

New evidence suggests that ALA might be a useful supplement for inflammation induced by oxidative stress in chronic diseases. This study investigated whether ALA has a protective role under oxidative stress induced inflammation in *Danio rerio*. Zebrafish has emerged as a powerful model system to examine mechanisms of human disease. The presence of both innate and adaptive utility in zebrafish allows its use as a tool to examine the role of immune cells in normal development and in the pathogenesis of disease states. A gene expression analysis of several proinflammatory marker genes (*il4*, *il13*, *tnfa*, *ifng1*, *nos2b*) was carried out in adult zebrafish gut after LPS (alone) and LPS plus ALA administration. Our preliminary data showed that ALA administration was capable to reduce ( $p < 0.001$ ) the inflammation induced by LPS treatment. Furthermore, we will also evaluate the effect of ALA on immunological aspects of chronic inflammation, using *spint1a* mutant zebrafish larval model which exhibit chronic skin inflammation characterized by epidermal hyperproliferation and neutrophil infiltration.

**keywords:** Chronic disease, inflammation, alpha Lipoic Acid, zebrafish, *spint1* mutant

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#### P-004.

##### Immune response assessment of Atlantic salmon against *P. salmonis* in sea-cage farming centers

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#### Abstract

The monitoring of sea farming centers at the south of Chile was performed to obtain data on the expression of 39 immune-related genes related of *Salmo salar* in three fish organs: gill, spleen and head kidney. The data was obtained from farms with and without occurrences of outbreaks of *Piscirickettsia salmonis*, and a generalized mixed linear model (GMLM) was established, considering the environmental variables and fish necropsy. This model allowed (i) the establishment of a baseline of expression of immune response genes, and (ii) molecular gene markers of susceptibility to the pathogen, which is, if fish are *P. salmonis* positive. For the identification of the baseline expression of immune genes, the condition of normal (healthy) fish was defined and differences in the expression of these genes were established in the gill, spleen and head kidney. In addition, genes exhibiting temporal variation in their expression were identified and therefore, an annual historical reference of this value was considered to the baseline determination. Regarding the genes proposed as markers of *P. salmonis* infection, it was determined that the expression of the genes coding for TNF- $\alpha$ , cathelicidin, NLRX1 and IL-1 $\beta$ , in gills; cathelicidin and hepcidin in anterior kidney; and hepcidin and IL-10 in spleen are indicators of infected fish, and thus, susceptible to *P. salmonis*. This result also highlights the data obtained at the gill level, an easy-sampling organ in the field with validated molecular indicators. While these studies were based on the genes expression levels, we also obtained result on the availability at the protein level of several of these molecules, using specific antibodies obtained in this project. The GMLM will also allow to propose molecules whose increase in expression over time can be

predictive indicators of *P. salmonis* infection. One of the molecular markers with the best application perspective and whose availability was also evaluated at the protein level is cathelicidin expressed in gills. The use of the proposed molecular tools in sea farming centers to evaluate the expression of gene markers will be useful

for the identification of critical windows for therapeutic treatment. Consequently, if the expression of gene markers is detected between reference values, it will indicate infection by *P. salmonis* with an associated probability, and industry could applied productive strategies such as the use of medicated diets.

This work was funded by the Program for Sanitary Management in Aquaculture of the Ministry of Economy, Development and Tourism of Chile (FIE-2015-V014 201708070149).

**keywords:** Immune response, *P. salmonis*, cathelicidin, gill immunity, culture center

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#### P-005.

##### Transcriptome profiling of Atlantic salmon kidney cells (ASK) after stimulation with poly (I:C) and infection with infectious Salmon Anemia Virus (ISAV)

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#### Abstract

Viral diseases are of great concern in fish farming. Close to 20% of salmon put out to sea are lost during production and a large part of this is due to infections. Oil-adjuvanted multivalent vaccines against bacteria/viruses are available and confer good protection against bacterial disease but efficiency against viral disease under field conditions has been questioned. Our goal is to test if known agonists for human Toll-like receptors (TLRs) can be used as adjuvants in vaccines formulations, against fish viruses. Poly (I:C) is a synthetic analog of double-stranded RNA (dsRNA) that mimics a viral infection and could be used for immunostimulation in therapeutics and vaccines. But before developing a vaccine it is necessary to understand more about how the immune system responds to these ligands.

Using ASK cells as an in vitro model, we have compared the transcriptome response of cells infected with ISAV and cells stimulated by poly (I:C) in different time points (12 and 48h). RNA sequencing analysis revealed a total of 3111 differential expressed genes (DEGs) in the treated groups compared to control. From these DEGs, 2815 and 1309 genes were differentially expressed in the poly (I:C) and, ISAV groups respectively. Poly (I:C) treated cells showed stronger response both at 12h and 48 hours when compared with ISAV infected cells. Using the recently annotated salmon genome, pathway and gene ontology (GO) enrichment analyses were performed using Ingenuity and the R package "ClusterProfiler". Most of the shared DEGs were immune-related and were overrepresented in pathways and GO terms related with immune response and response against virus. Some genes were only differentially expressed in one of the groups (e.g., CD28 – poly (I:C) group and interleukin 1 $\beta$  – ISAV group) while others were related only with early or late response against virus or poly I:C, for example interferon (IFN $\alpha$ 3) was only detected in early poly (I:C) group (12h) and late ISAV group (48h). Our results can help to comprehend the molecular mechanism of Atlantic salmon immune response against ISA virus infection. They can also help identify biomarkers for ISA virus early detection and to investigate the possible role of poly (I:C) as adjuvant for future vaccines in aquaculture.

**keywords:** Atlantic salmon, RNA sequencing, Poly (I:C), Toll-like receptor, ISA virus

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