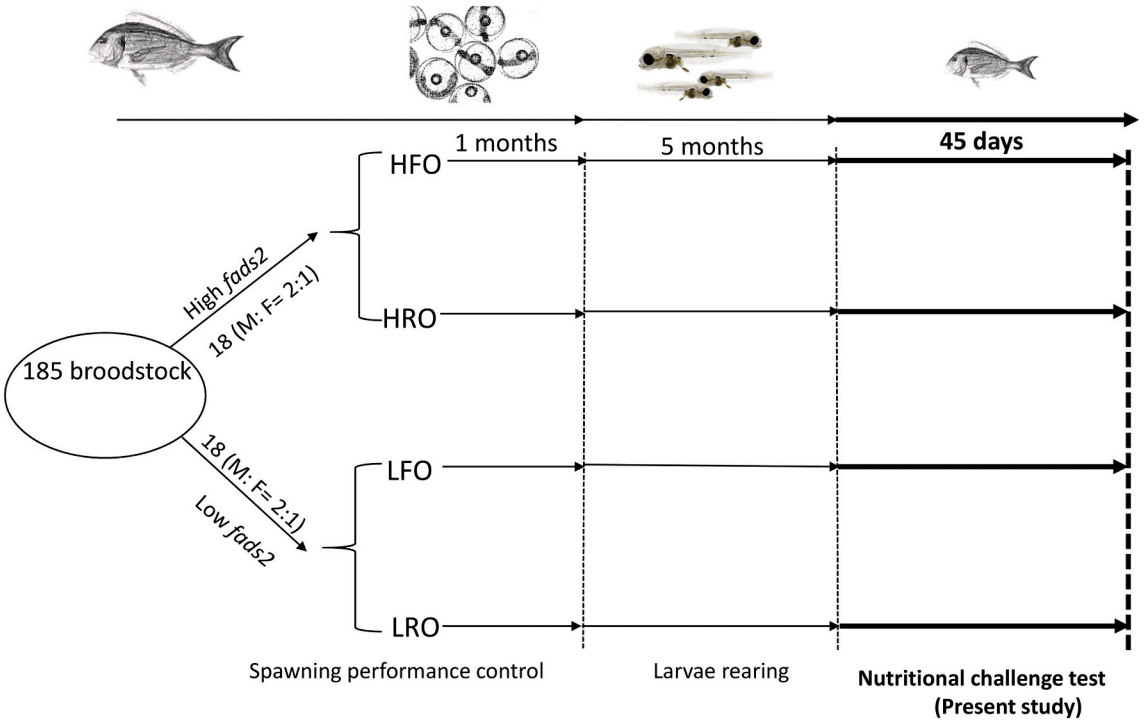

Fig. 1. Schematic view of the experimental design.

Fish were fed the experimental diet until apparent satiation 4 times per day (8:30, 10:30, 13:30 and 16:30) and 6 days per week for 45 days until fish body weight was tripled. Feed delivery was daily calculated, and uneaten pellets were collected in a net by opening the water outlet 30 min after each meal, dried in an oven for 24 h and weighed to estimate feed intake. All fish were weighted individually at day 30 and day 45 of the feeding trial. At the end of the trial, fish were fasted for 24 h, weighed and anesthetized with ethanol diluted clove oil (50:50) before samplings. The following performance parameters on mortality, growth (weight gain (WG), specific growth rate (SGR)), feed acceptance (feed intake (FI)), biological feed conversion ratio (FCR) (National Research Council, 2011), energy status (hepatosomatic index (HSI)) (Chellappa et al., 1995), lipid deposition (viscerosomatic index (VSI)), were calculated:

$$\text{Mortality}\ (\%) = 100^{*}(\text{n°dead fish}/\text{n°total fish})$$

$$\text{Weight gain}\ (\text{g}) = \text{final body weight}\ (\text{BW}_1)\text{–initial body weight}\ (\text{BW}_0)$$

$$\text{Specific growth rate}\ \left(\%\text{day}^{-1}\right) = 100^{*}(ln\ \text{BW}_1 - ln\ \text{BW}_0)/\text{n°days}$$

$$\text{Feedintake}\left(\text{FI,gfish}^{-1}\text{day}^{-1}\right) = \text{Feeddelivered}/(\text{numberoffish}^{*}\text{numberofdays})$$

$$\text{Biological feed conversion ratio (FCR)} = \text{Feed delivered}\ (t_1\text{–}t_0)/(\text{Biomass}\ t_1\text{–Biomass}\ t_0 + \text{Biomass}_{\text{harvested}} + \text{Biomass}_{\text{lost}})$$

$$\text{Hepatosomatic index (HSI)} = \text{Liver weight (g)}/\text{Body weight (g, weight of liver included)}$$

$$\text{Viscerosomatic index (VSI)} = \text{Visceral weight (g)}/\text{Body weight (g, viscerosomatic weight included)}$$

2.2. Histological study

The livers of 5 fish per tank were sampled and stored in 4% formalin. After embedded in paraffin wax, blocks were made and cut with a Leica microtome (Mod. Jung Autocut 2055; Leica, Nussloch, Germany) in 4 μm sections, which were placed in slides and stained with haematoxylin and eosin (H&E) (Martoja et al., 1970). Slides were studied and photos were taken by a light microscope (Olympus, Tokyo, Japan). Area, length of long and short axis of 60 hepatocyte per tank were analysed with ImagePro plus 6.0 (Media Cybernetics, Rockville, U.S.A.)

2.3. Biochemical analysis

2.3.1. Chemical composition and fatty acid analysis

At the end of the feeding trial, 5 fish per tank were euthanized by immersion in 10 ppm clove oil in methanol (50:50) and sampled to determine lipid content and fatty acid composition of whole-body, liver and muscle. All samples were frozen at −80 °C until analysis. All samples were homogenized immediately prior to analysis. Moisture was determined according to A.O.A.C.(Horwitz, 2002). Lipids were extracted with chloroform/methanol (2:1 *v*/v) (Folch et al., 1957) and then transmethylated to obtain the fatty acid methyl esters (FAMES) (Christie, 1989). FAMEs were then separated by gas liquid chromatography, quantified by flame ionization detector and identified by comparison with external standards and well-characterized FO (EPA 28, Nippai, Ltd. Tokyo, Japan) (Izquierdo et al., 1990; Masahiko and Watanabe, 1989).

Table. 1
Ingredients and proximate composition of the challenge diet used to feed the juveniles obtained from broodstock with different *fads2* expression (high H or low L) and fed either a FO or a RO broodstock diet.

Ingredient (% d.w.)	
Poultry oil[a]	6.14
Wheat[b]	9.46
Corn gluten[b]	10.00
Soybean meal[b]	5.00
Wheat Gluten[b]	19.18
Soya protein concentrate[b]	30.00
Faba beans[b]	5.00
Fish meal[b]	7.50
Rapeseed oil[b]	7.52
Premix vit[c]	0.10
Premix min[c]	0.10
Proximate composition (% d.w.)	
Crude protein	53.74
Crude lipid	22.90
Crude ash	4.71

[a] Poultry oil: Sonac. B.V. The Netherlands.
[b] Skretting AS (Norway).
[c] Trouw Nutrition, Boxmeer, the Netherlands, proprietary composition Skretting ARC (Stavanger, Norway).

2.4. RNA extraction and digital PCR

The liver and muscle from the juveniles after the nutritional challenge were kept at 4 °C overnight in 2.0 mL tubes with 1.5 mL of RNAlater and then transferred to −80 °C until molecular analysis. RNA from 100 mg sample was extracted using TRI Reagent (MilliporeSigma, Darmstadt, Germany) and then purified by RNeasy kit (Qiagen, Hilden, Germany). RNA quality was checked by 1.4% agarose electrophoresis and quantity was measured by Nanodrop 1000 (ThermoFisher, Waltham, U.S.A.). cDNA was synthesized from 1 μg of RNA in iCycler (Bio-rad, Hercules, U.S.A.), using the iScript cDNA synthesis kit (Bio-Rad). The expression of key enzymes related with growth and lipid metabolism were determined using the primers listed in Table 3 Digital PCR was performed as previous described in (Xu et al., 2019) using in QX200™ Droplet Digital™ PCR System (Bio-Rad). The amplification conditions of PCR were: 95 °C for 5 min, followed by 40 cycles at 95 °C for 30 s, elongation at Tm temperature for 1 min, and then stabilized the signal at 4 °C for 5 min, 90 °C for 5 min, finally the reaction was hold at 4 °C.

2.5. Data analysis

Two-way ANOVA was used to test for effects of broodstock *fads2* expression and broodstock diet, as well as their interaction, on the progeny's performance through SPSS 20.0 (IBM, New York, U.S.A.). Main effect analysis was performed for analyzing the effect of broodstock *fads2* expression or broodstock diet on offspring across the other factor. Besides, a pairwise comparison was used to test for the effect of one factor within one level of the other factor (simple effect analysis) (Enders, 2003). Post-hoc tests were adjusted using Bonferroni procedure through SPSS. Unless otherwise stated, data were shown as mean ± S.D. Normality was assessed by Shapiro-Wilk tests and homogeneity of variance was tested with Levene's test. Principal component analysis (PCA) was performed through Prism 9 for Mac (GraphPad Software, San Diego, USA). Gene expression was normalized by hepatic expression of *rpl27* (Park and Crowley, 2005). Correlations were analysed using Peason correlation coefficients calculated throught SPSS.

Table 2
Fatty acid composition (% total fatty acids) of the challenge diet used for juveniles obtained from broodstock with different *fads2* expression (high H or low L) and fed either a FO or a RO broodstock diet.

