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Arachidonic acid enriched live prey induces albinism in Senegal sole (*Solea senegalensis*) larvae

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Abstract

We examined the effect of dietary arachidonic acid (20:4n-6, ARA) on growth, survival, pigmentation and fatty acid composition of Senegal sole larvae using a dose–response design. From 3 to 37 days post-hatch (dph), larvae were fed live food (rotifers from 3 to 9 dph, *Artemia* nauplii from −37 dph) that had been enriched using one of three experimental emulsions containing 3 graduated concentrations (1.3, 68 and 120.1 mg ARA g^{-1} dry weight) of ARA and constant docosahexaenoic acid (22:6n-3, DHA). A commercial enrichment product (DHA-Protein Selco, ARA content 7.8 mg g^{-1} dry weight) was used as a reference diet. Final concentration of ARA in *Artemia* nauplii ranged from 0.2 to 17.5 mg g^{-1} lipids. Growth and survival were independent of dietary levels of ARA tested. However, there was a correlation between dietary ARA and a significant reduction in pigmentation leading to increased albinism. Tissue fatty acid concentrations reflected the corresponding dietary composition. Eicosapentaenoic acid (20:5n-3, EPA) levels in all the tissues examined were inversely related to dietary ARA. There was almost a 100-fold increase in the proportion of docosapentaenoic acid (22:5n-3, DPA) in the tissues relative to the diet, which might indicate chain elongation from EPA as a result of inadequate dietary DHA. A negative, linear correlation was found between the pigmentation rate and the ARA content in the head, as well as with dietary ARA/EPA ratio.

Keywords: Arachidonic acid; Albinism; Pigmentation; Fatty acid; Larvae; *Solea senegalensis*; Enrichment; Live food

1. Introduction

The Senegal sole is a flatfish found along the Mediterranean and South Atlantic coasts, and is a prime candidate for aquaculture in these areas (Dinis et al., 1999). The rearing of sole in Spain and Portugal began in the early 1980s using techniques employed in the culture of other commercial marine fish larvae (Dinis, 1986, 1992; Drake et al., 1984; Rodríguez, 1984), while the ongrowing of the species was carried out extensively in sea water ponds. The rearing methods for Senegal sole have been documented

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(Dinis et al., 1999), although high mortalities during the weaning phase due to inadequate nutrition and pathological problems (Padrós et al., 2003; Zarza et al., 2003), as well as problems of malpigmentation (Soares et al., 2001), are still substantial challenges to the successful culture of this species.

Although the mass production of juveniles occurs in Spain and Portugal, determination of the nutritional requirements of the larvae remains incomplete. Recent nutrition-related research has concentrated on weaning problems (Cañavate and Fernandez-Diaz, 1999; Day et al., 1999; Dinis et al., 2000; Engrola et al., 2001; Ribeiro et al., 2003) or on the amino acid requirements of larvae (Morais et al., 2004) and post larvae (Ronnestad et al., 2000, 2001; Aragao et al., 2004). Despite the importance of the essential fatty acids (EFA), ARA, EPA and DHA, in diets for marine fish larvae, no information exists on the requirements of these EFA in Senegal sole larvae. First-feeding Senegal sole larvae are briefly fed on rotifers (from 3 to 9 dph), followed by *Artemia* nauplii, or directly on *Artemia* from first feed (Villalta and Estévez, unpublished data). These live feeds are naturally deficient in EFA (Sargent et al., 1999), so it is necessary for the feeds to be enriched, either with algae, oil emulsions or other preparations rich in EFA and other essential nutrients. According to Dinis et al. (1999), different enrichment regimes in live feeds failed to demonstrate any clear effect of polyunsaturated fatty acids (PUFA) on larval growth or survival. Nevertheless, an adequate supply of essential PUFA during the early life stages may still affect growth and survival during later stages of development (Howell et al., 1995). Furthermore, Senegal sole, like all flatfish, undergo metamorphosis, with a transition from an upright swimming behaviour to the "flat" fish morphology of an adult. Migration of the eye also occurs at this time. The provision of adequate nutrition during major physiological events such as metamorphosis is particularly critical (Dhert et al., 1990). Estévez and Kanazawa (1995) reported that dietary EFA deficiency causes incomplete metamorphosis in other flatfish.

Despite the extensive research into larval requirement for n-3 PUFA, only recent consideration has been given to n-6 PUFA, in particular ARA. ARA is generally conserved during periods of starvation in marine fishes (see review by Izquierdo, 1996), including Senegal sole (Mourente and Vazquez, 1996), and serves as the preferred precursor for eicosanoid biosynthesis (Bell et al., 1994). High ARA levels, however, have been implicated in the malpigmentation of various flatfish species (McEvoy et al., 1998a; Estévez et al., 1999; Copeman et al., 2002; Bell et al., 2003). Malpigmentation is a major problem in the culture of many flatfish and dramatically reduces its marketability (Venizelos and Benetti, 1999). Thus, this study was designed to investigate the role of dietary ARA on growth, survival and pigmentation of Senegal sole larvae from 3 to 37 days post hatch (dph) using a dose–response design. In addition, a further aim of this study was to identify correlations between fatty acids in the head, gut and whole carcass with those found in the diet to determine whether particular fatty acids are being preferentially retained or utilised.

2. Material and methods

2.1. Experimental emulsions

Commercially available DHA and ARA oils obtained, respectively, from the heterotrophically grown algae *Crypthecodinium cohnii* (Neuromins®, Martek Bioscience, USA) and fungus *Mortierella alpina* (Vevodar®, DSM Food Specialties, Netherlands) were used. Vevodar® oil gradually replaced olive oil to produce emulsions with "low" (ARA-L), "medium" (ARA-M) or "high" (ARA-H) ARA contents. The components used in the formulation of each emulsion and the selected fatty acid composition is shown in Table 1. Oil mixtures were emulsified with equal amounts of distilled water by homogenising with an Ultra-turrax T25 at high speed for 60 s. The emulsion was then transferred to plastic syringes, the air removed, the syringes cooled in ice and kept in a refrigerator (4 °C), in an upright position. A commercial emulsion (DHA-Protein Selco, INVE) was used as a reference diet.

2.2. Live food enrichment

Rotifers were enriched in 10 l containers at a density of 500 rotifers ml^{-1} for 12 h at 20 °C using