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Is it possible to influence European sea bass (*Dicentrarchus labrax*) juvenile metabolism by a nutritional conditioning during larval stage?

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Abstract

The purpose of this study was to check if it is possible to influence sea bass juvenile metabolism by a conditioning of larvae from day 6 post hatching to day 45 to a low or a high HUFA compound diet (LH, 0.8% EPA+DHA and HH, 2.2% EPA+DHA) when reared at 16 or 22 °C. Following a 3-month intermediate period (at 19 °C using a commercial diet), the adaptability of the 4 initial larval groups to a HUFA experimental deprived diet (0.5% EPA+DHA) were tested at 19 °C in a 60 day-experiment (d-151–211). The four experimental duplicated conditions were ex-LH16 and ex-HH16 for the 2 groups previously reared at 16 °C (initial weight, 7.3±0.5 g) and ex-LH22 and ex-HH22 for the 2 groups previously reared at 22 °C (initial weight, 11.1±0.5 g). Survival was maximal and there was a 1.6–2 fold increase in mass during the experiment. Growth was similar in the 4 experimental groups: NS difference in growth curve slopes ($P=0.7$). At the end of the experiment (d-211), whole body fat levels were in the same range in all groups (13–15% WW). The fatty acid (FA) composition in polar lipids (PL) and total lipids (TL) were significantly affected by initial weight related to larvae conditioning, which can be mainly attributed to a dilution effect (impact of initial FA content on final FA content versus relative mass increase during the course of the experiment). Conversely to this trend, DHA content in PL was higher in the ex-LH groups than in the ex-HH groups whatever thermal conditioning of larvae was. This indicated that ex-LH groups had a better capacity to adapt to a deficient HUFA diet than ex-HH fish. The relative expression of the delta-6 desaturase (Δ6D) was significantly higher in ex-LH than in ex-HH groups ($P<0.001$) between d-151 and d-181, which suggested that Δ6D transcription in ex-LH groups was positively modulated by the HUFA-deprived diet. This stimulation of the first step of the desaturation/elongation pathway could allow synthesizing FA needed to compensate low dietary HUFA supply. This study shows for the first time that it seems possible to influence juvenile fish metabolism by a nutritional conditioning during the larval stage.

Keywords: Aquaculture; Delta-6 desaturase; *Dicentrarchus labrax*; HUFA; Programming concept

1. Introduction

In contrast to freshwater fish, marine fish require the presence of preformed highly unsaturated fatty acids (HUFA) in their diet as they have a low capacity to bioconvert 18 carbon fatty acids (linoleic 18:2n-6 and linolenic 18:3n-3) into HUFA with 20 or 22 carbons (arachidonic 20:4n-6, eicosapentaenoic EPA 20:5n-3 and docosahexaenoic acid DHA 22:6n-3), (Kanazawa et al., 1978; Mourente and Tocher, 1994). The first step of this bioconversion requires the presence of the delta-6 desaturase gene (Δ6D), which has been cloned in several

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freshwater species such as zebrafish (AF309556), common carp (AF309557), rainbow trout (Seiliez et al., 2001). Δ6D gene has also been cloned in two marine fish species: gilthead seabream and turbot (Seiliez et al., 2003; Zheng et al., 2004). In gilthead seabream, an enhanced expression of the gene was obtained by feeding juveniles a HUFA-free diet. It has been previously showed by Cho et al. (1999) and Seiliez et al. (2001) that dietary HUFA inhibits Δ6D gene expression in mammals and rainbow trout. The deficiency in Δ6D activity observed in marine fish can be related to the abundance of n-3 HUFA in the marine food chain, which has induced an adaptation (Sargent et al., 1995) or a repression of desaturase activity (Olsen et al., 1990).

