P-006.

Dietary arginine and citrulline supplementation during a short-term feeding period improves the gilthead seabream (*Sparus aurata*) immune status

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Abstract
Several amino acids (AA) are known to regulate key metabolic pathways that are crucial for immune response. In particular, arginine (ARG) appears to have important roles regarding immune modulation since it is required for macrophage responses and lymphocyte development. Moreover, citrulline (CIT) is a precursor of arginine, and it was reported as an alternative to ARG for improving macrophage function in mammals. The present study aimed to explore the effects of dietary ARG or CIT supplementation on the gilthead seabream immune status. Triplicate groups of fish (23.1 ± 0.4 g) were either fed a control diet (CTRL) with a balanced AA profile, or the CTRL diet supplemented with graded levels of ARG or CIT (0.5% and 1% of feed); ARG1, CIT1, ARG2 and CIT2, respectively.
After 2 and 4 weeks of feeding, fish were euthanized and blood was collected for blood smears, plasma for humoral immune parameters and shotgun proteomics, and head kidney for the measurement of health-related transcripts. A total of 94 proteins were identified in the plasma of all treatments. Among them, components of the complement system, apolipoproteins, as well as some glycoproteins were found to be highly abundant. After performing a PLS of the proteins of interest, differences between the two sampling points regardless dietary treatment were observed. In this regard, component 1 (61%) justified the effect of sampling time, whereas component 2 (18%) represents the individual variability within diet. It is particularly interesting that fish fed ARG2 and CIT2 at 4 weeks were more distant tan fish fed all dietary treatments at 2 weeks and fish fed the CTRL diet at 4 weeks , suggesting that the modulatory effects of AA supplementation at the proteome level were more effective after 4 weeks of feeding. The bactericidal activity increased in fish fed the highest supplementation level of both AAs after 4 weeks. A tendency of increased monocytes was observed for the relative proportion of peripheral blood leucocytes in fish fed diets with the highest supplementation level of both AAs after 2 weeks of feeding period, compared to their counterparts fed the lower supplementation level. Peripheral monocyte numbers also correlated positively with nitric oxide, which showed an increasing trend in a dose-dependent manner. The colony stimulating factor 1 receptor tended to be up-regulated at the final sampling point regardless of dietary treatments. These results suggest that dietary supplementation with ARG or its precursor (CIT) have an immunostimulatory effect after 4 weeks of feeding. More healthrelated biomarkers are being processed which will enlighten the effects of these functional diets.
keywords: Amino acids, immunology, aquaculture, functional feeds, gilthead seabream

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P-007.

Immune response of gilthead seabream (*Sparus aurata*) after experimental infection with lymphocystis disease virus (LCDV-Sa)

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Abstract
Lymphocystis disease (LCD) is caused by the lymphocystis disease virus (LCDV), a double-stranded DNA virus belonging to the genus *Lymphocystivirus* (family *Iridoviridae*), affecting more than 150 fish species from both marine and freshwater environments. A few studies have been focused on the immune defensive mechanisms of fish against LCDV, but only one was conducted during a natural LCD outbreak in gilthead seabream, which is one of the most important cultured fish species in the Mediterranean and the European Atlantic coasts. The aim of this study was the analysis of 23 genes related to the immune response in gilthead seabream specimens after experimental infection with LCDV-Sa using real-time PCR (qRT-PCR) in samples of head kidney and intestine at 1, 3, and 8 dpi. To study the progression of LCDV-Sa infection in gilthead seabreams, the number of viral DNA copies and the expression of *mcp* were determined in samples of caudal fin, head kidney and intestine. LCDV-Sa was detected by qPCR in all the samples from inoculated fish analysed, whereas no amplification was obtained in samples from the control group. Regarding the gene expression following LCDV-Sa infection, a total of 22 of the 23 genes studied were differentially expressed in head kidney or intestine samples at some time points analysed. The *pkr* was the only gene showing no differential expression compared to control samples through the entire experiment. Different gene expression profiles were obtained between the organs studied, detecting 18 differentially expressed genes (DEGs) in head kidney samples, four of them exclusively up- or down-regulated (*nccrp1*, *il10*, *mhcII*, and *tnfα* genes), and 5 genes with a significant change in the expression tendency from 1 to 8 dpi (*irf3*, *isg15*, *il10*, *ck10*, and *c3*). In the intestine, 18 DEGs were also detected (14 shared with head kidney), being *mx1*, *casp1*, *ck3* and *tlr9* genes exclusively detected in these samples, and *mx1*, *mx3*, *irf9* and *ighm* differentially regulated over time. The results obtained allow us to understand which genes are essential for host-pathogen interactions and could be used as molecular markers for vaccine efficacy evaluation.
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keywords: *Sparus aurata*, LCDV-Sa, experimental infection, immune response, differentially expressed genes

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P-008.

