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Nutritional programming through broodstock diets to improve utilization of very low fishmeal and fish oil diets in gilthead sea bream

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ABSTRACT

The complete removal of fishmeal (FM) and fish oil (FO) is required to promote the sustainable development of aquaculture and for that, fast growing high quality fish that are fed without FM and FO are necessary. Early nutritional programming may allow the production of fish better adapted to utilize diets with vegetable meals (VM) and oils (VO). The main objective of this study was to research in the potential value of fatty acids as modulators of early nutritional programming in marine fish for a better utilization of VO/VM. For that purpose gilthead sea bream (*Sparus aurata*) broodstock were fed four different replacement levels of FO by linseed oil (LO) and their effect on fecundity and spawn quality, egg composition, Δ-6-desaturase (Δ6D) gene expression, progeny growth performance and their growth response to a challenge with diets low in FO and FM, but high in VO and VM. The results showed that feeding gilthead sea bream broodstock with high LO diets had very long-term effects on the progeny. Thus, FO replacement by LO up to 80–100% in broodstock diets for gilthead sea bream not only reduced fecundity and spawn quality, but also growth of 45 dah and 4-month-old juveniles, as well as Δ6D gene expression. However, when the 4 month-old juveniles were fed with a low FM and FO diet, even those from broodstock fed only 60% replacement of FO by LO showed a higher growth and feed utilization than juveniles from parents fed FO. These results demonstrate the interesting potential of early nutritional programming of marine fish by broodstock feeding to improve long-term performance of the progeny. Further studies are being conducted to determine optimum nutrient levels in the broodstock diets and the molecular mechanisms implied to develop effective nutritional intervention strategies for this species.

Statement of relevance:

This study demonstrates for the first time in fish the potential of broodstock nutrition to conduct early nutritional programming of culture fish for a better utilization of low fish meal and fish oil diets by the progeny, showing its effect not only during reproduction and larval development but also during on-growing.

1. Introduction

World production of fishmeal (FM) and fish oil (FO) remains stagnant around 6 and 1 million tons, respectively (Kaushik and Troell, 2010). Accordingly, the continuous growth of global aquaculture, a main user of these commodities, requires the efficient use of such high quality products. Blends of different alternative protein and lipid sources allow substituting FM and FO in diets for salmonids and marine fish (Tacon and Metian, 2008). However, complete or very high replacement levels markedly reduce growth and alter fish metabolism and health (Bell and Waagbø, 2008; Izquierdo, 2005; Kaushik et al., 2004; Montero and Izquierdo, 2010; Torstensen et al., 2008). Therefore, improvements in the utilization of very low FM and FO diets by cultured fish, and the subsequently enhancement of health and growth performance, would be greatly beneficial for the further development of aquaculture.

During the last years, studies in mammals and other vertebrates have shown that nutrition during very early life history can permanently influence animal metabolism, the ability to effectively utilize nutrients later in life and the risk to suffer metabolic syndromes, a phenomenon termed "nutritional programming" (Symonds et al., 2009). These types of studies arise the prospect to apply nutritional stimuli during early life of cultured fish to program their metabolism for a

Abbreviations: ANOVA, analysis of variance; ARA, arachidonic acid (20:4n−6); dah, days after hatching; DHA, docosahexaenoic acid (22:6n−3); EPA, eicosapentaenoic acid (20:5n−3); FM, fishmeal; FO, fish oil; HUFA, highly unsaturated fatty acids with more than 20C and more than 2 double bonds; LO, linseed oil; VM, vegetable meal; VO, vegetable oil; Δ6D, delta-6-desaturase.