Until now, the main difficulty to meet the dietary n-3 HUFA requirement occurred in larvae fed living prey (Sargent et al., 1999). Specifically EPA and DHA are required for growth and play a major role in vision and brain development (Bell et al., 1996b; Sargent et al., 1997). As long as fish oil and fish meal represent the primarily ingredients of aquafeeds, juvenile HUFA requirements are easily covered. However, the rapid increase in farmed fish production in addition to the decline in natural stock has lead to look for substitutes for fish products commonly used in aquafeeds (Lodemel et al., 2001; Ringo et al., 2002). Incorporation of vegetable feedstuffs in fish feeds constitutes at the present time, the only solution in Europe, although these vegetable feedstuffs do not contain adequate n-3 HUFA to cover marine fish requirements except C18 HUFAs which may disturb fish physiology (Bell et al., 1996a,b; Parpoura and Alexis, 2001). In European sea bass juveniles, the minimal dietary n-3 HUFA requirement to sustain maximum growth is 0.7% dry matter (DM; Skalli and Robin, 2004), indicating that only some marine feedstuffs (fish meal or fish oil) can fulfil this requirement. There is also considerable interest in producing fish selected for their capacity to use vegetable feedstuffs as incorporation of these feedstuffs in high proportion in feeds usually lead to a decrease in fish dietetic quality expressed in terms of n-3 HUFA flesh content (Regost et al., 2003). This could be possible by applying a metabolic programming, using a nutritional and thermal conditioning during the young stage, as described in mammals (Lucas, 1998). The concept of metabolic programming is defined as an adaptive process at the cellular, molecular or biochemical level occurring during very young stages of organism development and which durably modify the genomic expression in the adult. In the rat, it has been shown that nutritional and thermal conditioning during young stages had consequences on survival, growth, learning process, lipid and glucoid synthesis (Lucas, 1998). In marine fish, several studies have shown the importance of temperature and trophic conditions on larval development, maturation of digestive functions, growth and larval quality (Bergeron and Person-Le Ruyet, 1997; Koumoundouros et al., 2001; Zambonino Infante and Cahu, 2001; Cahu et al., 2003; Lopez-Albors et al., 2003; Robin and Vincent, 2003). Since this adaptive process occurs during ontogenesis, the adaptability of juveniles could be modulated by larval "history".

The aim of this study was to determine if it is possible to influence European sea bass juvenile metabolism by a nutritional (HUFA) and thermal conditioning during larval development and specially if it is possible to modulate the Δ6D expression.

2. Materials and methods

2.1. Rearing conditions and experimental design

Replicated groups of European sea bass, *Dicentrarchus labrax*, larvae were initially reared at 16 or 22 °C and fed microparticulated diets from mouth opening (day 6 post-hatching, d-6), with a low (LH) or high (HH) HUFA content (0.8 and 2.2% EPA+DHA on dry matter basis, respectively). The four experimental conditions applied to larvae up to day 45 (d-45) were LH16, HH16, LH22 and HH22. The larval period was followed by an intermediate period of three months (d-46 to d-141) during which the four groups were separately held at 19 °C and fed a commercial diet with 2.7% EPA+DHA (DM basis).

At the end of this initial conditioning period, juveniles of each group were anaesthetised (ethylene-glycol-monophenylether, 0.15‰), individually weighed and then fish around the modal class were selected. At d-141, graded-fish were randomly distributed in square tanks, with an effective volume of 60 l, in order to obtain a similar biomass per condition: 5 tanks per condition with 75 fish per tank for the initial 16 °C groups (ex-HH16 and ex-LH16) and 40 fish per tank for the initial 22 °C group (ex-HH22 and ex-LH22). They were acclimated in the experimental unit for 10 days at the same temperature and diet as for the intermediate period (19 °C; commercial 2.7% EPA+DHA diet).

At d-151, 4 tanks per group were supplied with an experimental HUFA deprived diet (0.5% EPA+DHA, Table 1) and the last one was fed as previously in order to evaluate, from a qualitative point of view, the delay in growth induced by the deprived diet (this group was named qualitative reference, QR). Fish were hand fed to visual satiation at 09:00 and 15:00 h in order to avoid feed waste. All tanks were supplied with running