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Co-feeding of live feed and inert diet from first-feeding affects *Artemia* lipid digestibility and retention in Senegalese sole (*Solea senegalensis*) larvae

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ABSTRACT

The present study intended to evaluate the effects of early introduction of inert diet in lipid digestibility and metabolism of sole, while larval feed intake, growth and survival were also monitored. *Solea senegalensis* larvae were reared on a standard live feed regime (ST) and co-feeding regime with inert diet (Art R). Trials using sole larvae fed with *Artemia* enriched with two different lipid emulsions, containing glycerol tri [1-^{14}C] oleate (TAG) and L-3-phosphatidylcholine-1,2-di-[1-^{14}C] oleoyl (PL), were performed at 9 and 17 days after hatching (DAH) to study lipid utilization. Co-feeding did not affect sole survival rates (ST 59.1 ± 15.9%; Art R 69.56 ± 9.3%), but was reflected in significantly smaller final weight at 16 DAH (ST 0.71 ± 0.20; Art R 0.48 ± 0.14 mg). Higher feed intake was observed in sole larvae fed on *Artemia* enriched with labeled PL at 9 DAH but not at 17 DAH. At 17 DAH, the smaller larvae (Art R treatment) ingested proportionally more *Artemia* in weight percentage, independently of enrichment. At 9 DAH lipid digestibility was equal among treatments and higher than 90%, while at 17 DAH it was higher in ST treatment (around 73%) compared to the Art R group (around 66%). Lipid retention efficiency at 9 DAH was higher in the Art R treatment, reaching values of 50%, while these values almost duplicated at 17 DAH, ranging up to 80% in both treatments without significant differences. These results show that co-feeding of live feed and inert diet from first-feeding in Senegalese sole has a toll in terms of growth and lipid digestibility but does not seem to compromise lipid metabolic utilization.

1. Introduction

In order to successfully achieve the objective of a significant partial replacement of live feed by inert diets from first-feeding, a detailed understanding of the larval digestive physiology and how it may be influenced by the dietary components is indispensable (e.g., Cahu and Zambonino Infante, 2001; Morais, 2005; Engrola, 2008).

The Senegalese sole (*Solea senegalensis*, Kaup 1858) is a flatfish found along the Mediterranean and Atlantic coasts, and is a promising candidate for aquaculture in Europe since the nineties due to good market prices (Howell, 1997; Dinis et al., 1999; Imsland et al., 2003). Despite its high potential as an aquaculture species, only a few studies looking at sole larvae rearing conditions (Esteban et al., 1995; Dinis et al., 1999) and weaning strategies (Marin-Magan et al., 1995; Cañavate and Fernández-Díaz, 1999; Ribeiro et al., 2002; Engrola et al., 2005, 2007, 2009a) are available. Moreover, weaning success of Senegalese sole is still a critical step, with two strategies being possible: sudden weaning and weaning in co-feeding with *Artemia* metanauplii (Engrola et al., 2007). In spite of recent progress in sole larvae nutritional requirements and understanding of larval digestive physiology, weaning results obtained so far are variable and difficult to reproduce (Conceição et al., 2007b). Therefore, hatchery protocols for Senegalese sole still rely on live preys during the period before the metamorphosis, which occurs between 12 and 20 DAH, when they can be gradually substituted by frozen *Artemia* metanauplii. Recently it has been demonstrated that protein digestibility and retention are depressed by co-feeding with high levels of *Artemia* replacement by inert diet, and thereby lead to lower growth (Engrola, 2008). However, when a moderate level of *Artemia* replacement is used, sole are able to adapt their protein metabolism and enhance protein utilization in the long term, with a growth promoting effect at complete weaning (Engrola et al., 2009b).