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better utilization of low FM and FO diets during on-growing. Very recently, few very promising studies have been conducted in fish to determine the potential of nutritional programming (Fang et al., 2014; Geurden et al., 2013; Vagner et al., 2007) during first exogenous feeding. For instance, the potential long-term metabolic effects of early nutritional programming feeding high carbohydrates diets during first exogenous feeding have been recently studied in zebrafish (*Danio rerio*) (Fang et al., 2014). Nutritional stimulation with high carbohydrates during first feeding affected carbohydrates-related gene expression, digestion, transport and metabolism in the adult fish fed a high carbohydrate diet (Fang et al., 2014), while growth was not affected. Similarly, early short-term feeding of rainbow trout (*Oncorhynchus mykiss*) fry with a plant-based diet improved acceptance and utilization of the same diet when given at later life stages (Geurden et al., 2013).

However, prior to exogenous feeding, during peri-conception and embryogenesis, vertebrates show a great developmental plasticity, being very sensitive to nutritional changes allowing the organisms the adaptation to adverse post-natal conditions (Duque-Guimaraes and Ozanne, 2013). Nutrition alterations during these early critical periods may permanently influence the organism's metabolism in a process known in mammals as "fetal programming" (Reynolds and Caton, 2012). In fish, embryogenesis and development of most organs mainly occur before egg hatching and during the so-called "early larval development", before complete depletion of yolk-sac reserves, and is highly dependent on broodstock diet (Fernández-Palacios et al., 2011; Izquierdo et al., 2000). Up to date, no studies have been published on the effect of broodstock feeding on nutritional programming and the progeny performance during on-growing, although the importance of broodstock nutrition for embryogenesis and larval development is well documented (Fernández-Palacios et al., 2011). For instance, elevation of lipid levels in broodstock diets increased both growth and survival of 14 days after hatching (dah) rabbitfish (*Siganus guttatus*) larvae (Duray et al., 1994) and in several species, increase in n − 3 highly unsaturated fatty acids (HUFA), particularly docosahexaenoic acid (DHA), in broodstock diets enhanced growth of first feeding larvae (Abi-Ayad et al., 1997; Fernández-Palacios et al., 1995). However, little is known on the long-term effects of broodstock nutrition on progeny performance during juvenile or adult stages.

Whereas FM contents in on-growing diets can be almost completely replaced by vegetable meal (VM) without any adverse consequence in terms of somatic growth or nitrogen utilization (Kaushik et al., 2004), FO remains to be the key limiting factor for the future growth and sustainability of aquaculture development (De Silva et al., 2011). FO has a unique abundance of n − 3 HUFA, important fatty acids derived from linolenic acid that play an essential structural and functional role in fish metabolism. FO replacement relies mainly in vegetable oil (VO), which lack HUFA, but are frequently high in the linolenic acid precursor as it occurs in linseed oil (LO). Freshwater fish generally have sufficient elongase and desaturase activities to produce HUFA from the 18C precursors. On the contrary, marine fish have a very limited capacity to synthesize these fatty acids. Nevertheless, the gene of delta-6-desaturase (Δ6D), the key-limiting enzyme for HUFA synthesis, has been also characterized in marine fish (Li et al., 2014; Vagner and Santigosa, 2010) and its expression can be modulated through the diet (Izquierdo et al., 2008). For instance, in gilthead sea bream (*Sparus aurata*) high levels of dietary FO, rich in HUFA (end products of desaturation and elongation), down-regulated this gene, whereas reduction in FO and increase in LO, rich in the precursor 18:3n − 3, had an up-regulatory effect (Izquierdo et al., 2008). In one hand, polyunsaturated fatty acids are known to be regulators of gene transcription and expression in fish as in other animals (Benatti et al., 2004; Izquierdo and Koven, 2011) and in the other hand, delivery of nutrients to the embryo from the parental diet can interact with the genome, modify gene expression, and alter protein and metabolite composition in a long-term mode (Feil, 2006). However, it is unknown if fatty acids delivered through the broodstock diet are a suitable nutritional stimulus to modify fatty acid synthesis, the gene expression of key molecular markers such as Δ6D or improve utilization of VO during on-growing. Very few animal and human studies have research the potential of long chain fatty acids for nutritional programming and most of them have been conducted through first feeding (Bringhenti et al., 2011). However, few studies suggest a role of HUFA on nutritional programming in mammals. For instance, intake of oily fish during pregnancy has been associated with reduced risk of atopic or allergic outcomes in children (Kremmyda et al., 2011).

