Aquaculture 295 (2009) 250–265

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Effect of dietary vitamin A on Senegalese sole (*Solea senegalensis*) skeletogenesis and larval quality

Ignacio Fernández [a], Marta S. Pimentel [a], Juan B. Ortiz-Delgado [b], Francisco Hontoria [c], Carmen Sarasquete [d], Alicia Estévez [a], Jose Luis Zambonino-Infante [e], Enric Gisbert [a,*]

[a] IRTA, Centre d'Aqüicultura, Crta. de Poblenou km 5.5, 43540 Sant Carles de la Ràpita, Tarragona, Spain
[b] IFAPA—Centro el Toruño, CAP Junta de Andalucía, Ctra. N-IV, 11500 El Puerto de Santa María, Cádiz, Spain
[c] Instituto de Acuicultura de Torre de la Sal (CSIC), 12595 Torre de la Sal, Castellón, Spain
[d] Andalusian Institute of Marine Sciences (CSIC), Campus Universitario Rio San Pedro, Apdo. Oficial, 11510 Puerto Real, Cádiz, Spain
[e] Ifremer, UMR 1067 Nutrition Aquaculture et Génomique des Poissons, Ifremer Centre de Brest, BP 70, 29280 Plouzané, France

ARTICLE INFO

Article history:
Received 16 April 2009
Received in revised form 29 June 2009
Accepted 30 June 2009

Keywords:
Senegalese sole
Solea senegalensis
Larval quality
Vitamin A
Skeleton
Thyroid hormones
Deformities

ABSTRACT

The effects of different levels of vitamin A (VA) in Senegalese sole larval performance and development were evaluated by means of a dietary dose–response experiment using enriched *Artemia* metanauplii as a carrier of this micronutrient. Larvae were fed from 6 to 27 days post hatch (dph) with enriched *Artemia* containing graded levels of total VA (1.3, 2.1, 4.5 and 12.9 μg VA mg^{-1} DW). The content of VA in live prey directly affected its accumulation in larvae and early juveniles. Retinyl palmitate accumulated during larval ontogeny, whereas retinol showed the opposite trend, decreasing from hatching until 41 dph and then remaining constant until the end of the study.

In metamorphic larvae (10 and 15 dph), VA did not affect the number of thyroid follicles or the intensity of the immunoreactive staining of T_3 and T_4. However, at older stages of development (post-metamorphic larvae: 20, 30, 41 and 48 dph), VA decreased the number of thyroid follicles but increased their mean size and enhanced T_3 and T_4 immunoreactive staining. A dietary excess of VA did not affect either larval performance in terms of growth and survival or the maturation of the digestive system. However, the most remarkable impact of this morphogenetic nutrient was detected during skeletal morphogenesis. Dietary VA accelerated the intramembranous ossification of vertebral centrums, which led to the formation of a supranumerary haemal vertebra and a high incidence of fused and compressed vertebrae in fish fed 2.1, 4.5 and 12.9 mg VA mg^{-1} DW. In addition, VA also affected those structures from vertebrae and caudal fin formed by chondral ossification, leading to defects in their shape and fusions with adjacent skeletal elements. In particular, the caudal fin was the region most affected by the dietary treatments. In order of importance, the bones with more developmental anomalies were the modified neural and haemal spines, epural, hypurals and parahypural. The impact of systemic factors such as thyroidal hormones in skeletogenesis should not be neglected since present results revealed that an excess of dietary VA affected the levels of T_3 and T_4, which might have affected bone formation and remodelling, leading to skeletal deformities.

Published by Elsevier B.V.

1. Introduction

Since the nineties, Senegalese sole (*Solea senegalensis* Kaup, 1858) has been considered a promising flatfish species for diversifying European marine aquaculture (Dinis et al., 1999). Recently, as profit margins for the two main cultured Southern European fish species, gilthead sea bream and European sea bass, have decreased due to their overproduction, interest has increased in Senegalese sole farming in Mediterranean and Southern Atlantic waters. Some of the advantages of culturing Senegalese sole include its high market price, the natural spawning of wild broodstocks held in captivity and mass production of offspring, the rapid development of eggs and larvae, and the high growth rate exhibited by juveniles (see review in Dinis et al., 1999). However, several bottlenecks compromise the intensive culture of this flatfish species, such as the reproduction of F1 broodstock (Anguis and Cañavate, 2005), pathological outbreaks (Zarza et al., 2003), and the production of juveniles in proper quantity and quality to satisfy market demands (high incidence of pigmentary disorders and skeletal deformities) (Soares et al., 2002; Gavaia et al., 2002).

