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ScienceDirect
Aquaculture 267 (2007) 199–212
Aquaculture
www.elsevier.com/locate/aqua-online

# Combined replacement of fish meal and oil in practical diets for fast growing juveniles of gilthead sea bream (*Sparus aurata* L.): Networking of systemic and local components of GH/IGF axis

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Received 17 November 2006; received in revised form 10 January 2007; accepted 10 January 2007

**Abstract**

Growth performance and growth regulatory pathways were examined in juvenile gilthead sea bream fed diets containing largely plant-based ingredients. Four isonitrogenous and isolipidic extruded diets with a low level (20%) of fish meal inclusion were formulated with graded levels of a vegetable oil mixture (17:58:25 of rapeseed: linseed: palm oils) replacing fish oil at 33, 66 and 100% (33VO, 66VO and VO diets). All diets were supplemented with lysine (0.55%) and contained soy lecithin (1%). Daily growth coefficients and feed efficiency over the course of an 11-week trial were almost identical in fish fed the FO, 33VO and 66VO diets. The VO diet reduced feed intake and growth without significant effects in proximate whole body composition, nitrogen or energy retentions. The highest concentration of plasma levels of insulin-like growth factor-I (IGF-I) was found in fish fed the 33VO diet. The lowest concentration was attained in fish fed the VO diet, whereas intermediate values were found in fish fed FO and 66VO diets. An opposite trend was found for circulating levels of growth hormone (GH), probably as a result of a reduced negative feedback inhibition from circulating IGF-I. Hepatic expression of IGF-I and GH receptor type I (GHR-I) was regulated in concert and mRNA levels paralleled plasma levels of IGF-I. Hepatic IGF-II and GHR-II were expressed in a more constitutive manner and no changes at the mRNA level were detected. In the skeletal muscle, IGF-I and GHR-I mRNAs did not vary significantly among groups. By contrast, IGF-II mRNA was up-regulated in fish fed the control diet, whereas the highest amount of GHR-II mRNA was attained in fish fed the 66VO diet. All together, these results suggest different growth compensatory mechanisms mediated by IGF-II and GHR-II at the local tissue level. These new insights prompted us to propose that practical diets low in marine ingredients can be used over the productive cycle of gilthead sea bream when essential fatty acids are supplied above the requirement levels.


*Keywords:* Sparidae; Fish oil; Vegetable oil; Plant proteins; Growth hormone; Growth hormone receptors; Insulin-like growth factors; Endocrine disrupters; Contaminants

## 1. Introduction

Currently, aquaculture is the major consumer of fish meal, a protein-dense feedstuff that approximates the

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doi:10.1016/j.aquaculture.2007.01.011

ideal amino acid profile of most cultured livestock. However, fish meal is a limited resource whose availability has remained stable from the late 1980s at approximately 6 million metric tonnes per annum, which limits the continuous growth of aquaculture production (FAO, 2004). Furthermore, inherent variability in fish meal composition due to species, season, geographic origin and processing leads to variation in quality (Opstvedt et al., 2003; Bragadóttir et al., 2004), and most of the future changes in developing novel aquafeeds should be focused on alternative protein sources.

The n-3 long-chain highly unsaturated fatty acids (n-3 HUFA) are naturally abundant in the marine environment, and fish oil is the major source of eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 20:6n-3) for aquafeeds. Besides the scarcity of fish oil, which is of great concern for marine fish, these animals have a limited capacity to biosynthesize n-3 HUFA from the shorter chain linolenic acid (18:3n-3), and both EPA and DHA become critical dietary constituents to ensure successful survival, growth, and development of these fish (Sargent et al., 1999, 2002). At this standpoint, it must be noted that fish meal also contains certain amounts of oil rich in n-3 HUFA, and the fish oil added to energized diets can be totally replaced by vegetable oils when fish meal is included at a high level in diets for Atlantic salmon (Bell et al., 2003; Bransden et al., 2003; Torstensen et al., 2004), rainbow trout (Richard et al., 2006a), and the freshwater African catfish (Ng et al., 2004). Similar results have been achieved in a typically marine fish such as turbot (Regost et al., 2003). A high fish oil replacement is also feasible in the Murray cod using casein-based diets (Francis et al., 2006). Likewise, up to 60% of fish oil added to diets has been replaced successfully in juvenile European sea bass (Montero et al., 2005; Mourente et al., 2005) and gilthead sea bream (Izquierdo et al., 2005), but the diets used in these studies also contained 35 to 40% fish meal.

Marine derived feedstuffs are also possible vectors of contaminants, such as PCBs, dioxins and other harmful chemicals affecting the safety of farm-raised fish (Jacobs et al., 2002). It is clear that reduction in fish oil levels can lead to a decrease in the contaminant levels of feed and consequently on fish filets (Berntssen et al., 2005; Bethune et al., 2006). Thus, the general consensus is that alternative protein and oil sources are needed to supplement or replace fish meal and fish oil in aquafeeds, contributing to long-term sustainability of the aquaculture industry (Hardy, 2004). In the present study, our objective was hence to maximize the combined replacement of fish meal and fish oil in practical diets for fast growing juveniles of gilthead sea bream. In earlier studies, we had shown that a good proportion of fish meal can be replaced by a mixture of plant protein sources in gilthead sea bream diets (Gómez-Requeni et al., 2003, 2004; Sitjà-Bobadilla et al., 2005). Based on these results, we attempted here to replace fish oil by a blend of vegetable oils, which have been already shown to be very effective in other fish species (Torstensen et al., 2005; Mourente and Bell, 2006; Richard et al., 2006a,b). To address this issue, growth and nutrient retention were analyzed in a conventional manner. Circulating levels of growth hormone (GH) and insulin-like growth factor-I (IGF-I) were used as markers of growth and nutrient status (see Pérez-Sánchez and Le Bail, 1999; Dyer et al., 2004). Also, transcripts of IGFs and GH receptors (GHR) were measured in liver and skeletal muscle by means of real-time PCR assays.

## 2. Materials and methods

### 2.1. Diets

As shown in Table 1, three diets (33VO, 66VO and VO) with relatively low fish meal inclusion (20%) levels were formulated with practical plant protein ingredients for the graded replacement (33, 66 and 100%) of the added fish oil by a blend of vegetable oils (rapeseed oil: linseed oil: palm oil). A fish oil-based diet (FO diet) equal in lipid content (220 g $kg^{-1}$) was used as the reference diet. Diets were supplemented with lysine (0.55%) and contained soy lecithin (1%). EPA plus DHA content varied on a dry matter basis between 2.3% (FO diet) and 0.3% (VO diet), and the DHA/EPA ratio (1.1–1.2) remained constant. All diets were manufactured using a twin-screw extruder (Clextral, BC 45) in the INRA experimental research station of Donzacq (Landes, France), dried under hot air, sealed and kept in air-tight bags until use.

Diet samples were hydrolysed (6N HCl, 110 °C) and amino acid analysis was performed using high-performance liquid chromatography. Tryptophan was determined by the colorimetric method of Basha and Roberts (1977) after alkaline hydrolysis of each sample (see Table 2). Fatty acid methyl esters (FAME) were prepared from aliquots of total lipid by acid-catalysed transmethylation for 16 h at 50 °C (Christie, 1982) after the addition of non-adecaenoic fatty acid (19:0) as an internal standard. FAMEs were extracted and separated in a Fisons Instruments GC 8000 Series (Thermo Electron Co., Rodano, Italy) gas chromatograph, equipped with a fused silica 30 m × 0.25 mm open