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# Effects of graded levels of a blend of *Tisochrysis lutea* and *Tetraselmis suecica* dried biomass on growth and muscle tissue composition of European sea bass (*Dicentrarchus labrax*) fed diets low in fish meal and oil

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ABSTRACT

The aim of this study was to evaluate nutrient digestibility, growth response, carcass and fillet yields, muscle tissue composition and skin colour appearance of European sea bass (*D. labrax*) fed graded levels of a mixture of *Tisochrysis lutea* and *Tetraselmis suecica* freeze-dried biomass in partial substitution for fish meal and oil in diets containing substantial levels of vegetable oils and protein-rich derivatives. Five complete diets were formulated to be grossly isoproteic and isolipidic and prepared by including a blend of the two freeze-dried microalgae biomass in a 2:1 weight ratio, to replace approximately 15, 30 and 45% fish meal protein and 12, 24 and 36% fish lipid of a positive control diet. A negative control complete feed high in soybean meal was also prepared. Each diet was offered in triplicate, during 105 days until visual satiety of fish (204 ± 12.7 g) kept in a semi-closed recirculating marine water system ensuring optimal water quality to E. sea bass.

The results of the study have shown that replacing about 45% crude protein and 36% lipid from fish meal and lipid by a mixture of *Tisochrysis lutea* and *Tetraselmis suecica* dried biomass, did not adversely affect growth performance and feed conversion efficiency of European sea bass. A slight decline in dry matter, protein and energy digestibility occurred in response to graded levels of dietary microalgae biomass, which was compensated by increased feed intake. Moreover, the diet including the dried microalgae resulted in a higher nutritive value than that of the negative high-soybean meal control feed. No major changes were observed in biometry traits and slaughter yield while the nutritional properties of the edible muscle tissue were little affected in terms of n-3 PUFA. The presence of the dried microalgae in the diet resulted in a greenish pigmentation of the skin, with a slight tendency towards redness and diminished lightness and hue. Overall the results obtained here reveal a potential improvement in sustainability in terms of reduced reliance on halieutic feed resources in case of the microalgae-containing diets. Hence the sustainable use of the dried mixture of the 2 marine microalgae biomass as feed ingredients in diets for the E. sea bass seems to be mainly limited by the low availability and unaffordable market price which are both expected to improve over the next 10 years.

## 1. Introduction

Among feed resources quoted as possible candidate ingredients for more sustainable aquafeeds, single cell microorganisms have recently deserved renewed attention. Whole-cell dried microalgae biomass in particular, due to a minimal overall environmental impact compared to most conventional feed/food commodities, have been proposed as raw materials in partial substitution for fish-derivatives in fish diets, even if scarce availability and high market price still set a limit to their use in commercial aquafeeds (Muller-Feuga, 2004; Shields and Lupatsch, 2012; Zmora and Richmond, 2004). According to Draaisma et al. (2013) the feed-mill industry requires large quantities of biomass produced at low cost (< 1 € $kg^{-1}$) whereas independent economic estimates and analysis indicated the production costs of 1 kg of dried weight microalgae biomass to lie in a range of 4.14 to 5.96 € (Norsker et al., 2011), or 3.2 to 12.4 € (Tredici et al., 2016), depending on the species, cultivation system, geographic location and scale of production. Based on the potential improvements in productivity, it is assumed

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that over the next 5–10 years a sustainable reduction of microalgae production costs will be achieved.

As sources of nutrients, dry microalgae biomass are characterised by medium to high crude protein levels, ranging from 28 to 71% DM, depending on the species and culture/harvesting conditions, with an amino acid profile comparable with, or even better than that of conventional plant crops or vegetable feeds rich in protein (Becker, 2007). Cell wall structure and composition have been claimed to adversely affect bioavailability of nutrients supplied by certain microalgae species but, at the moment, this general assumption remains speculative in fish, where information on digestibility of different dried microalgae biomass or diets including them at substantial inclusion levels is still very limited (Burr et al., 2011; SØrensen et al., 2016; Tibaldi et al., 2015; Walker and Berlinski, 2011). Despite this, previous studies have shown dried biomass of a number of different microalgae species to be a valuable supplementary protein source or partial substitute for fish meal protein in the diet of various omnivorous and carnivorous fish species at the juvenile stage (Badawy et al., 2008; Belay et al., 1996; Hussein et al., 2013; Kiron et al., 2012, 2016; Nandeesha et al., 2001; Olvera-Novoa et al., 1998; Tulli et al., 2012; Vizcaìno et al., 2014, 2016; Walker and Berlinsky, 2011).

Certain marine microalgae are also rich in lipid and n-3 long chain polyunsaturated fatty acids (n-3 (LC)PUFAs) and dry preparations of docosahexaenoic acid (DHA)-rich *Schizochytrium* spp., *Crypthecodinium chonii, Nannochloropsis* sp., *Pavlova viridis* have proven successful as partial replacers for fish oil in starter diets for the juvenile stages of certain fish species (Atalah et al., 2007; Carter et al., 2003; Haas et al., 2015; Miller et al., 2007; Sarker et al., 2016b).