Fish larvae diets, and particularly enriched *Artemia*, tend to be rich in triacylglycerols (TAG) as lipid source (Morais et al., 2006), in an attempt to meet essential fatty acids (EFA) requirements, namely in terms of n-3 polyunsaturated fatty acids (PUFA) (Sargent et al., 1989). This can be a problem since the high levels of lipids as well as the

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unbalances in lipid class composition found occasionally in enriched live preys have been suggested to affect fatty acid (FA) digestion and absorption (Salhi et al., 1995, 1997, 1999; Díaz et al., 1997; Morais et al., 2007). In the marine environment, high levels of phospholipids (PL) are normally found in the total lipid fraction of phytoplankton and zooplankton ingested by fish larvae (Sargent et al., 1989). A beneficial effect of dietary PL supplementation in purified diets in terms of survival, growth, resistance to stress, and lower occurrence of deformities has been demonstrated in larval and juvenile stages of various species of fish and crustaceans (Geurden et al., 1995; Coutteau et al., 1997; Koven et al., 1998; Cahu and Zambonino Infante, 2001).

The present work intended to evaluate the effects of co-feeding live feed with inert diet from mouth opening on lipid digestive capacity and metabolism of sole larvae, compared to a standard feeding regime using only live feed. To achieve this, two metabolic trials using radiolabeled *Artemia* enriched with lipid emulsions containing either glycerol tri [1-^{14}C] oleate (TAG) or L-3-phosphatidylcholine-1,2-di-[1-^{14}C] oleoyl (PL) were performed with sole larvae before (9 days after hatching, DAH) and during the metamorphosis climax (17 DAH). The digestibility, retention and catabolism of the radiolabel incorporated in *Artemia*, as well as larval *Artemia* intake, were measured. In addition, the use of different sources of radiolabeled lipid (TAG or PL) enabled to verify whether these effects depend also on the molecular moiety in which the FA were supplied to *Artemia*.

2. Materials and methods

2.1. Larval rearing

Senegalese sole eggs used in the experiment were obtained from natural spawning of captive breeders maintained in IPIMAR-CRIPSul, Olhão, Portugal. The larvae were stocked in 100 L cylindro-conical tanks at a density of 100 larvae L^{-1}. The green water technique was used in the rearing tanks with a 1:1 mixture of *Tetraselmis chuii* and *Isochrysis galbana* in a recirculation system, at a temperature of 19.8 ± 0.4 °C and a salinity of 37.8 ± 1.5‰. Oxygen saturation was 96.4 ± 9.6% and a 12/12-h light/dark cycle was adopted. Water renewal was increased from 4 times/day from 0 DAH to 8 times/day from 13 DAH until the end of the experiment, which lasted 19 days.

2.2. Feeding regimes

Two different feeding regimes were randomly assigned in triplicate during the pelagic phase: standard live feed (Standard, ST) and live feed co-fed with inert diet from mouth opening (*Artemia* Replacement, Art R). The feeding was based on rotifers (*Brachionus rotundiformis*) enriched with Red Pepper (BernAqua, Olen, Belgium) from 2 to 4 DAH for both treatments; *Artemia* nauplii (INVE Aquaculture NV) from 4 to 9 DAH for both treatments; and *Artemia* metanauplii enriched for 12 h, at 250 nauplii mL^{-1}, with 0.4 g L^{-1} in two doses (at 3 and 6 h, following the manufacturer's instructions) of a 1:1 mixture (weight basis) of Easy DHA Selco® (INVE Aquaculture NV) and Micronised Fishmeal® (Ewos, Scotland) for both treatments until the end of experiment. The amount of *Artemia* supplied to the Art R treatments was gradually reduced during the experiment (see Table 1). At the end of the experiment Art R sole were being offered 45% frozen *Artemia* metanauplii and 55% inert diet (*Proton* 100–200 µm; INVE Aquaculture NV, Dendermonde, Belgium) in proportion (weight basis) to total daily ration. Between days 13 and 16, the *Artemia* metanauplii supply to both treatments was gradually changed from live to frozen *Artemia*. *Artemia* metanauplii were harvested, washed in seawater, counted, and frozen at −20 °C. Fifteen minutes before feeding, *Artemia* was thawed in seawater. Table 1 shows the feeding regimes in detail.