Fatty acid	%
14:0	0.39
14:1n-7	0.04
14:1n-5	0.02
15:0	0.05
15:1n-5	0.02
16:0 ISO	0.04
16:0	9.99
16:1n-7	1.52
16:1n-5	0.01
16:2n-4	0.02
17:0	0.03
16:3n-4	0.08
16:3n-3	0.02
16:3n-1	0.03
16:4n-3	0.05
16:4n-1	0.01
18:0	3.74
18:1n-9	42.03
18:1n-7	2.48
18.1n-5	0.06
18:2n-9	0.03
18.2n-6	25.09
18:2n-4	0.06
18:3n-6	0.16
18:3n-4	0.06
18:3n-3	5.43
18.3n-1	0.02
18:4n-3	0.21
18:4n-1	0.02
20:0	0.55
20:1n-9	0.15
20:1n-7	1.68
20.1n-5	0.07
20:2n-9	0.02
20:2n-6	0.15
20:3n-9	0.03
20:3n-6	0.11
20:4n-6	0.27
20:3n-3	0.10
20:4n-3	0.09
20:5n-3	0.85
22:1n-11	1.33
22:1n-9	0.34
22:4n-6	0.07
22:5n-6	0.20
22:5n-3	0.30
22:6n-3	1.98
n-6/n-3	2.88
18:2n-6/18:3n-3	4.62

3. Results

3.1. Feeding trial and fish performance

Feeds were well accepted by all fish groups and no significant differences were found in feed intake or mortality rates. Even after only 30 days of feeding, the highest body weight was found in juveniles from broodstock with high *fads2* and fed diet RO (HRO), which was significantly ($p<0.05$) higher than that of HFO and LRO (Fig. 2). Thus, at the end of the trial, the largest growth in terms of body weight, weight gain and SGR were found in juveniles from broodstock with high *fads2* and fed RO diet (HRO), whereas the lowest was found in juveniles obtained from broodstock with low *fads2* and fed RO diet (LRO) (Fig. 2, Table 4).. The two-way ANOVA showed a highly significant ($p=0.001$) interaction between the broodstock *fads2* expression (*H*,*L*) and the broodstock diet (LO, FO) on SGR (Table 4). According to the simple main effect analysis,

Table 3
Primers sequence for digital PCR and GeneBank accession numbers for sequences of target genes.

Gene	Forward	Reverse	GenBank accession
rpl-27	ACA ACT CAC TGC CCC ACC AT	CTT GCC TTT GCC CAG AA CTT	AY188520
fads2	CGA GAG CCA CAG CAG CAG GGA	CGG CCT GCG CCT GAG CAG TT	AY055749
elovl6	GTG CTG CTC TAC TCC TGG TA	ACG GCA TGG ACC AAG TAG T	JX975702
igf-1	GTG TGT GGA GAG AGA GGC TT	CTC TTG GCA TGT CTG TGT GG	AY996779.2
ghr-1	ACC TGT CAG CCA CCA CAT GA	TCG TGC AGA TCT GGG TCG TA	AH014067.4
ghr-2	GAG TGA ACC CGG CCT GAC AG	GCGGTGGTATCTGATTCATGGT	AH014068.4
cox-2	GAG TAC TGG AAG CCG AGC AC	GAT ATC ACT GCC GCC TGA GT	AM296029

SGR was 23% higher in HRO than in LRO juveniles (p=0.002), whereas it was 12% higher in LFO than in HFO juveniles (p=0.029) (Table 4). Comparing the juveniles from broodstock with the same *fads2* expression, SGR was 16% higher in HRO than in HFO juveniles (p=0.009), whereas it was 19% higher in LFO than in LRO juveniles (p=0.005). The FCR values followed a similar trend with a significant interaction between broodstock *fads2* expression and broodstock diet (p=0.023) (Table 4). The simple main effect analysis showed that the FCR was 17% lower in HRO than in LRO juveniles (p=0.033), whereas no significant differences were found between HFO and LFO juveniles (p>0.05). No significant differences were observed for HSI (p>0.05). Regarding VSI, increased *fads2* expression in broodstock or FO replacement by RO in broodstock diets significantly reduced VSI in juveniles (p=0.035, p=0.001, respectively) (Table 4). Thus, VSI was significantly lower in HRO than in HFO juveniles (p=0.007) and significantly lower in LRO than in LFO juveniles (p=0.014).

3.2. Histology study

The area, length and width of the hepatocyte of juveniles from broodstock fed with RO diet was 21%, 14% and 8% smaller than that from broodstock fed with FO diet (p_{area}=0.023, p_{length} =0.002, p_{width}=0.031) (Table 5). A positive correlation was found between the area of hepatocyte and the HSI of fish (r=0.96, p=0.043). The hepatocyte width in juveniles from broodstock with high *fads2* expression was 11% shorter than that from broodstock with low *fads2* expression (p=0.011). In the pairwise comparison, the width of HRO hepatocytes was significantly shorter than that of HFO and LRO hepatocytes (p = 0.038 and p = 0.018, respectively).

3.3. Biochemical analysis

No significant differences were found in lipid contents of whole fish, liver or muscle (p>0.05) (Table 6). Regarding the fatty acid profiles of liver, the two-way ANOVA showed the increase in hepatic PUFA contents in juveniles from broodstock with high *fads2* expression (p=0.014) and the interaction with the broodstock diet (p=0.015) (Table 7). Thus, the pairwise analysis showed that PUFA contents were higher in LRO than in LFO juveniles (p=0.036). Accordingly, hepatic n-6 PUFA were increased in juveniles from broodstock with high *fads2* expression (p=0.027), with higher values in HFO than in LFO juveniles (p=0.015). Also, hepatic n-3 fatty acids contents were significantly (p=0.017) affected by the interaction of broodstock *fads2* expression and

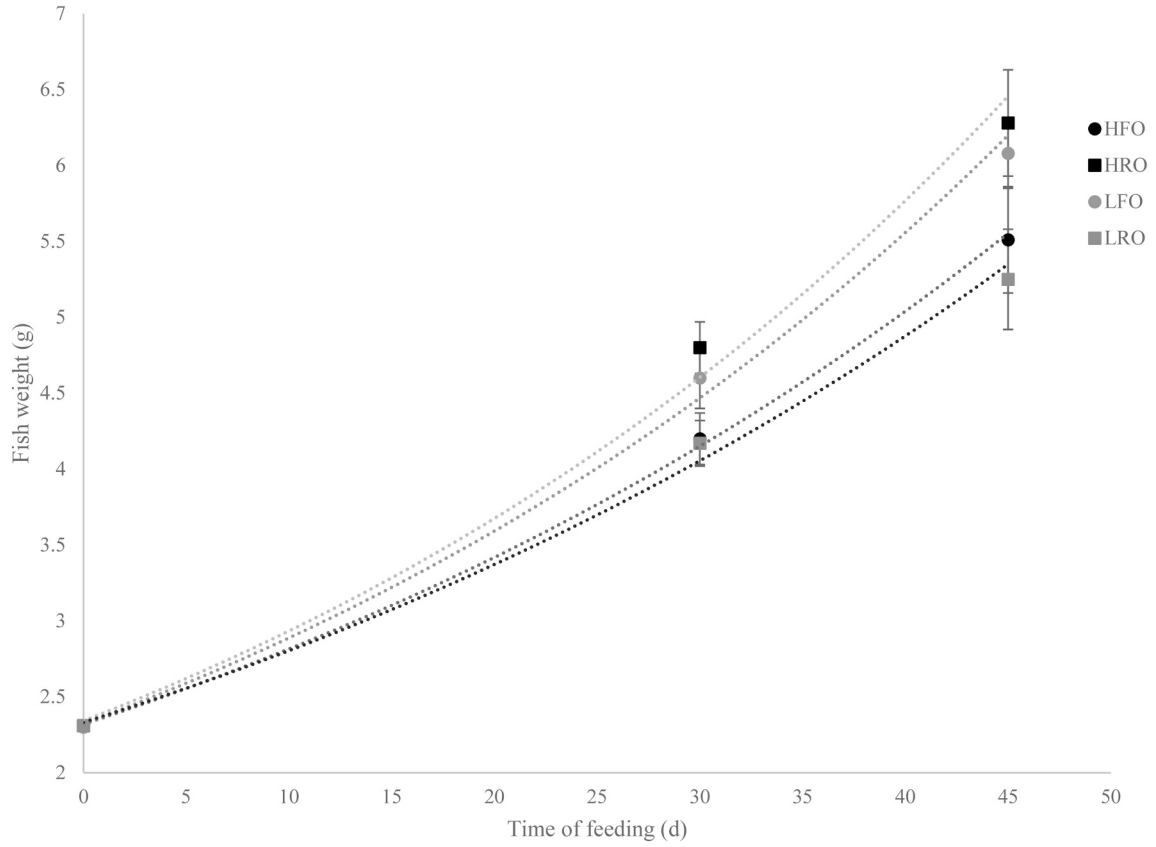

Fig. 2. Body weight of juveniles obtained from broodstock with different *fads2* expression (high H or low L) and fed either a FO or a RO broodstock diet, along the 45 days of feeding the challenge diet.

Table 4
Growth performance of gilthead seabream juveniles obtained from broodstock with different *fads2* expression (high H or low L) and fed either a FO or a RO broodstock diet, after 45 days of feeding the challenge diet.

