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ABSTRACTS

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METHIONINE AND TRYPTOPHAN DIFFERENTIALLY REGULATE THE EUROPEAN SEABASS *Dicentrarchus labrax* INFLAMMATORY RESPONSE

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Introduction

By providing nutritional supplements such as amino acids (AA), it is possible to increase the availability of key substrates for synthesis of specific protein related with the inflammatory process, thereby, improving the immune response. Methionine is the precursor of both polyamines and the antioxidant glutathione. Tryptophan, is precursor of serotonin, and has been shown to restrain stress response. We focused on evaluating the effect of the individual supplementation of diets with those AA on the European seabass inflammatory response.

Material and Methods

European seabass (*Dicentrarchus labrax*) weighing 274 ± 20g were maintained in a recirculated seawater system at the Estação de Zoologia Marítima "Dr. Augusto Nobre" facilities (Porto, Portugal) - Temperature: 25 ± 1°C; Salinity: 35 ppt; Photoperiod: 12h dark:12h light. Following two weeks of acclimation, fish were distributed into 6 tanks (300 l; n=15) and duplicate groups of these fish were fed three isonitrogenous (45% crude protein) and isolipidic (15% crude fat) practical diets for 15 days: a control diet (CTRL) formulated to meet the amino acid requirement, and the CTRL diet supplemented with $_{L}$-tryptophan (TRP) or $_{L}$-methionine (MET) at 2× the requirement level. Fish were fed twice a day until apparent satiety. At the end of the feeding trial fish were subjected to a peritoneal inflammation by intraperitoneally injecting UV killed *Photobacterium damselae* subsp. *piscicida* (*Phdp*) (10^6 CFU ml^{-1}). Fish injected with Hank's Balanced Salt Solution (HBSS) served as controls. Six fish per dietary treatment and stimulus were sampled at 4 and 24h post-injection. Fish were sacrificed by anesthesia with 2-phenoxyethanol and blood samples were taken to assess total and differential cell counts and humoral parameters as described by Costas et al. (2013). Head-kidney (HK) samples were kept in RNA*later* solution at 4°C overnight and then stored at -80°C until gene expression analysis. Brain was dissected into different sections and was then stored at -80°C. Brain samples were analyzed according to Gesto et al. (2006) for monoamine content.

Results

European seabass fed the CTRL diet showed significantly higher cell counts and immune humoral parameters 24h post-bacterial injection compared to HBSS-injected fish. Matrix metalloproteinase-9 (MMP-9) and interleukin-1β (IL1-β) gene expression were down-regulated in stimulated specimens at 4h after peritoneal inflammation. Moreover, brain monoamine levels followed the same pattern. Regarding the effect of dietary treatments, MET increased the cell-mediated response and plasma peroxidase and bactericidal activities at 24h post-bacterial injection compared to CTRL fish. Dietary MET also increased MMP-9 transcripts at both 4 and 24h post-injection. Regarding TRP treatment, diet significantly increased IL1-β gene expression and brain monoamine contents in the optic tectum at 4h post-injection.

Discussion

The peritoneal injection itself may have triggered both neuro-endocrine and immune responses, presenting HBSS-injected fish even higher monoamine and IL1-β mRNA levels in fish fed the CTRL and MET diets at 4h post-injection. Interestingly, fish fed dietary TRP presented higher levels of serotonin and its metabolite at 4h after peritoneal inflammation, while a direct enhancement of the immune system was not observed. By providing increased levels of serotonin precursor, this

(Continued on next page)

diet may have a soothing effect during an early phase of the inflammatory response. By enhancing the production of polyamines, MET supplementation increased immune-related cell proliferation, migration and, consequently, humoral response was amplified as well.

In conclusion, it is suggested that MET supplementation has a pronounced and direct influence on the innate immune response to a peritoneal inflammation. Despite the fact that TRP supplementation modulates brain serotonergic activity, it does not seem to improve the European seabass inflammatory response.

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