Analysis of gene expression in nodavirus-inoculated Senegalese sole using a new Openarray® platform

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Abstract
Nervous necrosis virus (NNV) is the causative agent of the viral encephalopathy and retinopathy, a disease that affects cultured Senegalese sole

(*Solea senegalensis*). A NNV reassortant (Ss160.03), combining genomic segments from red-spotted grouper nervous necrosis virus (RGNNV) and striped jack nervous necrosis virus (SJNNV) genotypes, has been previously isolated from Senegalese sole, being highly virulent to this fish species. The RNA-Seq technology has been used in a previous study to comparatively analyse Senegalese sole transcriptomes in two organs (head kidney and eye/brain) after infection with two NNV virus with different levels of virulence to that fish species, a highly virulent reassortant isolate (wSs160.03) and a less virulent mutant reassortant obtained by reverse genetics (rSs160.03247+270). To validate previous RNA-Seq results, a 112-assay OpenArray® platform (Thermofisher) has been designed. This platform included 89 genes chosen according to transcriptomic changes observed by RNA-Seq (covering PRRs, type I IFN response, signal transduction, inflammation, virus responsive genes, and apoptosis), 17 genes selected based on their previously described relation with the immune response against fish viral infections, and 6 control genes (including 3 endogenous genes and 3 viral genes). A total of 63.25% differentially expressed genes (DEGs) detected by RNA-Seq were validated by the OpenArray designed, showing similar expression levels and a 100% expression tendency accuracy. Furthermore, this tool brings new information about the infection process that was not shown by the RNA-Seq analysis, such as the expression profiles of *mda5*, *ifng*, *c9*, *c3*, *mx*, *ifit-1*, *myd88*, *tbkbp1*, and *ube1* genes in different samples at 48 h post-infection (pi). Moreover, a consistent decrease in the number of DEGs was observed at 72 hpi, confirming that 48 h is an adequate time point to study innate immune response of sole against NNV infection. In conclusion, this molecular platform has been confirmed as a good tool for further studies on the sole immune response against NNV mutant infections, which will contribute to the knowledge of the mechanisms of the pathogen-host interaction.

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keywords: *Solea senegalensis*, Reassortant Nervous Necrosis Virus, OpenArray®, differentially expressed genes (DEGs), Immune response.

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P-009.

Salmo Salar glucocorticoid receptors analysis of alternative splicing variants under stress conditions

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Abstract
Cortisol is the main glucocorticoid in teleost, where exerts multiple functions mediated through the glucocorticoid receptors (GR). Currently, it is known that many fish species have two GR genes, gr-1 and gr-2. Additionally, some teleost has also two different splice variants for GR1; gr-1a and gr-1b. In this study, we report for first time the identification of 2 gene copies for GR1 and GR2, located on chromosomes 4q-13q (gr1) and 5p-9q (gr2) of Salmo salar genome. Furthermore, our results describe gr1 splice variants in each chromosome, sharing typical teleost GR elements, such as the 9 amino acids insertion in DNA binding domain (DBD) and variations in length of the ligand binding domain (LBD). For GR2 gene copy on chromosome 5, three splice variants were predicted and differentiated by 5 amino acids insertion and length in the DBD. Also, we identified an uncommon truncated gr-2 gene copy on chromosome 9, lacking the DBD and LBD domains and expressing its mRNA in salmon. Finally, through of specific primers design for each predicted splice variants, we validate and determine the expression of its transcripts in S. salar subjected to stress by stoking density. The results showed differences in the expression of all identified mRNAs, revealing that gr1 and gr2 splice variants were upregulated in head kidney and gills of post-stressed fish. In conclusion, our findings suggest that from specific salmonid genomic duplication (125 MYA), two gene copies of each GR receptor were generated in S. salar and the splice variants identified, could contribute to the variability of the complex modulation of the receptors expression during stressful events, leading to different physiological responses in fish. Fondap 15110027.
keywords: Glucocorticoids Receptor, splicing variant, Salmo salar, cortisol, fish stress

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P-010.

MyD88 dependence on the activation of induced innate effector mechanisms by TLR5M and TLR5S in salmonids

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Abstract
The innate immune response (IIR) in teleosts is essential in the defense against pathogens because of adaptive immune response limitations. Also, the innate immune effector mechanisms are activated by the recognition of conserved structures among pathogens, through Pattern Recognition Receptors, such as Toll-like Receptors (TLRs). Specifically, the membrane-anchored and soluble Toll-like Receptor 5 (TLR5M and TLR5S, respectively) from teleosts recognize bacterial flagellin as do orthologs in mammals. However, it has not been demonstrated whether the induced signaling pathway by these receptors depends on the Myeloid Differentiation Protein 88 (MyD88) to generate a pro-inflammatory response, in addition to activating of IIR effector mechanisms in salmonids. Therefore, in this work we study the MyD88 dependence on the induction of TLR5M/TLR5S signaling pathway mediated by flagellin as ligand, at both predictive and experimental level. CellDesigner program was used for the construction and mathematical characterization of the TLRs model, and simulations were carried out to predict the its dynamics. On the other hand, at the experimental level, we studied the key components response of the TLR5M/TLR5S signaling pathway against to flagellin stimulation, as well as the functional participation of MyD88. Additionally, the activation of some RII effector mechanisms was evaluated against the induction of the signaling pathway under study and related dependence of MyD88. For these experimental assays, treatment kinetics were performed by immuno-stimulants and pre-treatments with a MyD88 inhibitor in *S. salar* Head Kidney Leukocytes (HKLs) primary culture, as cell model; and the key components expression of the signaling pathway was analyzed by RT-PCR. Moreover, the stimulation of some IIR effector mechanisms was evaluated (like Reactive Oxygen Species -ROS- production) in RT-S11 cells stimulated with flagellin and pre-treated with a MyD88 inhibitor.
Our results for the simulations predicted that the MyD88 inhibition produced a delayed response downstream of the signaling pathway against