

properties. In wildlife, and especially in jawed vertebrates, EE2 is classically recognized for its negative effects on the reproductive functions. As endogenous oestrogens, however, EE2 can also modulate the immune system. Consequently, EE2 may affect the individual fitness by altering the capacity to cope with pathogenic agents. Adverse effects of EE2 on immune system function and ontogenesis remain to be fully elucidated, both for mammals and teleost fish. Juvenile European sea bass (90 days post-hatch) were exposed to waterborne EE2 (5ng/L) for one month at 17°C in order to investigate the modes of actions of EE2 on the developing immune system. Exposure concentrations were verified by LC-MS/MS and the oestrogenic activity assessed by yeast estrogen screen assay. Following exposure, several lymphoid organs including the thymus, the head-kidney and the gills were sampled for analysis by qPCR, immunohistochemistry and flow cytometry on isolated leucocytes. The leucocytes were analyzed for their phagocytic capacity as well as their proportion of DLIg3+ and DLT15+ lymphocytes. DLIg3 and DLT15 are monoclonal antibodies, which specifically recognize sea bass IgM and a pan-T cell marker, respectively. First results validate the exposure, which did not significantly impact the biometric measurements of the fish (growth, spleno- and hepatosomatic indices). Considering the proportion of DLIg3+ and DLT15+ lymphocytes and the phagocytic capacity, the treatment increased significantly the proportion of DLIg3+ cells in the head-kidney only but had no effect on the other measured immune parameters. Ongoing work aims at evaluating the effect of EE2 on T cell and B cell differentiation in their respective primary organs (thymus and head-kidney) as well as the secondary immune organs in order to understand the capacity of EE2 on the establishment of the immunocompetence in sea bass.

keywords: Immune system; European sea bass; endocrine disruptor; T Cell; B Cell

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P-022.

Anti-inflammatory mediators and appetite regulatory neuropeptides are affected by chronic stress in *Salmo salar*

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Abstract

In fish farming, there are different long-term stress conditions, some of which are so severe that fish can no longer reestablish homeostasis. Factors that cause prolonged stress can drastically alter the defense against pathogens by upregulation of anti-inflammatory response. In addition, chronic stress modifies the fish energy balance diminishing the appetite. In vertebrates, the stimuli that generate stress are initially perceived by the central nervous system sensors of hypothalamus, stimulating the release of cortisol into the bloodstream. Interestingly, the feeding control center of fish, like in mammals, is also found in the hypothalamus. Thus, extensive cultivation under inappropriate conditions chronically can affect the inflammatory response and the energy uptake from the food. In this study,

the expression of the glucocorticoid-regulated protein Annexin A1 (AnxA1), an important endogenous anti-inflammatory mediator was analyzed in smolt salmon kept at different stocking densities for 40 days. The highest stocking density (HSD) (40 and 60 Kg/m³) simulated a chronic stress by crowding. In addition, the mRNA expression of NPY, substance P, VIP and CGRP appetite regulatory neuropeptides, and the anorexigenic hormone leptin were also analyzed. The results of ELISA assay showed that AnxA1 expression was significantly increased in the gill and muscle of specimens kept at highest stocking density. Moreover, gene expression analysis by Real-time PCR showed upregulation of VIP mRNA in gut of HSD group. Furthermore, the mRNA expression of SP neuropeptide increased three fold in liver of specimens held at 60 Kg/m³. For the other hand, downregulation of NPY mRNA in fish brain of HSD group was observed. Finally, leptin mRNA expression was maintained at high levels in the liver of specimens held at 60 Kg/m³. These alterations reflect the effect of high stocking density on inflammatory and appetite molecular signals. Therefore understanding how these signals are affected during the productive processes of fish farming is required. A reduced appetite involves a lower uptake of energy from food, which affects the functioning of different physiological processes such as the immune system.

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keywords: Anti-inflammatory, Annexin A1, appetite regulatory molecules, stock density, stress

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P-023.

Interleukin (IL-17) and receptors in European sea bass and gilthead seabream. Regulation by NNV infection

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Abstract

Interleukin-17 (IL-17) is a cytokine family composed of six ligands (A-F) being IL-17A and IL-17F the best characterized. These are produced by Th17 cells and induce the expression of many inflammatory mediators. In addition, several IL-17 receptors have been also identified with different cell distribution. The main objective of this work was to identify IL-17 forms, as well as some IL-17 receptors, in two fish species: the European sea bass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*). In addition, we have evaluated the transcription of IL-17 forms and their receptors in seabream and sea bass after the Nervous necrosis virus (NNV) infection, which is a disease that produces viral encephalopathy and retinopathy in fish, by real time PCR. This study revealed the presence of IL-17 and their receptors in both fish species. Moreover, a regulation of fish immune system was observed in fish exposed to NNV infection. To our knowledge, this is the first study addressing the IL-17 expression on the European sea bass and the gilthead seabream infected by NNV. This work was funded by projects from MINECO and FEDER (AGL2016-74866-C3-1-R) and Fundación Séneca (Grupo de Excelencia de la Región de Murcia 19883/GERM/15).

keywords: Interleukin IL-17, IL-17 receptors, sea bass, gilthead seabream, Nodavirus

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