Therefore, the main objective of this study was to research in the potential value of fatty acids as modulators of early nutritional programming in marine fish for a better utilization of VO. To achieve that aim, gilthead sea bream broodstock were fed four different combinations of FO/LO containing different ratios of HUFA/18:3n − 3 (Δ6D products/precursors). The effect of broodstock feeding on egg composition, Δ6D gene expression, progeny growth performance and their response to a challenge with diets low in FO and FM but high in VO and VM were investigated.

2. Materials and methods

2.1. Nutritional stimulus through broodstock diets

Thirty-six brood fish (2–4 year-old) from the gilthead sea bream (*S. aurata*) broodstock of Grupo de Investigación en Acuicultura (GIA-ULPGC) were randomly selected and distributed in twelve 1000 l fiberglass tanks. A 2:1 ratio of males to females (Fernández-Palacios et al., 1990) was maintained in each group. At the beginning of the trial, mean body weight and total length for females and males were 1.55 ± 0.40 kg and 1.04 ± 0.29 kg, and 41.25 ± 3.88 cm and 37.2 ± 3.01 cm, respectively (Table 1). Tanks were supplied with 16 l/min filtered seawater (37 ± 0.5‰ salinity) and strong aeration. Seawater temperature during broodstock feeding ranged between 19.41 ± 0.14 and 21.32 ± 0.25 °C, and fish were kept under a indirect natural light (12 h light photoperiod). At the beginning of the spawning season, from December 19th to January 15th, fish were fed a commercial diet to ensure that there was no significant differences in the spawning quality of different broodstock, since parent contribution to spawn quality is more important than time (Hamoutene et al., 2009). Afterwards, to conduct the nutritional stimulus trial, from January 16th to June 26th, brood fish were fed one of four experimental extruded diets produced by Biomar. The experimental diets were isoproteic and isolipidic (Table 2) and their common basal ingredients were 50% fishmeal (SA68 + NALT70), 13.2% sunflower cake, 10% soya cake 48 hi pro solvent extracted, 9.9% wheat, 7% corn gluten 60, and 1.07% vitamin and minerals premix. The diets only differed in their content in fish oil STD18 (FO) and linseed oil (LO): 100% FO, 40%FO/60%LO, 20%FO/80%LO and 100%LO (Table 2). Substitution of FO in broodstock diets by LO increased total n − 3 and n − 6 fatty acids (Table 3), mainly due to the increase in 18:3n − 3 (α-linolenic acid, LNA) and 18:2n − 6 (linoleic acid, LA). Besides, LO inclusion reduced saturated, monoenoic and n − 3 HUFA fatty acids, in relation to the lower levels of 14:0, 16:0, 16:1n − 7,

Table 1
Biometric characteristics of the gilthead sea bream broodstock at the beginning of the feeding trial.

Diet	Body weight (kg)		Total length (cm)	
	Males[a]	Females[b]	Males[a]	Females[b]
100FO	1.00 ± 0.20	1.69 ± 0.47	36.95 ± 1.78	42.50 ± 4.54
40FO/60LO	0.99 ± 0.45	1.42 ± 0.40	36.20 ± 4.56	39.40 ± 2.77
20FO/80LO	1.06 ± 0.23	1.39 ± 0.19	37.40 ± 2.79	39.80 ± 2.07
100LO	1.13 ± 0.25	1.79 ± 0.46	38.25 ± 2.27	43.30 ± 4.98

No significant differences were found.
[a] Mean ± SD, n = 6.
[b] Mean ± SD, n = 3.

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