The larvae were fed daily at 11:00 am, 14:00 pm and 17:00 pm. The first meal was composed by 50% of the daily feeding dose, and the remaining 50% was shared between the two following meals.

Table 1
Feeding regimes of Senegalese sole larvae from 2 to 19 days after hatching (DAH); ST — Standard live feed regime; Art R — *Artemia* replacement by inert diet (dry matter basis) from mouth opening.

	Treatments						
	ST			Art R			
DAH	Rot	Na AF	Meta EG	Rot	Na AF	Meta EG	Inert diet
2	3.0			3.0			0.1
3	5.0			5.0			0.1
4	8.0	2.0		8.0	2.0		0.1
5		4.0			4.0		0.1
6		6.0			5.0		0.2
7		8.0			6.0		0.2
8		8.0			6.0		0.3
9		4.0	4.0		3.0	3.0	0.4
10			6.0			3.0	0.5
11			8.0			4.0	0.6
12			10.0			5.0	0.8
13			12.0			6.0	0.9
14			12.0			6.0	1.1
15			14.0			7.0	1.2
16			14.0			7.0	1.5
17			16.0			8.0	1.8
18			16.0			8.0	2.2
19			18.0			9.0	2.7

Rot: Rotifers; Na AF: *Artemia* nauplii AF Strain; Meta EG: *Artemia* metanauplii EG Strain; and Inert diet: Proton diet. Rotifers and *Artemia* are expressed as 'number of prey/mL tank volume/day'; inert diet daily ration is expressed as 'mg/tank/day'.

2.3. Sampling

Samples were taken for the determination of individual dry weight (DW) at: 2 DAH, at mouth opening ($n = 30$ per treatment), 8 DAH ($n = 30$ for each replicate) and 16 DAH ($n = 15$ for each replicate). The larvae were stored at −20 °C and afterwards freeze-dried for 48 h in a Savant SS31 (Savant Instruments Inc., Hokbrook, NY, USA). The DW of the larvae was determined in a Sartorius type M5P scale (precision of 0.001 mg; Sartorius micro, Göttingen, Germany). Survival was determined at the end of the experiment, by counting the larvae remaining in the rearing tanks.

2.4. Lipid metabolism trials

Two trials were performed using *Artemia* labeled with different ^{14}C-lipid sources to analyze the effects of the feeding regimes on the digestive capacity and metabolism of sole larvae: the first at 9 DAH, in the pelagic phase, and the second at 17 DAH, during the metamorphosis climax.

2.4.1. *Artemia* [1-^{14}C] labeling

Two lipid emulsions were prepared using 0.09 g of Easy DHA Selco plus either 50 µL (50 µCi) of glycerol tri [1-^{14}C] oleate (TAG; 3·7 MBq/mL) or 100 µL (10 µCi) of L-3-phosphatidylcholine-1,2-di-[1-^{14}C] oleoyl (PL; 0·925 MBq/mL) (Amersham Pharmacia Biotech Ltd., UK). After mixing the radiolabeled lipids, the solvent in which the radiolabel came dissolved was evaporated by flushing N_2. The eppendorfs with the emulsions were covered with parafilm and submitted to 5 min of ultrasound bath at 30 °C, vigorously shaken for 2 min and then stored at −20 °C. The enrichment was made by adding and mixing the TAG or PL emulsions to 150 mL of seawater before introducing *Artemia* (200 metanauplii/mL). The incubation lasted 14 h to allow for complete lipid incorporation (Morais et al., 2004b), being also a common enrichment period used with several commercial products. After incubation, *Artemia* metanauplii was washed thoroughly, counted and samples were taken to measure the incorporated radiolabel.

2.4.2. Cold chase — set up

In both trials, approximately 14 h prior to the start of radiolabeled *Artemia* feeding and 30 min following their last meal, 10 larvae from