Skeletal deformities and pigmentary disorders are important factors affecting flatfish production costs and determining the fish external morphology, appearance, growth, survival rate, and final market price (Estévez and Kanazawa, 1996; Takeuchi et al., 1998; Gavaia et al., 2002; Hamre et al., 2005). The development of these abnormalities is linked to a poorly understood relationship between nutritional, environmental, and

* Corresponding author. Tel.: +34 977745427; fax: +34 977443138.
E-mail address: enric.gisbert@irta.cat (E. Gisbert).

0044-8486/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.aquaculture.2009.06.046

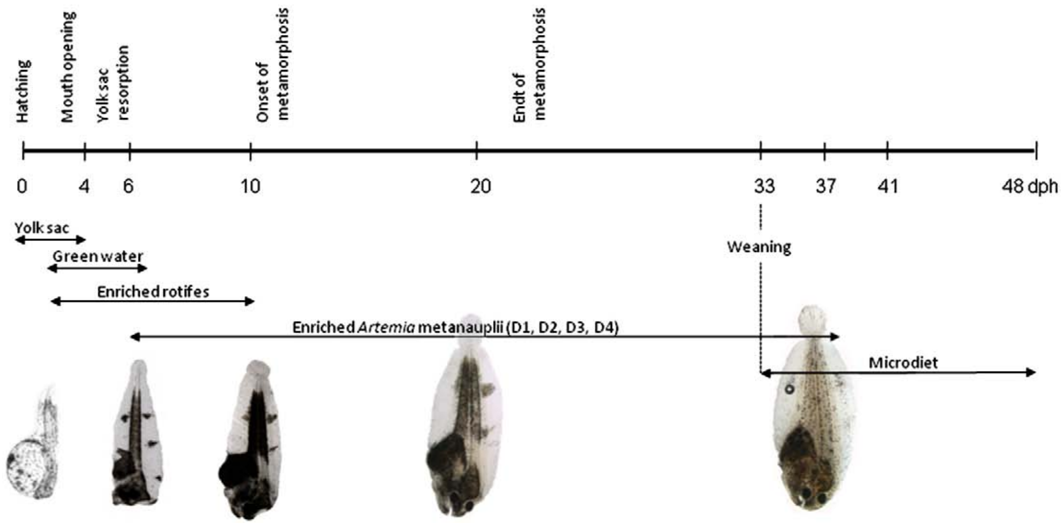

Fig. 1. Feeding protocol of Senegalese sole. *Artemia* metanauplii were enriched with experimental emulsions containing 500 (D1), 1000 (D2), 2100 (D3) and 4000 (D4) retinol equivalents g^{-1}.

genetic factors. Among them, larval nutrition at first feeding is one of the key parameters that affect skeletogenesis and pigmentation processes during early development. In this regard, several studies have shown that nutrients are responsible for the appearance of skeletal deformities and pigmentation disorders when their level and/or form of supply in the diet are inappropriate or unbalanced (see review in Lall and Lewis-McCrea, 2007; Hamre et al., 2005). Several authors have indicated that colour abnormalities in Japanese flounder could be effectively reduced by feeding larvae with high doses of vitamin A (VA) (Estévez and Kanazawa, 1995; Dedi et al., 1997; Takeuchi et al., 1995; Haga et al., 2002; Tarui et al., 2006). However, larvae fed high levels of VA showed a high incidence of skeletal deformities (Estévez and Kanazawa, 1995; Dedi et al., 1997; Takeuchi et al., 1998; Martinez et al., 2007) due to the morphogenetic action of this nutrient, which is known to have teratogenic effects in vertebrates at inappropriate dietary levels (Ross et al., 2000). Thus, in a situation in which a given nutrient exerts positive and negative effects simultaneously on different quality parameters, it is very important to determine a safe level that assures a normal skeletal development (minimum incidence of skeletal deformities) while preventing pigmentary disorders (pseudoalbinism and/or ambicolouration). The rapid physiological changes that Senegalese sole larvae undergo throughout development, reaching a fully metamorphosed morphology at an age of 20 days at 20 °C (Fernández-Díaz et al., 2001), make this species of particular interest for studying the dietary effects of vitamin A on skeletogenesis and metamorphosis.

