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Establishment of Farmed Fish Welfare Biomarkers Using an Omics Approach

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Abstract : Farmed fish welfare is a very recent concept, widely discussed among the scientific community. Consumers' interest regarding farmed animal welfare standards has significantly increased in the last years posing a huge challenge to producers in order to maintain an equilibrium between good welfare principles and productivity, while simultaneously achieve public acceptance. The major bottleneck of standard aquaculture is to impair considerably fish welfare throughout the production cycle and with this, the quality of fish protein. Welfare assessment in farmed fish is undertaken through the evaluation of fish stress responses. Primary and secondary stress responses include release of cortisol and glucose and lactate to the blood stream, respectively, which are currently the most commonly used indicators of stress exposure. However, the reliability of these indicators is highly dubious, due to a high variability of fish responses to an acute stress and the adaptation of the animal to a repetitive chronic stress. Our objective is to use comparative proteomics to identify and validate a fingerprint of proteins that can present an more reliable alternative to the already established welfare indicators. In this way, the culture conditions will improve and there will be a higher perception of mechanisms and metabolic pathway involved in the produced organism's welfare. Due to its high economical importance in Portuguese aquaculture Gilthead seabream will be the elected species for this study. Protein extracts from Gilthead Seabream fish muscle, liver and plasma, reared for a 3 month period under optimized culture conditions (control) and induced stress conditions (Handling, high densities, and Hipoxia) are collected and used to identify a putative fish welfare protein markers fingerprint using a proteomics approach. Three tanks per condition and 3 biological replicates per tank are used for each analisys. Briefly, proteins from target tissue/fluid are extracted using standard established protocols. Protein extracts are then separated using 2D-DIGE (Difference gel electrophoresis). Proteins differentially expressed between control and induced stress conditions will be identified by mass spectrometry (LC-Ms/Ms) using NCBInr (taxonomic level - Actinopterygii) databank and Mascot search engine. The statistical analysis is performed using the R software environment, having used a one-tailed Mann-Whitney U-test ($p < 0.05$) to assess which proteins were differentially expressed in a statistically significant way. Validation of these proteins will be done by comparison of the RT-qPCR (Quantitative reverse transcription polymerase chain reaction) expressed genes pattern with the proteomic profile. Cortisol, glucose, and lactate are also measured in order to confirm or refute the reliability of these indicators. The identified liver proteins under handling and high densities induced stress conditions are responsible and involved in several metabolic pathways like primary metabolism (i.e. glycolysis, gluconeogenesis), ammonia metabolism, cytoskeleton proteins, signalizing proteins, lipid transport. Validition of these proteins as well as identical analysis in muscle and plasma are underway. Proteomics is a promising high-throughput technique that can be successfully applied to identify putative welfare protein biomarkers in farmed fish.

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