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# Influence of conjugated linoleic acid on growth, lipid composition and hepatic lipogenesis in juvenile European sea bass (*Dicentrarchus labrax*)

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#### Abstract

Dietary conjugated linoleic acids (CLA) can be successfully incorporated in several fish species but have not been evaluated in marine fish. A 12-week growth trial was conducted with European sea bass juveniles, having an initial weight of 13.96±0.16 g (S.D.). Fish were fed to satiation, twice a day, with diets containing graded amounts of CLA (0, 0.5, 0.75, 1.0 or 2%). At the end of the experiment, the daily growth index, feed intake, feed efficiency and whole body composition and tissue fatty acid profile were determined. The activity of some enzymes (glucose-6-phosphate dehydrogenase, G6PD, malic enzyme, ME, and fatty acid synthetase, FAS,) was also measured in the liver. Digestibility of the diets was determined, using chromic oxide as inert tracer, and the Guelph System for faecal collection. No significant differences were detected (P>0.05) in growth parameters, feed conversion, body composition or nutrient retention among treatments. Dietary CLA did not affect significantly the activity of the lipogenic enzymes when expressed as mIU/mg protein, but when the HSI variations were taken into consideration (IU/100 g fish) the activities of both G6PD and ME seemed to be depressed in fish fed increasing CLA levels. Dietary inclusion of CLA had no effect on tissue lipid content, but significantly affected the total percentages of polyunsaturated (PUFA) and monounsaturated fatty acids (MUFA) in both muscle and liver tissues. Dietary CLA supplementation resulted in a significant increase of the biologically active trans-10, cis-12 and cis-9, trans-11 CLA isomers in both tissues. The total accumulation of CLA was clearly higher in muscle than in liver, reaching 5.61% and 3.00% of total lipids, respectively, in fish fed 2% CLA. The results obtained in this experiment clearly suggest European sea bass can successfully incorporate CLA in both liver and muscle up to 2%, contributing to the production of a functional food.

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#### 1. Introduction

Conjugated linoleic acids (CLA) is a collective term for 18 carbon fatty acids that differ in the geometry and

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position of two conjugated double bonds. CLA isomers, in particular cis-9, trans-11 and trans-10, cis-12 CLA, have received much attention for their health promoting effects (Jahreis et al., 2000; Pariza et al., 2001; Roche et al., 2001). They were shown to inhibit atherosclerosis (Lee et al., 1994), chemically induced carcinogenesis (Ha et al., 1990; Ip et al., 1999), obesity (Park et al., 1997), and to enhance feed efficiency (Li and Watkins, 1998) in several rodent models. In addition, dietary CLA has been reported to decrease body fat in mice (Park et al., 1997), rats (Sisk et al., 2001), pigs (Dugan et al., 1997), and chickens (Badinga et al., 2003). The exact mechanism for the reduced fat accumulation by dietary CLA is not clear, but it can be related to the inhibition of lipid absorption and lipogenesis and the promotion of lipid oxidation (Park et al., 1997; Takahashi et al., 2003).

The current trend in fish production is to increase the lipid content in diets to spare protein, to improve feed conversion and to decrease the amount of waste produced by fish (Hillestad and Johnsen, 1994; Perez et al., 1997; Vergara et al., 1999). However, these diets change the body composition and slaughter quality, particularly through an increase of lipid deposition (Cowey, 1993; Hillestad and Johnsen, 1994). The farming industry is very interested in finding methods that control body lipid deposition and/or increase the market value of cultured fish, when such diets are used. Fish are not a natural rich source of CLA, but in several species the dietary supplementation results in high muscle deposition levels (Twibell et al., 2000, 2001; Berge et al., 2004; Kennedy et al., 2005; Bandarra et al., 2006; Valente et al., 2007). Moreover, dietary CLA may reduce growth response but improve feed efficiency (Twibell et al., 2000), and reduce tissue total lipids in some fish species (Twibell et al., 2000, 2001; Yasmin and Takeuchi, 2002).