The objective of the present study was to evaluate the effect of graded levels of dietary VA administered to Senegalese sole larvae during the *Artemia* feeding phase on larval performance (growth, survival, maturation of the digestive function, and metamorphosis success) and quality (incidence and typology of skeletal deformities).

2. Materials and methods

2.1. Larval rearing and experimental diets

Newly hatched larvae of Senegalese sole were obtained from Stolt Sea Farm SA (Cambre, La Coruña, Spain) and shipped by road to IRTA facilities. After their acclimation, larvae were distributed (initial density: 50 larvae l^{-1}) in 12 cylindrical tanks (100 l) connected to a recirculation unit (Carbó et al., 2003). Water conditions were as follows: 18 ± 1 °C, 35 ppt salinity, pH between 7.8 and 8.2, and daily exchange of water (20%) in the recirculation system with gentle aeration and oxygenation (>4 mg l^{-1}). Photoperiod was 12 L:12D, and light intensity was 500 lx at water surface.

Fig. 1 shows the feeding protocol for *S. senegalensis* used in the present study. In detail, larvae were fed from day 3 post hatch (dph) to 10 dph with rotifers (*Brachionus plicatilis*) enriched with Easy Selco™ (ES, INVE, Belgium) following manufacturer's instructions. Rotifer density was 10 rotifers ml^{-1} from 3 to 4 dph and gradually reduced to 5 rotifers ml^{-1} at 10 dph. Rotifer density was adjusted twice a day in order to assure the optimal prey density. Enriched *Artemia* metanauplii (EG, INVE, Belgium) were offered to larvae from 6 to 37 dph at increasing densities from 0.5 to 12 metanauplii ml^{-1}. *Artemia* metanauplii density was adjusted four times per day (at 9, 12, 15 and 18 h) to assure the optimal prey density and nutritional VA value; adjustments were conducted according to Cañavate et al. (2006). The retention of VA in enriched *Artemia* metanauplii in larval rearing tanks during the first 4 h of starvation post-enrichment did not change (Fernández, unpublished data). From 33 dph to the end of the experiment (48 dph), larvae were progressively weaned onto dry feed (Gemma Micro 150–300© Skretting, Spain).

The effect of VA in Senegalese sole skeletogenesis was evaluated by means of four different dietary regimes containing graded levels of VA and using enriched *Artemia* metanauplii as carrier; each regime was done in triplicate. As live preys (rotifers and *Artemia* nauplii) accumulate VA in different patterns (Giménez et al., 2007), we could not maintain the same levels of VA during the whole live prey-feeding period. Thus, we decided to focus our study only during the *Artemia* feeding phase. The graded levels of VA in *Artemia* metanauplii were obtained by adding different amounts of retinyl palmitate (1,600,000 IU g^{-1}, Sigma-Aldrich, Spain) to a commercial enriching emulsion, Easy Selco™. Experimental emulsions were designed to contain 500 (D1), 1000 (D2), 2100 (D3) and 4000 (D4) retinol equivalents g^{-1} (Table 1). For comparative purposes, the emulsion containing 500 retinol equivalents g^{-1} (1666 IU VA g^{-1}) was considered as the control group (ES without retinyl palmitate). Both live preys were enriched as previously described in Fernández et al. (2008).

Table 1
Total lipid and retinoid content (retinyl palmitate, retinol and total VA) in experimental *Artemia* enriching emulsions.

Emulsion	Total lipids	Retinyl palmitate	Retinol	Total VA
D1	84.3 ± 2.94	1.23 ± 0.010a	0.0051 ± 0.0005a	1.32 ± 0.030a
D2	81.7 ± 3.31	2.07 ± 0.440ab	0.0057 ± 0.0003ab	2.09 ± 0.123b
D3	87.8 ± 6.25	4.47 ± 0.830b	0.0079 ± 0.0005b	4.50 ± 0.249c
D4	82.7 ± 3.01	12.87 ± 0.198c	0.013 ± 0.002c	12.91 ± 0.059d

Total lipid content is expressed as % DW and retinoid content in emulsions is expressed as µg mg^{-1} DW. Different letters within the same column show significant differences between emulsions (ANOVA, $P<0.05$).