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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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