RECOMBINANT FERRITIN-H INDUCES IMMUNOSUPRESSION IN EUROPEAN SEA BASS LARVAE (*DICENTRARCHUS LABRAX*) RATHER THAN IMMUNOSTMULATION AND PROTECTION AGAINST *VIBRIO ANGUILLARUM*

Eamy Nursaliza Yaacob^{1, 5}, Bruno G. De Geest², Jens Goethals³, Aline Bajeck⁴, Kristof Dierckens¹, Peter Bossier^{1*}, Daisy Vanrompay^{3*§}

¹Laboratory of Aquaculture & Artemia Reference Center, Ghent University, Ghent, Belgium

²Department of Pharmaceutics, Ghent University, Ghent, Belgium

³Laboratory of Immunology and Animal Biotechnology, Ghent University, Ghent, Belgium

⁴Ecloserie Marine de Gravelines, France

⁵School of Fisheries and Aquaculture Sciences, Universiti Malaysia Terengganu,

Terengganu, Malaysia

ABSTRACT

Studies in aquatic animals including European sea bass (*D. labrax*) suggested a role for ferritin in innate immune defence as they demonstrated a significant increase in ferritin gene transcript levels in several tissues upon administering microbial pathogens. At present, we examined the use of recombinant sea bass ferritin-H to protect European sea bass larvae against *Vibrio anguillarum* infection.

The ferritin-H gene *Dlfer* was cloned into pcDNA4/V5-His B. Ferritin-H was expressed in transfected COS-7 cells, purified using the ProBond[™] purification kit and examined by SDS-PAGE and Western blotting using an anti-V5 FITC-labelled mouse monoclonal antibody.

Axenic sea bass larvae were fed alginate microparticles (1.0 mg) containing a high (1.0 mg) or low dose (0.5 mg) of the recombinant ferritin-H at day 7 after hatching. Simultaneously, two groups were either fed with empty alginate microparticles or were receiving no

microparticles (unfed) (negative controls). Larvae were infected with V. anguillarum after

18 h of feeding. Negative controls showed high mortality. V. anguillarum significantly

upregulated the expression of the tlr3, tlr5, cas1, il1\beta, tnf\alpha, mif, il10, cc1, cxcl8, cxcr4 and

ccr9 genes. Sea bass larvae fed with recombinant ferritin-H prior to infection were not

significantly protected. Gene expression points more in the direction of immuno-suppression

by ferritin-H, as in humans instead of immuno-stimulation, as described for shrimp. Further

research is needed to design innovative protective measures against vibrio infections in sea

bass.

KEYWORDS

Dicentrarchus labrax, sea bass, axenic, larvae, ferritin, innate immunity, Vibrio anguillarum

*These authors have contributed equally to this work.

§Corresponding author. Tel.: +32 2645972; Fax: +32 092648721

E-mail address: Daisy. Vanrompay@ugent.be