EFFECTS OF DIETARY IRON SUPPLEMENTATION ON BIOSYNTHESIS OF LONG-CHAIN POLYUNSATURATED FATTY ACIDS IN THE NEREID POLYCHAETE *HEDISTE DIVERSICOLOR*

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Introduction

Multiple aquatic invertebrates have the necessary enzymatic machinery for *de novo* biosynthesis of long-chain ($\geq C_{20}$) polyunsaturated fatty acids (LC-PUFA) including the so-called "omega-3" EPA (20:5n-3) and DHA (22:6n-3). Two distinct types of fatty acyl desaturases, namely methyl-end (ω des) and front-end desaturases (Fed), are known to be involved in animals' LC-PUFA biosynthesis. Iron (Fe) has a prominent role in LC-PUFA biosynthesis since it is a cofactor of desaturases, and is present in the active di-iron centres of all aerobic desaturases [1]. Since expression of desaturases involved in LC-PUFA biosynthesis can be increased by feeding a low LC-PUFA diet, an innovative strategy to enhance the endogenous production of LC-PUFA in aquatic organisms consists of dietary Fe supplementation to guarantee adequate supply under conditions resulting in desaturase activation. Supplementation of Fe has been shown to positively influence LC-PUFA biosynthesis in salmonids [2] but, to the best of our knowledge, has not been yet investigated in invertebrates. The present study aimed to assess dietary Fe supplementation as an enhancer of LC-PUFA biosynthesis in the nereid polychaete *Hediste diversicolor*, a commercially important species with great interest for aquaculture.

Materials and Methods

First, an *in vitro* trial was carried out by growing transgenic yeast expressing the *H. diversicolor* desaturases (two ω des and two Fed) in the presence of specific fatty acid (FA) substrates. For each desaturase, the following treatments were tested: no Fe supplementation (control), supplementation with $FeSO_4$ (inorganic Fe), supplementation with ProPath® Fe (organic Fe), and supplementation with an Fe chelating agent (chelator). The desaturase activity of transgenic yeast was estimated by calculating the conversion of the FA substrate into the FA product. Second, an *in vivo* trial with *H. diversicolor* juveniles was carried out. Briefly, 20 worms (25-50 mg ww) were randomly distributed in nine experimental units (3 units x 3 diets). The worms were fed for 7 weeks on an experimental diet with low LC-PUFA (control), which was supplemented with either $FeSO_4$ (inorganic Fe) or ProPath® Fe (organic Fe). Worms were fed to 4% of the biomass 5 d per week. Survival and specific growth rate (SGR) were recorded. After 7 weeks, the animals were starved for 24h prior sampling for lipid analysis. Total lipids were extracted and quantified gravimetrically, with an aliquot being transmethylated to fatty acid methyl esters (FAME), and analysed using gas chromatography. The results were processed using principal component analysis (PCA). FA analyses from yeast (*in vitro* assay) were carried out as described above for worm samples.

Results and Discussion

In the *in vitro* trial all the *H. diversicolor* desaturases converted the specific FA substrate into the corresponding product with inorganic and organic Fe treatment exhibiting higher enzyme activity than control. The Fe chelating agent reduced the activity of al desaturases (Figure 1a). These results suggest that Fe can effectively enhance desaturase activity as previously reported in yeast [3]. At the end of the experiment, no significant differences ($p>0.05$) in SGR (average of 0.084) nor survival (96.1±4.2 %) were found among treatments. Moreover, the results from the *in vivo* trial did not show any clear effect of organic and inorganic Fe supplementation on the FA composition of *H. diversicolor*

whereas a clear segregation of the day 0 samples was found (Figure 1b). High levels of 18:1n-9 and 18:2n-6 (23.6 and 19.3%, respectively) on the FA profile of all treatments were found, suggesting a strong dietary effect (34.4 and 15.4%, respectively). Besides, in all treatments, the FA composition of polychaetes showed high levels of PUFA (33.4%) such as 20:4n-6 (ARA), EPA and DHA, indicating bioconversion and trophic upgrading. The reasons underlying the apparent discrepancy between the enhanced desaturase activity observed *in vitro* and the lack of increased LC-PUFA biosynthesis in worms fed on Fe supplemented diets (*in vivo* trial) remain unclear. However, it is reasonable to speculate that the Fe enhancing effect on LC-PUFA biosynthesis could not be detected in the present study due to an insufficient capacity of experimental diet to increase the expression of fatty acyl desaturases in vivo. Further analyses on gene expression, as well as FA composition of the polar and neutral lipid fractions, will contribute to clarify the role that dietary Fe supplementation may play as enhancer of LC-PUFA biosynthesis in *H. diversicolor*.

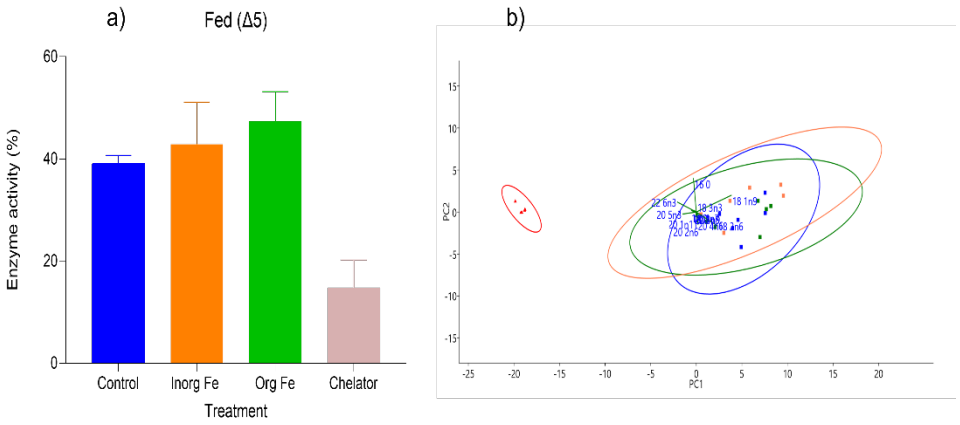

Figure 1. a) *In vitro* assay testing the desaturation capacity of the *H. diversicolor* Fed (Δ5 desaturase) towards 20:3n-6 under no supplementation (Control), supplementation with inorganic and organic iron (Inorg Fe, Org Fe), and supplemented with an Fe chelator. b) PCA of the fatty acid profiles of *H. diversicolor* fed with different supplemented diets (*in vivo* trial). Blue (control), orange (inorganic Fe) green (organic Fe) and red (day 0). Fatty acids responsible for the grouping pattern are displayed in the biplot (blue vectors); 95% confidence ellipses are shown.

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