	HFO		HRO		LFO		LRO		Two-way ANOVA		
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	*Bfads2*	*Bdiet*	*Bfads2*Bdiet*
Initial weight (g)	2.31	0.01	2.31	0.00	2.30	0.01	2.31	0.01	*n.s.*	*n.s.*	*n.s.*
Weight gain (g)	$^{b}3.20^{B}$	0.35	$^{a}3.98^{I}$	0.03	$^{1}3.78^{A}$	0.23	$^{2}2.94^{II}$	0.33	*n.s.*	*n.s.*	*0.001*
Mortality (%)	5.67	6.43	2.66	0.58	2.66	0.58	3.33	1.15	*n.s.*	*n.s.*	*n.s.*
SGR (%day)	$^{b}1.97^{B}$	0.15	$^{a}2.28^{I}$	0.02	$^{1}2.21^{A}$	0.09	$^{2}1.86^{II}$	0.14	*n.s.*	*n.s.*	*0.001*
Feed intake (g fish^{-1} day^{-1})	0.12	0.01	0.12	0.01	0.12	0.01	0.11	0.01	*n.s.*	*n.s.*	*n.s.*
FCR (%)	1.61	0.11	1.37^{II}	0.06	1.47	0.15	1.64^{i}	0.2	*n.s.*	*n.s.*	*0.023*
HSI (%)	2.31	0.17	2.21	0.25	2.41	0.11	2.22	0.11	*n.s.*	*n.s.*	*n.s.*
VSI (%)	$^{a}7.59$	0.64	$^{b}6.08$	0.62	$^{1}8.25$	0.5	$^{2}6.92$	0.16	*0.035*	*0.001*	*n.s.*

SGR: Specific growth rate; FCR: Feed conversion rate; HSI: Hepatic somatic index; VSI: Visceral somatic index; Bfads2 refers to the effect of the broodstock *fads2* expression (H or L); Bdiet refers to the effect of the broodstock diet (FO or RO).
[a, b]In front of the value mean there is significant difference between the offspring come from same selection broodstock group (High) but the broodstock were fed with different diet.
[1,2]In front of the value mean there is significant difference between the offspring come from same selection broodstock group (Low) but the broodstock were fed with different diet.
[A, B]In the back of the value mean there is significant difference between the offspring come from broodstock fed with diet FO but with different *fads2* expression.
[I, II]In the back of the value mean there is significant difference between the offspring come from broodstock fed with diet VO but with different *fads2* expression.
n.s. No statistical significance ($p>0.05$).

broodstock diets, with higher n-3 PUFA contents in HFO than in LFO (p=0.007) and in HFO than in HRO (p=0.032). The same trend was observed for ARA (p=0.012), with higher contents in HFO than in LFO (p=0.005) and in HFO than in HRO (p=0.009), and for EPA (p=0.036), with higher contents in HFO than in LFO (p=0.038).

SFA was 21% lower in HFO than LFO (p=0.019). MUFA showed a complementary trend to the PUFA contents, with lower values for HFO than for LFO juveniles (p_{MUFA}=0.007) and lower values for LRO than for LFO juveniles (p_{MUFA}=0.042). Besides, 16:1/16:0 ratios were higher in juveniles from broodstock with higher *fads2* expression than in those from low *fads2* expression broodstock (p=0.015). Also, juveniles from broodstock fed with RO diet showed significantly higher 18:3n-6/18:2n:6, 18:2n-9/18:1n-9 ratio than that from broodstock fed with FO diet ($p_{18:3n-6/18:2n:6}$ =0.033, $p_{18:2n-9/18:1n-9}$ =0.033). A negative correlation was found between 18:3n-6/18:2n:6 and the hepatocyte area (r =−0.999, p=0.001).

In agreement with these results the PCA of hepatic fatty acids profiles showed that PC1 accounted for most of the variance found, and together with PC2 explained up to 71.97% of the differences. Thus, a clear separation was found between HFO and LFO regarding PC1 (Fig. 3a and b) this separation was led in a higher extend by certain fatty acids such as 18:1n-9, 16:0, 16:1n-7 and 18:0, in agreement with the significant difference found for these fatty acids between HFO and LFO by the pairwise comparison by the simple effect analysis (Table 7).

In muscle, the n-6 fatty acid content and, particularly, 18:2n-6, were significantly higher in juveniles from high *fads2* expression broodstock than in those from broodstock with low *fads2* expression ($p_{n\text{-}6\ FA}$ =0.002; $p_{18:2n\text{-}6}$ =0.027). Juveniles from broodstock fed with RO diet had a greater ratio 16:1n-7/16:0 than those from broodstock fed with FO diet (p=0.039) (Table 8). In whole body, there was also an interaction with broodstock diets (p=0.046) and therefore, n-6 content was significantly higher in HRO than in LRO juveniles (p=0.012), and also higher in LFO than in LRO juveniles (p = 0.031) (Table 9). Besides, there was a combined effect of both factors, *fads2* expression and broodstock diet, on EPA (p=0.002), which was significantly higher in HFO than in HRO (p=0.033) or LRO (p=0.022). The PCA of fatty acid profiles in muscle and whole body did not showed marked differences among the 4 groups studied.

3.4. Gene expression

Hepatic *fads2* expression was 38% higher in juveniles from broodstock with high *fads2* expression (p =0.044) than in juveniles from broodstock with low *fads2* expression (Table 10). Moreover, a significant linear regression was found between the *fads2* expression in broodstock and in the juvenile progeny (r=0.97, p=0.01). Hepatic *fads2* expression in juveniles was also correlated to the SGR (r=0.580, *p=0.048*). The expression of hepatic *fads2* showed the interaction of broodstock *fads2* expression and broodstock diets (p = 0.049). Simple main effect analysis showed that expression of *elovl6* was 3.69 times higher in HFO than in HRO juveniles (p=0.019). *Elovl6* expression in liver was correlated to the EPA content in liver lipids (r=0.67, p=0.06). No significant differences were found in the expression of the other genes studied in the liver, including *cox-2*, *igf-1*, *srebp* or *ppara.* Nevertheless, *igf-1* in liver was negatively correlated to the HSI (r=−0.92, p=0.08) and the n-3 fatty acid content in muscle (r=−0.95, p=0.05). In muscle, the ratio *ghr-1/ ghr-2* showed a significant interaction of the broodstock *fads2* expression and the broodstock diet (p=0.039). Thus, the muscle *ghr1/ghr2* was significantly higher (p=0.04) in HFO juveniles than in HRO ones. Juveniles from broodstock fed with FO diet had a significantly higher *ghr1/ghr2* ratio than those from broodstock fed with RO (p=0.014).

Table 5
Morphometry of hepatocytes from gilthead seabream juveniles obtained from broodstock with different *fads2* expression (high H or low L) and fed either a FO or a RO rich diet, after 45 days of feeding the experimental diet.

	HFO		HRO		LFO		LRO		Two-way ANOVA		
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	*Bfads2*	*Bdiet*	*Bfads2*Bdiet*
Area (μm^2)	80.45	11.51	65.76	8.29	87.72	10.2	72.88	5.19	*n.s.*	*0.023*	*n.s.*
Long axis (μm)	11.11	0.86	9.53	0.33	11.54	0.51	10.3	0.21	*n.s.*	*0.002*	*n.s.*
Short axis(μm)	6.88	0.46	6.09	0.58	7.41	0.19	7.03	0.17	*0.011*	*0.031*	*n.s.*

n.s. No statistical significance ($p>0.05$).

Table 6
Lipid content of different tissues from gilthead seabream juveniles obtained from broodstock with different fads2 expression (high H or low L) and fed either a FO or a RO broodstock diet, after 45 days of feeding the challenge diet.

(% wet weight)	HFO		HRO		LFO		LRO		Two-way ANOVA		
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	*Bfads2*	*Bdiet*	*Bfads2*Bdiet*
Whole fish	10.46	1.23	10.75	1.21	11.07	0.47	11.24	1.55	*n.s.*	*n.s.*	*n.s.*
Liver	32.56	0.31	33.89	2.77	33.47	1.57	35.47	2.31	*n.s.*	*n.s.*	*n.s.*
Muscle	7.25	2.63	7.57	1.10	8.62	0.87	8.08	0.44	*n.s.*	*n.s.*	*n.s.*

n.s. No statistical significance ($p>0.05$).

4. Discussion

The results of the present study showed the trans-generational effect of both the broodstock *fads2* expression and the type of lipid in the broodstock diet on the metabolism and performance of the juvenile progeny challenged with a diet low in FM and FO.

In marine fish, the limited ability to synthesize LC-PUFAs, due to insufficient expression of key genes such as *fads2*, constrains their capacity to use low FM and FO diets. This gene codes for the enzyme Δ6 desaturase, which initiates the synthesis of LC-PUFA from the 18-carbon atom fatty acid precursors. In the present study, high *fads2* expression in broodstock markedly increased hepatic *fads2* expression in juveniles, affecting fatty acid composition of different tissues, and reducing VSI ($p<0.05$). In agreement, PUFA content of the liver of juveniles from high *fads2* broodstock significantly increased due to the elevation of n-6 PUFA content, particularly ARA, the end product of n-6 PUFA biosynthesis initiated by Fads2 on 18:2n-6. In muscle, n-6 PUFA were also significantly increased. This was due to the increase in 18:2n-6, which was higher than in liver since this fatty acid is preferentially incorporated into PL in this species (Caballero et al., 2006), and muscle has a proportionally larger content of PL (Izquierdo et al., 2005). Indeed, this is one of the reasons for the persistence of 18:2n-6 in muscle of seabream fed vegetable oils, even several months after being fed with a "wash out" diet containing low levels of 18:2n-6 (Izquierdo et al., 2005). On the other hand, a 25% increase in the ARA content of muscle of juveniles from broodstock with high *fads2* expression contributed to the increase in n-6 PUFA, in agreement with the higher *fads2* expression in these juveniles. All these results agree well with the increase in n-6 PUFA found in muscle of chicken expressing high *fads2* (Boschetti et al., 2016). The lack of effect on the n-3 PUFA content of liver, muscle or whole body would be related to the low 18:3n-3 content in the diet fed to the juveniles, in comparison to the high dietary 18:2n-6 content. Besides, the high *fads2* expression in the broodstock was also associated with a significant ($p<0.05$) reduction in VSI of juveniles. These results agree well with the tendency to a higher *fads2* expression found in livers of gilthead seabream juveniles from parents with high *fads2* expression (Turkmen et al., 2019a). However, in that study no significant differences were observed in *fads2* expression, which could be related to the shorter duration of the trial (fish only doubled their weight) and the larger initial weight of the juveniles (20 g). In comparison, the present study was conducted until fish from the all experimental groups had at least tripled their initial weight.