In this context, the dry biomass of *Tisochrysis lutea,* formerly *Isochrysis galbana* (T-iso), has been poorly studied so far, but it deserves attention as it combines medium-high levels of protein with high lipid and DHA contents (Ben-Amotz et al., 1987; Sánchez et al., 2000). Furthermore, *Tisochrysis lutea* being small in size (4–6 µm) and lacking a structured cell wall (naked) is expected to be readily digested (Vizcaìno et al., 2016). All these attributes make it a potential candidate ingredient for diets low in fish meal and fish oil for the E. sea bass which, besides requiring high protein diets, is notably incapable of significant n-3-(LC)PUFAs biosynthesis (Mourente et al., 2005) and where a huge substitution of fish oil by vegetable counterparts has been shown to be detrimental in terms of healthy attributes of the flesh due to a marked reduction of the n-3(LC)PUFA content (Montero et al., 2005; Mourente and Bell, 2006). Encouraging results in this direction have been recently observed in this marine fish species (Tibaldi et al., 2015), in that the use of dried *Tisochrysis lutea* biomass to replace up to 20% crude protein from fish meal and up to 36% fish lipid in a diet with reduced level of fish oil, did not adversely affect feed intake or growth performance. However, this resulted in a slight decline of energy digestibility and n-3(LC)PUFA content in muscle tissue.

*Tetraselmis suecica* (Prasinophyceae, Chlorophyta) is also of particular interest being medium-high in protein and relatively easily mass-cultivable at low cost, when produced in a new generation of photobioreactors (Tredici et al., 2015). *Tetraselmis* has a pretty thin cell wall and is widely used in hatcheries for feeding juvenile bivalve molluscs, penaeid shrimp larvae and rotifers as a source of nutrients and n-3(LC) PUFA being relatively rich in eicosapentaenoic acid (EPA) (Brown et al., 1997). When E. sea bass were fed to satiety complete diets where *T. suecica* was up to 16% by weight, a linear decline in nutrient digestibility was observed althought without adversely affecting the growth performance of the fish (Tulli et al., 2012).

It should be noted that, in most of the studies carried out so far to evaluate fish response to variable levels of different microalgae dried biomass in feeds, the test diets were compared to control preparations largely based on fish meal and oil as major protein and lipid sources. This could have led to underestimate the nutritive value of microalgae when compared with that of protein-rich plant and oil sources which are currently increasingly being used as major alternatives to fish meal and oils in commercial aquafeeds.

Besides the nutritive value to fish, microalgae are also a source of pigments and their inclusion in the diet has been shown to affect the colour appearance of a range of fish species including marine ones (Chatzifotis et al., 2011; Ribeiro et al., 2017; Tulli et al., 2012; Tibaldi et al., 2015; Walker and Berlinski, 2011). This is particularly important since colour and visual appearance are known to influence market value, flavour perception and acceptability in market size specimens of fish food products (Spence et al., 2010; Vasconcellos et al., 2013).

On this basis, the aim of this study was to evaluate growth response, dressing out yield, muscle tissue composition and skin colour appearance of E. sea bass (*D. labrax*) fed graded levels of a mixture of freeze-dried *T Isochrysis lutea* and *Tetraselmis suecica* in partial substitution for fish meal and oil in diets containing substantial amounts of vegetable oils and protein-rich plant derivatives.

## 2. Materials and method

### *2.1. Test ingredients and diets*

The chemical composition and fatty acid profile of the freeze-dried *T. lutea* and *T. suecica* are shown in Tables 1 and 2, respectively.

Five diets were formulated to be grossly isoproteic (49.3% DM) and isolipidic (18.5% DM). The ingredient and proximate composition of the diets and fatty acid profile are shown in Tables 3 and 4, respectively. A positive control diet (C+) was prepared in order to have

**Table 1**
Chemical composition of *T. lutea* and *T. suecica* freeze-dried biomass (data expressed on dry matter basis).

| | *T. lutea* | *T. suecica* |
|---|---|---|
| Proximate composition (%) | | |
| Water | 10.0 | 5.9 |
| Crude protein | 46.3 | 48.7 |
| Total lipid | 26.0 | 8.0 |
| Ash | 11.3 | 17.5 |
| Phosphorous (g/kg) | 0.8 | 1.1 |
| ß-carotene (mg/kg) | 761.7 | 267 |
| Essential AA (%) | | |
| Arginine | 2.52 | 2.05 |
| Histidine | 0.91 | 0.72 |
| Isoleucine | 1.76 | 1.41 |
| Leucine | 3.92 | 3.27 |
| Lysine | 2.46 | 2.27 |
| Methionine + cysteine | 1.41 | 1.66 |
| Phenylalanine + tyrosine | 3.75 | 3.98 |
| Threonine | 2.38 | 1.81 |
| Tryptophan | 0.56 | 0.30 |
| Valine | 2.37 | 1.87 |
| Non essential AA (%) | | |
| Alanine | 3.17 | 2.86 |
| Aspartic acid | 4.19 | 3.44 |
| Glutamic acid | 4.58 | 5.06 |
| Glycine | 2.64 | 2.52 |
| Proline | 2.36 | 1.80 |
| Serine | 2.17 | 1.67 |
| Fatty acid composition[a]% | | |
| SFA | 5.66 | 1.03 |
| MUFA | 3.89 | 1.07 |
| n-6 PUFA | 1.98 | 2.41 |
| n-3 PUFA | 5.52 | 0.16 |
| 20:5n-3 | 0.19 | 0.26 |
| 22:6n-3 | 1.81 | – |
| n-3/n-6 | 2.79 | 0.07 |

[a] The following fatty acids were considered in the respective composite fractions but are not shown in the table: 10:0, 11:0, 12:0, 13:0, 14:0, 15:0, 16:0, 18:0, 20:0; 14:1n-5; 16:1n-7, 16:1n-9, 17:1, 18:1n-9, 18:1n-7, 20:1n-9, 20:1n-11, 22:1n-9, 22:1n-11; 16:2n-4, 18:2n-6, 18:3n-6, 20:4n-6, 22:5n-6; 16:3n-4, 18:3n-3; 16:4n-1, 18:4n-1, 18:4n-3, 20:4n-3, 22:4n-6, 21:5n-3; 22:5n-3.