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Effect of partial substitution of dietary fish oil by vegetable oils on desaturation and β-oxidation of [1-^{14}C]18:3$n-3$ (LNA) and [1-^{14}C]20:5$n-3$ (EPA) in hepatocytes and enterocytes of European sea bass (*Dicentrarchus labrax* L.)

G. Mourente[a,*], J.R. Dick[b], J.G. Bell[b], D.R. Tocher[b]

[a]*Departamento de Biología, Facultad de Ciencias del Mar y Ambientales, Universidad de Cádiz, E-11510 Puerto Real (Cádiz), Spain*
[b]*Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland, UK*

Abstract

The increasing worldwide aquaculture output and concomitant decrease in the stocks of feed-grade fish used for fish oil production has made fish oil replacement in feeds a priority for the aquaculture industry. The regulation of fatty acid metabolism in fish is important in order to determine strategies for the best use of plant oils in diets for commercially important cultured fish species. We have studied the desaturation/elongation and β-oxidation of ^{14}C-linolenic (LNA) and ^{14}C-eicosapentaenoic (EPA) acids in hepatocytes and pyloric caecal enterocytes in European sea bass fed diets with partial substitution (60%) of fish oil (FO) with vegetable oils (rapeseed, linseed and palm oil) blended in different proportions, for 64 weeks. The rate of desaturation of ^{14}C-LNA was very low in hepatocytes from all treatments and no significant differences were observed among treatments. The rate of desaturation of ^{14}C-LNA in enterocytes was higher than that in hepatocytes but still low (less than 5% of total radioactivity recovered). The desaturation of ^{14}C-EPA in enterocytes was also higher than in hepatocytes, but again was low and no significant differences were found among treatments. The rates of β-oxidation of ^{14}C-LNA and ^{14}C-EPA were much higher than the rates of desaturation in both hepatocytes and enterocytes; however, no significant differences were observed in either hepatocytes or enterocytes among treatments. The rates of β-oxidation of ^{14}C-LNA were considerably higher than those of ^{14}C-EPA in both hepatocytes and enterocytes. In conclusion, European sea bass (a carnivorous marine fish) showed very low desaturation and elongation of LNA to EPA and DHA, and EPA to DHA, higher β-oxidation of LNA than EPA, and all desaturation and oxidation activities were significantly higher in enterocytes than in hepatocytes. A second major conclusion is that no clear quantitative nutritional

Abbreviations: AA, arachidonic acid (20:4$n-6$); BHT, butylated hydroxytoluene; CPO, crude palm oil; DHA, docosahexaenoic acid (22:6$n-3$); EFA, essential fatty acid; EPA, eicosapentaenoic acid (20:5$n-3$); FAF-BSA, fatty acid-free bovine serum albumin; FO, fish oil; HBSS, Hanks balanced salt solution; HPTLC, high performance thin layer chromatography; HUFA, highly unsaturated fatty acids (carbon chain length ▲C_{20} with ▲3 double bonds); LA, linoleic acid (18:2$n-6$); LNA, α-linolenic acid (18:3$n-3$); LO, linseed oil; PUFA, polyunsaturated fatty acid; RO, rapeseed oil; TLC, thin layer chromatography.

* Corresponding author. Tel.: +34 956 016013; fax: +34 956 016019.
E-mail address: gabriel.mourente@uca.es (G. Mourente).

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effects on the desaturation/elongation and β-oxidation activities in either hepatocytes or enterocytes of sea bass were observed upon the inclusion of vegetable oils in the diet.

Keywords: European sea bass; Enterocytes; Hepatocytes; PUFA; Desaturation; β-Oxidation; Rapeseed oil; Linseed oil; Palm oil; Fish oil

1. Introduction

Aquaculture has been successful in converting low value fish meal and oil, derived from industrial fisheries, into high value food for the human consumer. An increasing proportion of fish for human consumption is provided by aquaculture, which is expanding at 10% per year (Tidwell and Allan, 2002). As a consequence, demand for fish oil is increasing rapidly and current estimates suggest aquaculture feeds will consume around 90% of the world fish oil supplies by 2010 (Barlow, 2000). Marine fish are traditionally fed relatively high lipid diets using ingredients of marine origin containing high levels of $n-3$ fatty acids, particularly $n-3$ highly unsaturated fatty acids (HUFA) such as eicosapentaenoic (20:5$n-3$, EPA) and docosahexaenoic (22:6$n-3$, DHA) acids. However, stagnating worldwide supplies of marine oils and fish meal (Barlow, 2000) has forced the industry to investigate alternative lipid sources for use in marine fish diets. The only sustainable alternative to fish oils are plant (vegetable) seed oils, which are rich in C_{18} polyunsaturated fatty acids (PUFA) but lack the $n-3$ HUFA abundant in fish oils. Vegetable oils such as rapeseed, linseed and olive are potential candidates to partially replace fish oil, and blends of these oils could also be an alternative in marine aquaculture diets. It is also of interest to investigate palm oil as a dietary oil source for marine fish as palm oil production is predicted to exceed soybean oil production within the next 10 years making it the most abundant vegetable oil in the world (Gunstone, 2001), and it has been used successfully in diets for salmonid species (Torstensen et al., 2000; Bell et al., 2002). Therefore, there is currently great interest in the regulation of HUFA biosynthesis in fish to determine the effectiveness with which vegetable oils can be utilized to replace FO in the diets of commercially important cultured fish species (Sargent et al., 2002).

PUFAs, linoleate (18:2$n-6$, LA) and linolenate (18:3$n-3$, LNA) cannot be synthesized de novo by animals, including fish, and therefore are termed essential fatty acids (EFA) (Burr and Burr, 1929; Holman, 1986). The EFA requirement of fish differs between species. In freshwater fish, LA and/or LNA can satisfy the EFA requirement, whereas marine fish require the longer chain HUFA, EPA and DHA in the diet for optimal growth and health (Sargent et al., 1995, 1999). Supporting this, the conversion of LA to arachidonic acid (20:4$n-6$, AA) and LNA to EPA and DHA is well established for many freshwater species of fish, but the marine fish species studied so far cannot perform the conversions at a significant or appreciable rate (Sargent et al., 2002). The European sea bass, a strict carnivorous marine fish, is a highly prized species. Reared to a range of sizes, usually around 400–600 g, the fish is provided fresh, on ice to the market. With more than 50,000 tons coming from aquaculture, the supply of sea bass to Europe has increased and provides a high quality product. Although production of this species is a well-controlled process, knowledge of its nutritional requirements is still incomplete compared to other fish species, such as salmonids and carp (Oliva-Teles, 2000). In consequence, neither the EFA requirements or capacity of bioconversion of LNA to EPA and DHA, and EPA to DHA, nor the β-oxidation processes have been investigated in this species (Sargent et al., 2002). In addition, the replacement of dietary fish oil with vegetable oils resulted, in salmonids at least, in significantly increased activities of the fatty acyl desaturation/elongation pathway in hepatocytes and enterocytes, although flesh and liver $n-3$ HUFA levels decreased significantly (Bell et al., 2001a, 2002; Tocher et al., 2002, 2003, 2004). Moreover, recent in vivo studies using stable isotopes (deuterium labelled LNA) had shown that dietary LNA was readily and substantially oxidized (Bell et al., 2001b) and that intestine was a tissue with high fatty acyl desaturation activity (Bell et al., 2003). In a previous study, we determined desaturation/elongation and β-oxidation of ^{14}C-LNA in hepatocytes of European sea bass fed diets with 60% substitution of