To our knowledge, this is the first study that shows a degree of inheritance in the ability to express *fads2* in fish, and particularly in gilthead seabream. Even though it could seem obvious that the progeny from a high *fads2* expression broodstock should have a high *fads2* expression, heritability of the *fads2* expression has been suggested to be very low in fish. Moreover, most studies have focused on the potential heritability of DHA and EPA content in muscle but have not directly targeted *fads2* expression heritability. For instance, in salmonids, a moderate cross-validation accuracy for selection for DHA and EPA has been recently demonstrated, opening the possibilities for selection of these traits (Horn et al., 2018). However, the DHA and EPA content of fish are regulated by a number of factors apart from *fads2* expression. In gilthead seabream, preliminary studies with a reduced but representative number of genetically characterized fish suggest an estimation of the *fads2* expression heritability in this species of 0.08±0.20 (Afonso and Izquierdo, unpublished data), in agreement with the high correlation between *fads2* expression in broodstock and juveniles in the present study. This increased *fads2* expression in the progeny of high *fads2* broodstock, in comparison to the low *fads2* broodstock, could be related to genetic or epigenetic factors. For instance, in humans, various genotypes are responsible for differences in *FADS2* expression and Δ6 desaturase activity (Howard et al., 2014), which are affected by single nucleotide polymorphisms (Schaeffer et al., 2006; Xie and Innis, 2008). Besides, DNA methylation of enhancer regions of the FADS are associated with Δ6 desaturase activity in humans (Howard et al., 2014). Increased methylation of specific CpG sites in the promoter region of *fads2* has been also found in the progeny of gilthead seabream from broodstock with low *fads2* expression (Turkmen et al., 2019a). Nevertheless, it cannot be dismissed that those differences in *fads2* expression or the products of Fads2 activity could be related to or mediated by other factors associated to the selection of high *fads2* expression broodstock. For instance, in the seabream females used in the present study, *fads2* expression was positively related to plasma 17β-estradiol levels (Ferosekhan et al., 2020). Also in female rats, estradiol increases DHA tissue contents through the up-regulation of Δ6 desaturase gene (Kitson et al., 2013). Nevertheless, before having a proterandry development of gonads in sea bream, aromatase activity to produce 17β-estradiol would be extremely low until the fish reach at least 300 g and would become females (Zohar et al., 1984). Since the juveniles in the present study weighed only few grams, this steroid hormone would not likely be the cause of the *fads2* up-regulation.

Feeding broodstock with the RO diet significantly ($p<0.05$) increased the hepatic 18:3n-6/18:2n-6 and 18:2n-9/18:1n-9 ratios, which are indicators of Fads2 activity (Vagner and Santigosa, 2011), in the juvenile offspring. These results denote a significant nutritional programing effect of the broodstock diet on the juvenile progeny, especially on promoting the Fads2 activity. These results are in agreement with previous studies where the seabream juvenile progeny of broodstock fed a linseed oil rich diet showed increased contents of Fads2 products in comparison to those from broodstock fed a FO diet (Turkmen et al., 2019b). Similarly, in red drum (*Sciaenops ocellatus*) nutritional programming by essential fatty acids increased Fads2 products in the 21-day-old larvae progeny challenged with a low LC-PUFA diet (Fuiman and Perez, 2015). In the present study, the increase in these hepatic indicators of Fads2 activity, together with the lack of a significant effect on the *fads2* expression, suggest that a post-transcriptional factor could mediate this nutritional programing effect. This hypothesis is in agreement with the down-regulation of microRNAs related with lipid metabolism in offspring of mice fed a high fat diet from conception to lactation, in comparison to those fed a chow diet (Zhang et al., 2009). Interestingly, this enhancement of Fads2 activity was not observed in previous studies when broodstock was fed a diet high in LNA but not sufficiently low in LC-PUFA (Turkmen et al., 2019a). In that study, the difference in the n-3 LC-PUFA content between the control FO and the high VO broodstock diets was only of 2.6%, in comparison to the 8.1% difference between the broodstock diets of the present study (Ferosekhan et al., 2020). Thus, the high n-3 LC-PUFA content of the previous study (Turkmen et al., 2019a) could have inhibited nutritional

Table 7
Fatty acid composition (% total fatty acids) of liver from gilthead seabream juveniles obtained from broodstock with different *fads2* expression (high H or low L) and fed either a FO or a RO broodstock diet, after 45 days of feeding the challenge diet.

