

Two microalgae *Cryptocodinium cohnii* and *Phaeodactylum tricornutum* as alternative source of essential fatty acids in starter feeds for seabream (*Sparus aurata*)

E. Atalah^{a,*}, C.M. Hernández Cruz^a, M.S. Izquierdo^a, G. Rosenlund^b, M.J. Caballero^a,
A. Valencia^a, L. Robaina^a

^a Grupo de Investigación en Acuicultura (ICCM and IUSA), P.O. Box 56, 35200, Telde, Las Palmas de Gran Canaria, Spain

^b Skretting Aquaculture Research Centre A/S, P.O. Box 48, N-4001 Stavanger, Norway

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Abstract

Despite oils extracted from algae and other microorganisms that may constitute excellent sources of HUFAs, few studies have determined the nutritional value of different microalgal species for young marine fish. Six thousand gilthead seabream (*Sparus aurata*) postlarvae (73 mg body weight) were fed for 57 days diets containing either fish oil as a single lipid source or 2 and 4% of *Cryptocodinium cohnii* or 5% *Phaeodactylum tricornutum*. Fish oil substitution by *C. cohnii* resulted in improved fish survival and a very good growth performance, in agreement with a higher proportion of DHA in diets and in total lipids of fish. Incorporation of DHA and other fatty acids was proportional to their contents in diet suggesting the good nutritional utilization of homogenized *C. cohnii*. Lower survival rates were found in fish fed *P. tricornutum* and could be related to an epithelial degeneration observed in the anterior intestine. This degeneration could be related to a higher lipid content in these fish or to the strong hornlike comutate processes found in the valves of the diatom *P. tricornutum*.

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1. Introduction

Aquaculture has traditionally used certain fisheries products such as fish meal and oil in a very efficient way (Bell et al., 2003). However, fisheries constitute a declining resource and the continued exploitation of

fish-stocks in order to meet the demands of an expanding market implies important environmental consequences (Tonon et al., 2002). Since the late 1990s, industrial fisheries that supply the world's fish oil demand have been stagnant or even declined, particularly during years under “El Niño” effect (Sargent and Tacon, 1999). This restriction, together with the increasing fish oil demand, not only to sustain the spectacular world aquaculture development but also for terrestrial animal feed and human dietary supplements, have converted fish oil into a highly valuable but limited resource (Harel et al., 2002). Furthermore, supplies of

Abbreviations: ARA, arachidonic acid; DHA, docosahexaenoic acid; EPA, essential fatty acids; EPA, eicosapentaenoic acid; FO, fish oil; HUFA, high unsaturated fatty acid; LP, lamina propria; PUFA, polyunsaturated fatty acid; SGR, specific growth rate.

* Corresponding author. Tel.: +34 928132900; fax: +34 928132908.

E-mail address: eyadatalah@gmail.com (E. Atalah).

fish oils for aquaculture production are expected to become critical before 2010 (Bell and Sargent, 2003), when more than 85% of the world supply will be required for aquaculture feeds (Barlow, 2000). Hence, the actual growth rate of aquaculture production would only be possible if alternatives to fish oil are developed and introduced (Izquierdo et al., 2005). Moreover, the use of fish oil in fish feeds encounters some other constraints such as its poor oxidative stability (Swaaf et al., 1999), pollutants and heavy metal accumulation (Tonon et al., 2002) or a variable and complex fatty acid profile (Medina et al., 1998).