The effects of dietary CLA in the growth performance and flesh quality of marine species of interest for European aquaculture have not been reported. Since fish are considered an important source of protein and n-3 PUFA (polyunsaturated fatty acids), a further increase in its CLA content could be of interest to enhance the nutritional value of fish for human consumption. Thus, the overall objective of this study was to evaluate the effects of graded dietary levels of CLA (0, 0.5, 0.75, 1 and 2%) on growth performance, body composition, tissue fatty acid deposition and lipogenic enzymes activities (glucose-6-phosphate dehydrogenase, G6PD, malic enzyme, ME, and fatty acid synthetase, FAS) of European sea bass juveniles.

#### 2. Materials and methods

#### 2.1. Experimental diets

A commercial extruded diet for European sea bass was supplied by Sorgal (Ovar, Portugal). Before oil coating, the extruded pellets (2 mm of diameter) were analyzed for fat composition and then coated with 13% oil containing the different CLA levels (0, 0.5, 0.75, 1 or 2%) to obtain five isonitrogenous (48% crude protein) and isolipidic (22% total lipid) diets (Table 1). The CLA supplement was added to the diets at the expense of fish oil to maintain a constant energy level among dietary treatments. The CLA mixture (>70% CLA) was a gift from Bioriginal Food and Science Corp., Saskatoon SK, Canada. The fatty acid profiles of the experimental diets are presented in Table 2.

Table 1 Ingredients and proximate composition of the experimental diets

	Dietary CLA treatments (%)				
	0	0.5	0.75	1	2
Ingredients (%)					
Fish meal LT (68-70) SP	43.65	43.65	43.65	43.65	43.65
Fish meal 60	12.50	12.50	12.50	12.50	12.50
Fish meal LT	5.00	5.00	5.00	5.00	5.00
Extruded peas meal <sup>1</sup>	10.00	10.00	10.00	10.00	10.00
Yeast	7.39	7.39	7.39	7.39	7.39
Corn gluten	2.55	2.55	2.55	2.55	2.55
Wheat	2.30	2.30	2.30	2.30	2.30
Fish oil	12.61	11.94	11.61	11.28	9.94
CLA <sup>2</sup>	0.00	0.67	1.00	1.33	2.67
Vitamin <sup>3</sup> and mineral mix <sup>4</sup>	4.00	4.00	4.00	4.00	4.00
Proximate composition					
Dry matter (DM, %)	89.18	89.08	90.90	89.32	89.41
Protein $(N \times 6.25)$ (% DM)	48.95	48.95	48.14	48.50	48.34
Fat (% DM)	22.32	21.98	22.70	21.12	21.66
Gross energy (kJ g <sup>-1</sup> DM)	24.24	24.23	24.24	24.27	24.65
Ash (% DM)	11.31	11.03	11.11	11.05	10.81

<sup>1</sup>Aquatex (20.5 Crude protein); Sotexpro, Bermericourt, France.

<sup>4</sup>Minerals (g or mg/kg diet): manganese oxide, 20 mg; potassium iodide, 1.5 mg; copper sulphate-5-hydrate, 5 mg; cobalt sulphate-7 hydrate, 0.1 mg; zinc oxide 30 mg; sodium selenite, 0.25 mg; iron sulphate monohydrate, 60 mg.

<sup>&</sup>lt;sup>2</sup>CLA Mixture: total CLA>75%; 18:2 (*cis*-9, *trans*-11), 46.5%; 18:2 (*trans*-10, *cis*-12), 40.2%; Unsaponifiable matter, 0.4%.

<sup>&</sup>lt;sup>3</sup>Vitamins (mg or IU/kg diet): DL-alpha tocopheryl acetate, 50 mg; menadione sodium bisulfitete, 10 mg; retinyl acetate, 8000 IU; DL-cholecalciferol, 1700 IU; thiamin hydrochloride, 8 mg; riboflavin, 20 mg; pyridoxine hydrochloride, 10 mg; cyanocobalamin, 0.02 mg; nicotinic acid, 70 mg; folic acid, 6 mg; ascorbic acid (Lutavit C monophosphate 35), 500 mg; inositol, 300 mg; biotin, 0.7 mg; calcium pantothenate, 30 mg; choline chloride, 1000 mg.

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