Name	HFO		HRO		LFO		LRO		Two-way ANOVA		
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Selection	Diet	Selection* Diet
14:0	0.85	0.23	0.93	0.42	^{1}1.10	0.03	^{2}0.69	0.07	*n.s.*	*n.s.*	*n.s.*
14:1n-7	^{a}0.25^{A}	0.11	^{b}0.08	0.08	0.02^{B}	0.00	0.13	0.10	*n.s.*	*n.s.*	*0.024*
14:1n-5	0.24^{A}	0.11	0.08	0.09	0.01^{B}	0.00	0.12	0.10	*n.s.*	*n.s.*	*0.027*
15:0	0.21^{A}	0.60	0.12	0.07	0.07^{B}	0.01	0.16	0.08	*n.s.*	*n.s.*	*0.038*
15:1n-5	^{a} 0.21^{A}	0.09	^{b}0.06	0.06	0.02^{B}	0.00	0.12	0.10	*n.s.*	*n.s.*	*0.017*
16:0 ISO	^{a}0.22^{A}	0.10	^{b}0.06	0.07	0.00^{B}	0.00	0.10	0.08	*n.s.*	*n.s.*	*0.018*
16:0	10.04^{B}	0.25	10.90	2.64	13.66^{A}	0.35	11.24	2.23	*n.s.*	*n.s.*	*n.s.*
16:1n-7	1.86^{B}	0.15	2.03	0.42	2.16^{A}	0.04	1.97	0.25	*n.s.*	*n.s.*	*n.s.*
16:1n-5	0.21^{A}	0.08	0.09	0.03	^{2}0.05^{B}	0.01	^{1}0.16	0.09	*n.s.*	*n.s.*	*0.016*
16:2n-4	^{a}0.23^{A}	0.11	^{b}0.06	0.04	0.02^{B}	0.00	0.12	0.09	*n.s.*	*n.s.*	*0.012*
17:0	^{a}0.27^{A}	0.13	^{b}0.09	0.03	0.05^{B}	0.01	0.11	0.07	*0.048*	*n.s.*	*0.021*
16:3n-4	^{a} 0.32^{A}	0.12	^{b}0.15	0.01	0.13^{B}	0.01	0.18	0.06	*n.s.*	*n.s.*	*0.02*
16:3n-3	0.28^{A}	0.17	0.08	0.07	0.03^{B}	0.01	0.14	0.12	*n.s.*	*n.s.*	*0.044*
16:3n-1	^{a}0.28^{A}	0.17	^{b}0.07	0.08	0.02^{B}	0.00	0.12	0.11	*n.s.*	*n.s.*	*0.036*
16:4n-3	^{a} 0.44^{A}	0.18	^{b}0.11	0.11	0.03^{B}	0.01	0.15	0.13	*0.037*	*n.s.*	*0.035*
18:0	6.06^{B}	0.74	6.29	0.41	^{1}7.45^{A}	0.45	^{2}6.64	0.17	*0.014*	*n.s.*	*n.s.*
18:1n-9	37.46^{B}	3.31	41.67	2.40	43.71^{A}	0.38	40.08	2.19	*n.s.*	*n.s.*	*0.019*
18:1n-7	2.55	0.27	2.77	0.07	2.70	0.04	3.44	0.97	*n.s.*	*n.s.*	*n.s.*
18:1n-5	0.28^{A}	0.10	0.12	0.11	0.07^{B}	0.01	0.11	0.13	*n.s.*	*n.s.*	*n.s.*
18:2n-9	^{b}2.57	0.50	^{a}3.54	0.54	2.85	0.63	3.24	0.10	*n.s.*	*0.042*	*n.s.*
18:2n-6	14.07	1.66	15.44	0.97	14.33	0.79	14.73	1.69	*n.s.*	*n.s.*	*n.s.*
18:2n-4	^{a}0.25^{A}	0.06	^{b}0.09	0.09	0.04^{B}	0.01	0.09	0.12	*n.s.*	*n.s.*	*n.s.*
18:3n-6	^{b}3.38	0.35	^{a}4.45	0.27	^{2}3.11	0.46	^{1}3.96	0.21	*n.s.*	*0.001*	*n.s.*
18:3n-4	0.23^{A}	0.08	0.12	0.07	0.05^{B}	0.01	0.09	0.11	*0.048*	*n.s.*	*n.s.*
18:3n-3	2.37	0.27	2.33	0.24	2.13	0.16	2.24	0.33	*n.s.*	*n.s.*	*n.s.*
18:4n-3	0.99^{A}	0.13	0.95	0.04	^{2}0.68^{B}	0.07	^{1}0.86	0.30	*0.047*	*n.s.*	*0.013*
18:4n-1	^{a}0.31^{A}	0.15	^{b}0.09	0.08	0.03^{B}	0.01	0.18	0.15	*n.s.*	*n.s.*	*0.023*
20:0	^{a}0.36^{A}	0.03	^{b}0.23	0.10	0.22^{B}	0.02	0.27	0.07	*n.s.*	*n.s.*	*0.048*
20:1n-9	0.37^{A}	0.11	0.22	0.09	0.18^{B}	0.02	0.28	0.10	*n.s.*	*n.s.*	*0.034*
20:1n-7	0.86	0.13	0.68	0.23	0.69	0.02	0.76	0.17	*n.s.*	*n.s.*	*n.s.*
20:1n-5	0.13	0.11	0.15	0.11	0.08	0.02	0.15	0.08	*n.s.*	*n.s.*	*n.s.*
20:2n-9	0.61	0.11	0.52	0.28	0.56	0.07	0.40	0.10	*n.s.*	*n.s.*	*n.s.*
20:2n-6	0.47	0.09	0.28	0.13	0.27	0.03	0.33	0.19	*n.s.*	*n.s.*	*n.s.*
20:3n-9	0.50	0.21	0.32	0.20	0.19	0.15	0.03	0.25	*n.s.*	*n.s.*	*n.s.*
20:3n-6	0.35	0.16	0.14	0.21	0.12	0.15	0.27	0.04	*n.s.*	*n.s.*	*n.s.*
20:4n-6	^{a}0.65^{A}	0.24	^{b}0.26	0.10	0.22^{B}	0.01	0.34	0.08	*n.s.*	*n.s.*	*0.012*
20:3n-3	^{a}0.47^{A}	0.17	^{b}0.21	0.20	0.09^{B}	0.02	0.18	0.09	*0.032*	*n.s.*	*n.s.*
20:4n-3	0.44^{A}	0.14	0.20	0.17	0.12^{B}	0.02	0.29	0.24	*n.s.*	*n.s.*	*n.s.*
20:5n-3	0.86^{A}	0.22	0.45	0.22	0.36^{B}	0.05	0.67	0.38	*n.s.*	*n.s.*	*0.037*
22:1n-11	0.55	0.51	0.37	0.24	0.27	0.04	0.45	0.28	*n.s.*	*n.s.*	*n.s.*
22:1n-9	0.38	0.34	0.35	0.21	0.30	0.04	0.37	0.21	*n.s.*	*n.s.*	*n.s.*
22:4n-6	0.91	0.59	0.32	0.47	0.08	0.03	0.50	0.68	*n.s.*	*n.s.*	*n.s.*
22:5n-6	1.26	1.10	0.33	0.40	0.13	0.02	0.42	0.37	*n.s.*	*n.s.*	*n.s.*
22:5n-3	1.18^{A}	0.89	0.31	0.31	0.10^{B}	0.01	0.41	0.33	*n.s.*	*n.s.*	*0.075*
22:6n-3	3.23	1.72	1.88	1.16	1.43	0.03	2.30	0.23	*n.s.*	*n.s.*	*n.s.*
∑SFA	17.78^{B}	0.75	18.55	3.31	^{1}22.55^{A}	0.05	^{2}19.12	2.02	*n.s.*	*n.s.*	*n.s.*
∑MUFA	45.15^{B}	2.86	48.61	1.64	^{1}50.25^{A}	0.50	^{2}48.02	1.06	*n.s.*	*n.s.*	*0.023*
∑PUFA	36.64^{A}	2.80	32.71	4.11	^{2}27.17^{B}	0.06	^{1}32.64	1.88	*0.014*	*n.s.*	*0.015*
∑n-3 FA	^{a}10.25^{A}	2.30	^{b}6.51	2.40	5.02^{B}	0.21	7.38	1.14	*n.s.*	*n.s.*	*0.017*
∑n-6 FA	21.10^{A}	1.01	21.23	1.57	^{2}18.27^{B}	0.39	^{1}20.55	1.19	*0.027*	*n.s.*	*n.s.*
18:1/18:0	0.42	0.01	0.44	0.03	0.36	0.02	0.52	0.16	*n.s.*	*n.s.*	*n.s.*
18:4n-3/18:3n-3	0.42	0.08	0.41	0.04	0.34	0.06	0.45	0.09	*n.s.*	*n.s.*	*n.s.*
18:3n-6/18:2n-6	0.24	0.03	0.29	0.02	0.22	0.05	0.27	0.03	*n.s.*	*0.03*	*n.s.*
20:3n-6/20:2n-6	0.78	0.45	0.37	0.47	0.46	0.56	1.03	0.59	*n.s.*	*n.s.*	*n.s.*
18:2n-9/18:1n-9	0.07	0.01	0.09	0.01	0.07	0.02	0.08	0.01	*n.s.*	*0.03*	*n.s.*
16:1/16:0	0.19^{A}	0.02	0.19	0.01	0.16^{B}	0.01	0.18	0.02	*0.02*	*n.s.*	*n.s.*
18:0/16:0	0.60	0.08	0.59	0.10	0.54	0.04	0.61	0.12	*n.s.*	*n.s.*	*n.s.*
18:1n-7/16:1n-7	1.39	0.24	1.41	0.29	^{2}1.25	0.03	^{1}1.73	0.27	*n.s.*	*n.s.*	*n.s.*
DHA/EPA	4.13	2.55	3.95	0.64	3.97	0.44	4.25	2.35	*n.s.*	*n.s.*	*n.s.*

SFA: Saturated fatty acid; MUFA: Monounsaturated fatty acid; PUFA: Polyunsaturated fatty acid. Bfads2 refers to the effect of the broodstock *fads2* expression (H or L); Bdiet refers to the effect of the broodstock diet (FO or RO).

[a, b]In front of the value mean there is significant difference between the offspring come from same selection broodstock group (High) but the broodstock were fed with different diet.

[1,2]In front of the value mean there is significant difference between the offspring come from same selection broodstock group (Low) but the broodstock were fed with different diet.

[A, B]In the back of the value mean there is significant difference between the offspring come from broodstock fed with diet FO but with different *fads2* expression.

n.s. No statistical significance ($p>0.05$).

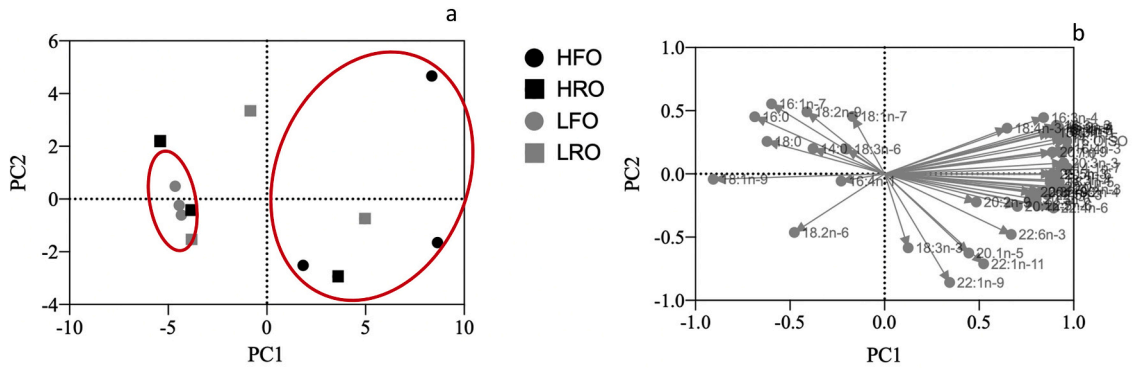

Fig. 3. Principal component analysis of hepatic fatty acid profiles in juveniles obtained from broodstock with different *fads2* expression (high H or low L) and fed either a FO or a RO broodstock diet, after 45 days of feeding the challenge diet. Scores (a) and variables of PC1 and PC2 (b).

programing. In agreement with this hypothesis, in Atlantic Salmon the VO diet used to effectively induce a nutritional programing effect was 22.3% lower in n-3 LC-PUFA than the control FO diet (Clarkson et al., 2017).

Feeding broodstock a diet with partial replacement of FO by RO led to a marked ($p<0.001$) reduction in the VSI of juveniles and the size of hepatocytes, which was significantly correlated to the HSI ($r = 0.96$, $p<0.05$). However, liver lipid content were not affected, suggesting that the reduced hepatocyte size would be related to the catabolism of glycogen rather than lipid, since hepatocytes are energy reservoirs in these types of fish species (Chellappa et al., 1995). HSI was negatively correlated with the expression of liver *igf-1*. However, no significant differences were found in *igf-1* expression values, which could have been related to the low number of copies/μL obtained. *Igf-1* is preferentially expressed in the liver, by stimulation of growth hormone (GH), and promotes systemic body growth, although is not always directly correlated to fish growth (Beckman, 2011). *Igf-1* is regulated by different factors including fish nutritional status (Pérez-Sánchez et al., 2018) and it is down-regulated in gilthead seabream fed low LC-PUFA diets (Escobar-Aguirre et al., 2020). Thus, it is possible that the very low LC-PUFA contents in the diet for juveniles would have also inhibited *igf-1*expression, leading to the low number of copies/μL obtained in present study. Nevertheless, the juveniles from broodstock fed the RO diet seemed to be more resilient to this inhibition, since they showed *igf-1*expression values that were 20% higher than those of juveniles from broodstock fed the FO diet.