Besides providing energy, fish oils are the traditional source of highly unsaturated fatty acids (HUFA) which are indispensable in fish nutrition, being necessary for fish growth and survival (Watanabe, 1982; Sargent et al., 1999) where they play many different structural and physiological functions (Izquierdo, 1996). Oils extracted from algae and other microorganisms may constitute excellent sources of HUFAs, being widely used as nutritional supplements in human infant formulas (Cohen et al., 1995). Microalgae have also frequently been employed in aquaculture feeds (Day et al., 1990; Zhou et al., 1991; Laing and Millican, 1992; Day and Tsavalos, 1996; Langden and Onal, 1999), marine species being considered as a key diet component in finfish and shellfish aquaculture (De Pauw and Persoone, 1988). Besides, microalgae have a simpler fatty acid profile which facilitates purification (Medina et al., 1998) and constitute a non limited resource which can be produced in industrial quantities under environmentally safe conditions (Harel et al., 2002), controlling the species and culture media to design particular fatty acid compositions (Medina et al., 1998). The HUFA content of microalgae depend not only on the species, but also on factors related to culture condition including composition of the medium, pH, aeration, light intensity, temperature, age of culture (Tonon et al., 2002) and duration of the photoperiod (Medina et al., 1998). The marine dinoflagellate *Cryptocodinium cohnii* constitutes an excellent heterotrophic producer of DHA. *C. cohnii* is a chloroplastless heterotrophic marine microalgae, which shows two different phases: swarming flagellated cells and cysts. Utilizing several carbon sources *C. cohnii* accumulates lipid over 40% of its biomass dry weight, with up to 30 of total lipids being DHA (Swaaf et al., 2003a,b). Another interesting single cell source of lipids is the diatom *Phaeodactylum tricornutum* which utilizes lipids as main storage products (Reis et al., 1996; Mirón et al., 2002), producing large quantities of polyunsaturated fatty acids, including considerable amounts of EPA (Arao et al.,

Table 1

Ingredients and proximate composition of the experimental diets (%)

	Diets			
	FO	C	2C	P
Fish meal LT ¹	80.7	79.9	79.12	77.37
Suprex corn ²	4	3.96	3.92	3.84
Whey powder ³	2.5	2.48	2.45	2.40
Premix ⁴	5.5	5.45	5.39	5.27
Fish oil ⁵	5.3	6.24	5.20	6.33
EPAX ⁶	2.0			
Dry algae	–	1.98	3.92	4.79
Algal type	–	<i>C. cohnii</i>	<i>C. cohnii</i>	<i>P. tricornutum</i>
Crude protein	61.4±2.3	62.6±0.9	62.4±0.7	61.6±0.4
(% dry weight)				
Crude lipids	19.7±0.4	19.8±0.6	18.4±0.6	19.0±0.5
(% dry weight)				

¹Nordsilme, Norway.²Codrico, Rotterdam, The Netherlands.³Tine Meierier, Klepp, Norway.⁴Constant ingredients, g kg⁻¹: 4.27 g vitamin and mineral premix (according to NRC, 1993), proprietary composition (Skretting ARC, Stavanger, Norway), 0.73 g Lutavit C (BASF, Ludwigshafen, Germany), 40.0 g lecithin (Yelkinol, Trouw Nutrition, Putten, The Netherlands), 10 g betaine (Cultor, Helsinki, Finland).⁵Fish oil (capelin oil, Denofa, Fredrikstad, Norway).⁶EPAX 1040 (Pronova, Sandefjord, Norway).

1987; Dunstan et al., 1994). In *P. tricornutum* the fatty acids constitute between 8 and 10% of the algal cell biomass, where EPA constitute between 27 and 30% of the total fatty acids present, or 2.6–3.1% of the dry biomass.

However few studies have determined the nutritional value of different microalgal species included in diets for young marine fish. Hence, the objective of the present study was to evaluate the potential inclusion of two marine microalgae (*C. cohnii* and *P. tricornutum*) in starter diets for seabream determining its effect on fish performance and health.

2. Materials and methods

Four thousand eight hundred gilthead seabream (*Sparus aurata*) postlarvae (68 day-old post-hatch, 73 mg body weight) were randomly distributed in 12 tanks (150 l at the beginning and 500 l in the experimental second half period, 400 larvae/tank), submitted to a natural photoperiod regime (12 h dark: 12 h light with an intensity of 500 lx) and fed one of the four experimental diets for 57 days. Seawater temperature ranged between 21 and 24 °C during the experiment.

Four isonitrogenous and isolipidic experimental diets with different lipid sources were produced by Skretting Aquaculture Research Centre (Stavanger, Norway). Diet

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