In juveniles coming from broodstock with high *fads2* expression, FO replacement by RO in the broodstock diets, led to a significant ($p<0.05$) reduction in the *ghr-1/ghr-2* ratio in muscle as well as a significant ($p<0.01$) increase in SGR. The growth hormone receptors (Ghrs) are main components of the somatotropic axis that mediate the action of Gh (Sakamoto et al., 1993). Doubled Ghrs are actively transcribed in fish (Saera-Vila et al., 2007) and their expression can be regulated by nutrition and season, among other factors (Pérez-Sánchez et al., 2018). For instance, in gilthead sea bream, *ghr-1* and *ghr-2* have a protective and/or growth promoting action and *ghr-2* expression in muscle is up-regulated when the fish are fasted or fed with a low FO diet (Benedito-Palos et al., 2007; Saera-Vila et al., 2005). In comparison with *ghr-2*, *ghr-1* is more actively transcribed in liver and adipose tissue than in muscle of gilthead sea bream and remains stable in muscle when fish are fasted or fed with different lipid sources (Benedito-Palos et al., 2007; Saera-Vila et al., 2005). Thus, the ratio between *ghr-2* and *ghr-1* in muscle could be used to evaluate the ability of muscle maintenance and growth in gilthead sea bream (Benedito-Palos et al., 2007). In the present study, the ratio of *ghr-1/ghr-2* expressions in muscle was significantly reduced in juveniles coming from broodstock with high *fads2* expression and fed the RO diet instead of the FO diet. These results demonstrate the significant effect of nutritional programing on *ghr-1/ghr-2* expression in muscle and support their value as a mechanism of growth regulation to confront poor nutritional conditions previously suggested (Pérez-Sánchez et al., 2018). This increased SGR found in juveniles coming from broodstock with high *fads2* expression and fed the RO diet instead of FO is in agreement with other studies regarding the nutritional programing effect of feeding plant ingredients to broodstock or first feeding fish. For instance, 70% replacement of dietary fish oil by a combination of vegetable oils on diets for broodstock gilthead seabream promotes growth performance in offspring juveniles (Izquierdo et al., 2015; Turkmen et al., 2017a, 2017b; Turkmen et al., 2019a; Turkmen et al., 2019b). Among freshwater fish, feeding plant-based diets in early life stages improves growth later in life (Geurden et al., 2013; Clarkson et al., 2017). In general, the plasticity of the fish during the developmental stages of rapid growth facilitates the regulation of metabolism by nutritional factors. Specifically, ingestion, digestion, absorption and biosynthesis pathways can be regulated by nutritional programming (reviewed in Hou and Fuiman, 2019). However, in marine fish such as European sea bass, feeding larvae with low dietary LC-PUFA from first exogenous feeding for 39 days does not improve growth performance or fatty acid biosynthesis capacity when fish are later challenged with a low n-3 LC-PUFA diet (Vagner et al., 2009). Similarly, nutritional programing was not successful in gilthead seabream when larvae were fed a low n-3 LC-PUFA diet (Turkmen et al., 2017b). In both species, and generally in marine fish, larval stages are weak and very sensitive to low dietary n-3 LC-PUFA levels, which constrains the use of these nutrients for nutritional programming during larval development. The mechanisms underlying these nutritional programing effects are not yet clearly understood. In fish, the development of embryos relies on nutrients deposited in the yolk sac, which depend on the maternal intake of nutrients during and before oogenesis (Hou et al., 2020). In addition, evidence suggests a high maternal (Rauwerda et al., 2016) and paternal (Otero-Ferrer et al., 2020) influence on the transcriptome of the developing embryos and the transfer of gene expression regulating RNAs, lncRNA and miRNA (Sullivan et al., 2015) or specific proteins (Lubzens et al., 2017).

Despite the increased growth found in juveniles coming from broodstock with high *fads2* expression and fed RO diet instead of FO, the ARA and n-3 PUFA content in the liver were significantly reduced. Since none of these LC-PUFA were significantly reduced in whole body or muscle, their lower levels in liver suggest the mobilization of these PUFAs from liver to other tissues to support increased growth. Only EPA levels in whole body of juveniles from broodstock with high *fads2* expression was reduced when FO was replaced by RO in the broodstock diets. Expression of *elovl6* in liver of juveniles from broodstock with high *fads2* expression, was down regulated by the replacement of FO by RO in broodstock diets, denoting a strong nutritional programing effect. Indeed, hepatic *elovl6* expression was inversely correlated to SGR ($r=-0.62$, $p=0.05$). These results are in agreement with our previous studies in gilthead seabream where broodstock nutrition induced a strong nutritional programing effect on the expression of certain energy

Table 8
Fatty acid composition of muscle of juveniles obtained from broodstock with different *fads2* expression (high H or low L) and fed either a FO or a RO broodstock diet, after 45 days of feeding the challenge diet.

Name	HFO		HRO		LFO		LRO		Two-way ANOVA		
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Selection	Diet	Selection* Diet
14:0	0.83	0.2	0.72	0.13	0.74	0.06	0.79	0.13	*n.s.*	*n.s.*	*n.s.*
14:1n-7	0.05	0.01	0.05	0.01	0.08	0.05	0.06	0.02	*n.s.*	*n.s.*	*n.s.*
14:1n-5	0.03	0.03	0.02	0.03	0.05	0.06	0.03	0.02	*n.s.*	*n.s.*	*n.s.*
15:0	0.12	0.03	0.10	0.01	0.11	0.04	0.1	0.01	*n.s.*	*n.s.*	*n.s.*
15:1n-5	0.03	0.02	0.04	0.00	0.06	0.04	0.05	0.04	*n.s.*	*n.s.*	*n.s.*
16:0 ISO	0.03	0.03	0.03	0.01	0.05	0.06	0.03	0.03	*n.s.*	*n.s.*	*n.s.*
16:0	10.95	0.97	10.62	1.06	10.91	1.37	11.01	0.49	*n.s.*	*n.s.*	*n.s.*
16:1n-7	2.34	0.23	2.42	0.28	2.31	0.24	2.41	0.05	*n.s.*	*n.s.*	*n.s.*
16:1n-5	0.06	0.04	0.04	0.01	0.08	0.03	0.06	0.02	*n.s.*	*n.s.*	*n.s.*
16:2n-4	0.05	0.02	0.04	0.01	0.07	0.03	0.05	0.00	*n.s.*	*n.s.*	*n.s.*
17:0	0.08	0.03	0.06	0.01	0.09	0.03	0.08	0.01	*n.s.*	*n.s.*	*n.s.*
16:3n-4	0.14	0.03	0.13	0.02	0.15	0.02	0.14	0.02	*n.s.*	*n.s.*	*n.s.*
16:3n-3	0.04	0.03	0.04	0.02	0.06	0.05	0.04	0.02	*n.s.*	*n.s.*	*n.s.*
16:3n-1	0.11	0.02	0.08	0.03	0.1	0.04	0.11	0.03	*n.s.*	*n.s.*	*n.s.*
16:4n-3	0.06	0.01	0.1	0.076	0.12	0.11	0.08	0.02	*n.s.*	*n.s.*	*n.s.*
16:4n-1	0.01	0.02	0.02	0.03	0.00	0.02	0.02	0.00	*n.s.*	*n.s.*	*n.s.*
18:0	4.06	0.18	3.84	0.15	3.98	0.2	4.08	0.13	*n.s.*	*n.s.*	*n.s.*
18:1n-9	38.29	0.99	39.67	1.37	39.63	1.3	38.99	0.73	*n.s.*	*n.s.*	*n.s.*
18:1n-7	2.58	0.06	2.57	0.38	2.55	0.07	2.59	0.04	*n.s.*	*n.s.*	*n.s.*
18.1n-5	0.09	0.03	0.08	0.02	0.14	0.13	0.09	0.02	*n.s.*	*n.s.*	*n.s.*
18:2n-9	1.28	0.23	1.00	0.31	1.09	0.10	1.03	0.13	*n.s.*	*n.s.*	*n.s.*
18.2n-6	21.3	0.65	21.09	0.24	20.39	0.46	20.65	0.22	*0.026*	*n.s.*	*n.s.*
18:2n-4	0.05	0.02	0.06	0.01	0.09	0.09	0.06	0.03	*n.s.*	*n.s.*	*n.s.*
18:3n-6	1.58^{A}	0.16	1.29	0.4	1.19^{B}	0.12	1.24	0.06	*n.s.*	*n.s.*	*n.s.*
18:3n-4	0.09	0.03	0.09	0.03	0.11	0.05	0.1	0.02	*n.s.*	*n.s.*	*n.s.*
18:3n-3	3.88	0.13	4.00	0.03	3.95	0.03	3.89	0.07	*n.s.*	*n.s.*	*n.s.*
18:4n-3	0.5^{A}	0.01	0.46	0.09	0.46^{B}	0.15	0.47	0.04	*n.s.*	*n.s.*	*n.s.*
18:4n-1	0.07	0.07	0.04	0.01	0.09	0.07	0.05	0.00	*n.s.*	*n.s.*	*n.s.*
20:0	0.34	0.04	0.33	0.07	0.42	0.14	0.33	0.01	*n.s.*	*n.s.*	*n.s.*
20:1n-9	0.21	0.01	0.21	0.03	0.24	0.1	0.21	0.01	*n.s.*	*n.s.*	*n.s.*
20:1n-7	1.17	0.04	1.26	0.11	1.32	0.32	1.22	0.03	*n.s.*	*n.s.*	*n.s.*
20.1n-5	0.1	0.02	0.18	0.11	0.18	0.16	0.13	0.02	*n.s.*	*n.s.*	*n.s.*
20:2n-9	0.24^{B}	0.08	0.33	0.07	$^{1}0.46^{A}$	0.03	$^{2}0.3$	0.02	*0.018*	*n.s.*	*0.006*
20:2n-6	0.36	0.01	0.37	0.01	0.45	0.17	0.37	0.01	*n.s.*	*n.s.*	*n.s.*
20:3n-9	0.29	0.07	0.33	0.00	0.34	0.01	0.29	0.04	*n.s.*	*n.s.*	*n.s.*
20:3n-6	0.08	0.04	0.09	0.02	0.08	0.04	0.06	0.06	*n.s.*	*n.s.*	*n.s.*
20:4n-6	0.46	0.06	0.39	0.06	0.36	0.06	0.43	0.06	*n.s.*	*n.s.*	*n.s.*
20:3n-3	0.17	0.08	0.16	0.03	0.16	0.05	0.13	0.02	*n.s.*	*n.s.*	*n.s.*
20:4n-3	0.20	0.03	0.20	0.02	0.24	0.03	0.20	0.01	*n.s.*	*n.s.*	*n.s.*
20:5n-3	1.12	0.12	1.14	0.29	1.03	0.19	1.29	0.18	*n.s.*	*n.s.*	*n.s.*
22:1n-11	0.66	0.02	0.68	0.05	0.79	0.23	0.65	0.00	*n.s.*	*n.s.*	*n.s.*
22:1n-9	0.46	0.02	0.42	0.02	0.54	0.14	0.41	0.06	*n.s.*	*n.s.*	*n.s.*
22:4n-6	0.19	0.07	0.15	0.02	0.24	0.2	0.19	0.06	*n.s.*	*n.s.*	*n.s.*
22:5n-6	0.43	0.13	0.39	0.11	0.40	0.09	0.41	0.07	*n.s.*	*n.s.*	*n.s.*
22:5n-3	0.49	0.60	0.49	0.11	0.55	0.32	0.52	0.08	*n.s.*	*n.s.*	*n.s.*
22:6n-3	4.33	1.2	4.18	1.31	3.56	0.81	4.54	0.82	*n.s.*	*n.s.*	*n.s.*
∑SFA	16.37	1.16	15.66	1.04	16.25	1.42	16.39	0.50	*n.s.*	*n.s.*	*n.s.*
∑MUFA	46.04	1.14	47.61	1.44	47.90	0.61	46.85	0.66	*n.s.*	*n.s.*	*n.s.*
∑PUFA	37.53	1.65	36.66	2.43	35.74	1.75	36.67	0.98	*n.s.*	*n.s.*	*n.s.*
∑n-3 PUFA	10.79	1.23	10.76	1.76	10.14	1.55	11.16	0.99	*n.s.*	*n.s.*	*n.s.*
∑n-6 PUFA	24.41^{A}	0.53	23.77	0.39	$^{2}23.09^{B}$	0.03	$^{1}23.35$	0.02	*0.002*	*n.s.*	*0.046*
18:1/18:0	0.64	0.04	0.67	0.04	0.64	0.03	0.64	0.01	*n.s.*	*n.s.*	*n.s.*
18:4n-3/18:3n-3	0.13	0.01	0.12	0.03	0.12	0.00	0.12	0.01	*n.s.*	*n.s.*	*n.s.*
18:3n-6/18:2n-6	0.074	0.008	0.061	0.020	0.058	0.005	0.060	0.003	*n.s.*	*n.s.*	*n.s.*
20:3n-6/20:2n-6	0.23	0.11	0.24	0.06	0.17	0.03	0.16	0.16	*n.s.*	*n.s.*	*n.s.*
18:2n-9/18:1n-9	0.034	0.007	0.025	0.009	0.028	0.002	0.027	0.003	*n.s.*	*n.s.*	*n.s.*
16:1/16:0	$^{b}0.21$	0.01	$^{a}0.23$	0.00	0.21	0.01	0.22	0.01	*n.s.*	*0.039*	*n.s.*
18:0/16:0	0.37	0.04	0.36	0.05	0.37	0.03	0.37	0.03	*n.s.*	*n.s.*	*n.s.*
18:1n-7/16:1n-7	1.11	0.08	1.07	0.13	1.11	0.11	1.08	0.03	*n.s.*	*n.s.*	*n.s.*
DHA/EPA	3.83	0.66	3.63	0.22	3.44	0.16	3.51	0.23	*n.s.*	*n.s.*	*n.s.*

SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids, Bfads2 refers to the effect of the broodstock *fads2* expression (H or L); Bdiet refers to the effect of the broodstock diet (FO or RO).

[a, b]In front of the value mean there is significant difference between the offspring come from same selection broodstock group (High) but the broodstock were fed with different diet.

[1,2]In front of the value mean there is significant difference between the offspring come from same selection broodstock group (Low) but the broodstock were fed with different diet.

[A, B]In the back of the value mean there is significant difference between the offspring come from broodstock fed with diet FO but with different *fads2 e*xpression.

[I, II]In the back of the value mean there is significant difference between the offspring come from broodstock fed with diet RO diet but with different *fads2* expression.

n.s. No statistical significance ($p>0.05$).

Table 9
Whole body fatty acid composition of seabream juveniles obtained from broodstock with different *fads2* expression (high H or low L) and fed either a FO or a RO broodstock diet, after 45 days of feeding the challenge diet.

Name	HFO		HRO		LFO		LRO		Two-way ANOVA		
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Selection	Diet	Selection* Diet
14:0	0.96	0.39	1.17	0.52	1.20	0.14	1.43	0.38	*n.s.*	*n.s.*	*n.s.*
14:1n-7	0.11	0.10	0.07	0.31	0.08	0.01	0.06	0.02	*n.s.*	*n.s.*	*n.s.*
14:1n-5	0.18	0.26	0.03	0.01	0.06	0.06	0.03	0.01	*n.s.*	*n.s.*	*n.s.*
15:0	0.20	0.14	0.13	0.04	0.12	0.00	0.14	0.02	*n.s.*	*n.s.*	*n.s.*
15:1n-5	0.13	0.14	0.04	0.03	0.06	0.02	0.03	0.01	*n.s.*	*n.s.*	*n.s.*
16:0 ISO	0.13	0.17	0.03	0.03	0.05	0.04	0.02	0.01	*n.s.*	*n.s.*	*n.s.*
16:0	10.25	2.76	11.85	3.00	11.97	0.91	12.94	0.87	*n.s.*	*n.s.*	*n.s.*
16:1n-7	2.46	0.62	2.88	0.59	2.62	0.16	2.99	0.57	*n.s.*	*n.s.*	*n.s.*
16:1n-5	0.11	0.10	0.08	0.03	0.13	0.07	0.04	0.01	*n.s.*	*n.s.*	*n.s.*
16:2n-4	0.34	0.10	0.10	0.03	0.06	0.06	0.10	0.03	*n.s.*	*n.s.*	*n.s.*
17:0	0.12	0.04	0.09	0.03	0.10	0.03	0.11	0.03	*n.s.*	*n.s.*	*n.s.*
16:3n-4	0.15	0.00	0.15	0.01	0.15	0.03	0.15	0.02	*n.s.*	*n.s.*	*n.s.*
16:3n-3	0.10	0.09	0.03	0.02	0.06	0.03	0.04	0.01	*n.s.*	*n.s.*	*n.s.*
16:3n-1	0.16	0.07	0.13	0.05	0.14	0.02	0.12	0.03	*n.s.*	*n.s.*	*n.s.*
16:4n-3	0.24	0.14	0.20	0.07	0.16	0.02	0.14	0.02	*n.s.*	*n.s.*	*n.s.*
16:4n-1	0.09	0.09	0.07	0.04	0.03	0.04	0.02	0.01	*n.s.*	*n.s.*	*n.s.*
18:0	3.91	0.22	3.63	0.42	4.11	0.18	4.17	0.24	*n.s.*	*n.s.*	*n.s.*
18:1n-9	36.88	3.03	37.46	1.97	39.04	1.33	39.24	2.62	*n.s.*	*n.s.*	*n.s.*
18:1n-7	2.67	0.06	5.20	4.63	2.63	0.05	2.61	0.01	*n.s.*	*n.s.*	*n.s.*
18.1n-5	0.15	0.09	0.06	0.05	0.14	0.07	0.07	0.01	*n.s.*	*n.s.*	*n.s.*
18:2n-9	1.29	0.33	1.23	0.19	1.13	0.06	1.20	0.11	*n.s.*	*n.s.*	*n.s.*
18.2n-6	18.49	1.30	20.15	1.58	19.14	0.95	18.3	0.76	*n.s.*	*n.s.*	*n.s.*
18:2n-4	0.13	0.09	0.05	0.03	0.1	0.06	0.07	0.02	*n.s.*	*n.s.*	*n.s.*
18:3n-6	1.44	0.15	1.53	0.08	1.35	0.07	1.40	0.14	*n.s.*	*n.s.*	*n.s.*
18:3n-4	0.21	0.18	0.08	0.03	0.15	0.07	0.08	0.02	*n.s.*	*n.s.*	*n.s.*
18:3n-3	3.59	0.28	3.91	0.26	3.73	0.11	3.51	0.18	*n.s.*	*n.s.*	*n.s.*
18:4n-3	0.74	0.03	0.58	0.05	0.5	0.02	0.59	0.05	*n.s.*	*n.s.*	*n.s.*
18:4n-1	0.16	0.16	0.05	0.04	0.10	0.05	0.04	0.02	*n.s.*	*n.s.*	*n.s.*
20:0	0.42	0.16	0.26	0.06	0.33	0.06	0.30	0.01	*n.s.*	*n.s.*	*n.s.*
20:1n-9	0.33	0.21	0.17	0.05	0.20	0.03	0.19	0.02	*n.s.*	*n.s.*	*n.s.*
20:1n-7	1.62	0.43	1.09	0.18	1.11	0.07	1.20	0.05	*n.s.*	*n.s.*	*n.s.*
20.1n-5	0.31	0.31	0.16	0.06	0.14	0.05	0.11	0.02	*n.s.*	*n.s.*	*n.s.*
20:2n-9	0.39	0.10	0.33	0.04	^{1}0.44	0.08	^{2}0.26	0.05	*n.s.*	*0.02*	*n.s.*
20:2n-6	0.44	0.17	0.27	0.05	^{1}0.38	0.03	^{2}0.29	0.02	*n.s.*	*0.035*	*n.s.*
20:3n-9	0.10	0.16	0.03	0.03	0.35	0.08	0.14	0.12	*0.022*	*n.s.*	*n.s.*
20:3n-6	0.35	0.18	0.26^{I}	0.05	0.08	0.10	0.09^{II}	0.07	*0.009*	*n.s.*	*n.s.*
20:4n-6	0.47	0.17	0.37	0.13	0.37	0.10	0.37	0.04	*n.s.*	*n.s.*	*n.s.*
20:3n-3	0.26	0.26	0.14	0.09	0.20	0.09	0.12	0.03	*n.s.*	*n.s.*	*n.s.*
20:4n-3	0.32	0.15	0.19	0.05	0.23	0.06	0.20	0.06	*n.s.*	*n.s.*	*n.s.*
20:5n-3	1.62	0.37	1.03	0.28	0.98	0.21	1.33	0.23	*n.s.*	*n.s.*	*0.02*
22:1n-11	1.12	0.53	0.57	0.25	0.75	0.15	0.74	0.09	*n.s.*	*n.s.*	*n.s.*
22:1n-9	0.55	0.24	0.36	0.11	0.48	0.08	0.38	0.05	*n.s.*	*n.s.*	*n.s.*
22:4n-6	0.39	0.29	0.15	0.13	0.72	0.93	0.12	0.01	*n.s.*	*n.s.*	*n.s.*
22:5n-6	0.47	0.30	0.27	0.15	0.51	0.39	0.26	0.04	*n.s.*	*n.s.*	*n.s.*
22:5n-3	0.76	0.32	0.37	0.13	0.51	0.25	0.51	0.11	*n.s.*	*n.s.*	*n.s.*
22:6n-3	4.50	1.53	3.13	1.18	3.08	0.59	3.74	0.52	*n.s.*	*n.s.*	*n.s.*
∑SFA	15.86	3.02	17.11	3.98	17.83	1.06	19.10	1.09	*n.s.*	*n.s.*	*n.s.*
∑MUFA	46.40	1.29	48.13	2.26	47.38	0.91	47.67	2.10	*n.s.*	*n.s.*	*n.s.*
∑PUFA	37.46	3.90	34.69	2.57	34.67	1.87	33.18	1.78	*n.s.*	*n.s.*	*n.s.*
∑n-3 FA	12.58	2.80	9.48	1.86	9.45	1.11	10.17	1.08	*n.s.*	*n.s.*	*n.s.*
∑n-6 FA	22.04	0.35	23.00^{I}	1.32	^{1}22.56	0.51	^{2}20.83^{II}	0.72	*n.s.*	*n.s.*	*n.s.*
18:1/18:0	0.68	0.05	1.55	1.55	0.64	0.04	0.63	0.04	*n.s.*	*n.s.*	*n.s.*
18:4n-3/18:3n-3	0.21	0.10	0.15	0.02	0.13	0.01	0.17	0.01	*n.s.*	*n.s.*	*n.s.*
18:3n-6/18:2n-6	0.078	0.013	0.076	0.007	0.071	0.003	0.077	0.011	*n.s.*	*n.s.*	*n.s.*
20:3n-6/20:2n-6	0.53	0.35	0.48	0.23	0.53	0.23	0.43	0.08	*n.s.*	*n.s.*	*n.s.*
18:2n-9/18:1n-9	0.036	0.012	0.033	0.004	0.029	0.001	0.031	0.002	*n.s.*	*n.s.*	*n.s.*
16:1/16:0	0.24	0.01	0.25	0.01	0.22	0.00	0.23	0.03	*n.s.*	*n.s.*	*n.s.*
18:0/16:0	0.40	0.09	0.31	0.05	0.34	0.02	0.32	0.04	*n.s.*	*n.s.*	*n.s.*
18:1n-7/16:1n-7	1.14	0.31	2.08	2.21	1.01	0.08	0.90	0.18	*n.s.*	*n.s.*	*n.s.*
DHA/EPA	3.02	0.42	2.98	0.39	3.17	0.14	2.85	0.39	*n.s.*	*n.s.*	*n.s.*

SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids, Bfads2 refers to the effect of the broodstock *fads2* expression (H or L); Bdiet refers to the effect of the broodstock diet (FO or RO).

[a,b]In front of the value mean there is significant difference between the offspring come from same selection broodstock group (High) but the broodstock were fed with different diet.

[1,2]In front of the value mean there is significant difference between the offspring come from same selection broodstock group (Low) but the broodstock were fed with different diet.

[A, B]In the back of the value mean there is significant difference between the offspring come from broodstock fed with diet FO but with different *fads2* expression.

[I, II]In the back of the value mean there is significant difference between the offspring come from broodstock fed with diet RO diet but with different *fads2* expression.

n.s. No statistical significance ($p>0.05$).

Table 10
Gene expression of seabream juveniles obtained from broodstock with different *fads2* expression (high H or low L) and fed either a FO or a RO broodstock diet, after 45 days of feeding the challenge diet.

	HFO		HRO		LFO		LRO		Two-way ANOVA		
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	*Bfads2*	*Bdiet*	*Bfads2*Bdiet*
Liver (copies/μL)											
fads2	683.13	177.46	793.87	145.51	491.17	101.10	426.45	105.16	*0.014*	*n.s.*	*n.s.*
cox-2	1.19	0.55	1.65	0.40	1.52	0.88	1.81	1.42	*n.s.*	*n.s.*	*n.s.*
elovl6	^{a}144.52^{A}	17.27	^{b}39.18^{II}	32.52	^{2}69.31^{B}	11.77	119.40^{I}	45.22	*n.s.*	*n.s.*	*0.005*
igf-1	20.79	4.53	22.46	6.24	18.34	1.80	24.65	0.61	*n.s.*	*n.s.*	*n.s.*
srebp	59.91	12.63	60.43	24.52	52.46	18.82	50.86	10.29	*n.s.*	*n.s.*	*n.s.*
ppara	55.43	23.23	74.97	4.84	62.00	22.57	74.07	3.84	*n.s.*	*n.s.*	*n.s.*
Muscle											
ghr-1	14.82	3.55	10.04	1.11	13.33	2.06	13.49	5.33	*n.s.*	*n.s.*	*n.s.*
ghr-2	22.06	3.99	31.65	10.84	23.73	4.54	25.61	9.81	*n.s.*	*n.s.*	*n.s.*
ghr-1/ghr-2	^{a}0.67	0.10	^{b}0.35	0.14	0.56	0.02	0.53	0.10	*n.s.*	*0.014*	*0.039*

Bfads2 refers to the effect of the broodstock *fads2* expression (H or L); Bdiet refers to the effect of the broodstock diet (FO or RO).
$^{a,\ b}$In front of the value mean there is significant difference between the offspring come from same selection broodstock group (High) but the broodstock were fed with different diet.
$^{I,\ II}$In the back of the value mean there is significant difference between the offspring come broodstock fed with diet RO diet but with different *fads2* expression.
n.s. No statistical significance ($p>0.05$).

and lipid metabolism-related genes such as *elovl6* (Izquierdo et al., 2015; Turkmen et al., 2017a, 2017b). In those studies, *elovl6* in the progeny was down-regulated by the FO replacement by vegetable oils in the broodstock diet, in agreement with the improved utilization of dietary lipids and carbohydrates found in *Elovl6* disrupted mice models (Matsuzaka and Shimano, 2009).

In conclusion, the results of the present study have shown that it was possible to up-regulate the *fads2* expression of juvenile gilthead seabream by using broodstock with inherently high *fads2* expression, which led to increased PUFA content in liver and muscle. Nutritional programing through FO replacement by RO in broodstock diets increased the Fads2 activity (based on the ratio of fatty acid products and substrates for Fads2), reduced VSI, hepatocyte size and expression of *elovl6* in liver and *ghr-1/ghr-2* in muscle. Moreover, the combination of both broodstock with high *fads2* expression and nutritional programing with RO produced gilthead seabream juveniles that showed a faster growth when challenged with a low FM and low FO diet. Further studies are being conducted to better understand the regulation of nutritional programing through broodstock nutrition in gilthead seabream.

Author statement

Hanlin Xu: design of the experiment, feeding experiments, biological, biochemical and molecular analyses, manuscript writing. Shajahan Ferosekhan: design of the experiment. Serhat Turkmen: design of the experiment. Maria Jesus Zamorano and Juan Manuel Afonso: supervision of molecular samples analysis. Marisol Izquierdo: design of the experiment, supervision of the entire work and manuscript writing.

CRediT authorship contribution statement

Hanlin Xu: Data curation, Formal analysis, Investigation, Visualization, Writing - original draft. **Shajahan Ferosekhan:** Conceptualization, Writing - review & editing. **Juan Manuel Afonso:** Methodology, Supervision, Writing - review & editing. **María Jesús Zamorano:** Methodology, Supervision, Writing - review & editing. **Marisol Izquierdo:** Conceptualization, Data curation, Methodology, Project administration, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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