Selective breeding influences innate immune responses of Rainbow trout (*Onchorhynchus mykiss*) challenged with *Lactococcus garvieae* in controlled conditions

Tofael Ahmed Sumon[a,b], Md. Abdullah-Al-Mamun[a,b], Elena Sacca[a], Marco Galeotti[a], Donatella Volpatti[a]

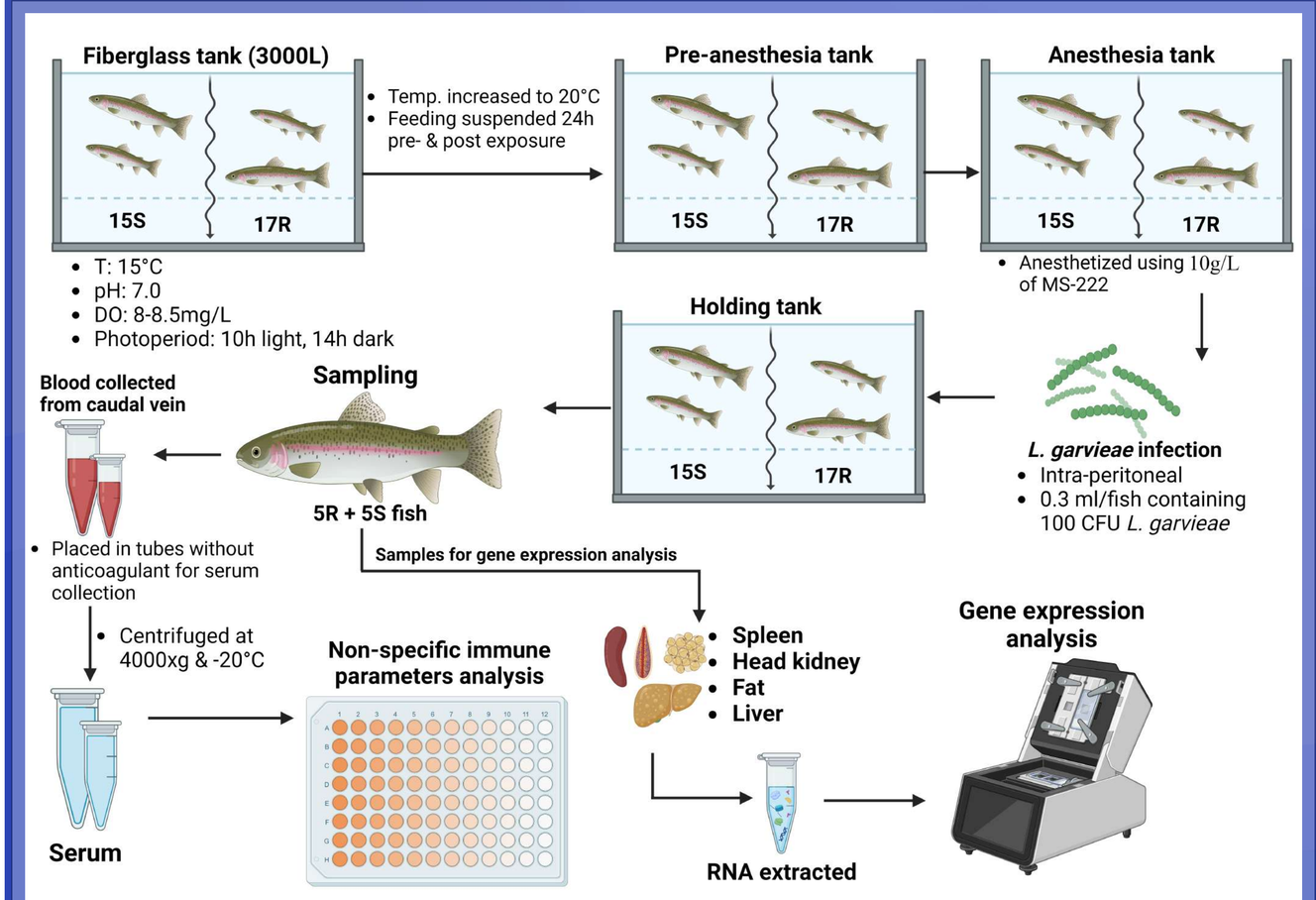
a Veterinary pathology unit, Department of Agricultural, Food, Environmental and Animal Sciences (DI4A), University of Udine, Udine 33100, Italy.

b Department of Fish Health Management, Faculty of Fisheries, Sylhet Agricultural University, Sylhet 3100, Bangladesh.

Background

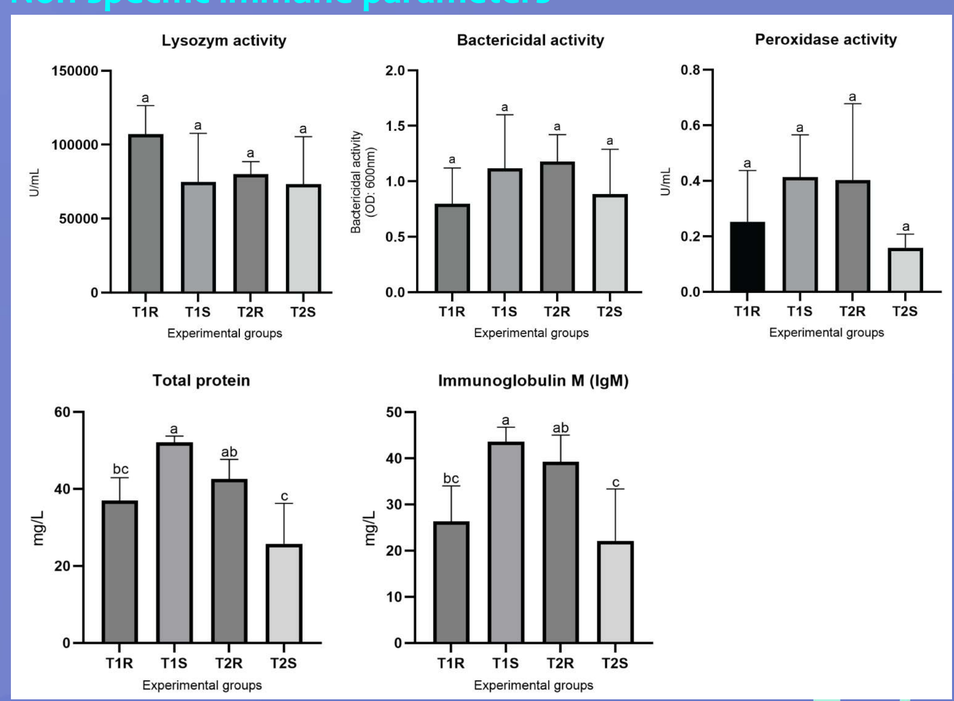
Rainbow trout (*Oncorhynchus mykiss)* is the most widely cultured species among freshwater fish in Europe. According to the European Aquaculture Production Report, Italy has been one of the major contributors to production of portion and large size rainbow trout (17%) in the European Union during the year 2019. However, rainbow trout farms outbreaks of many bacterial diseases nowadays. In particular, they are affected by Lactococcosis which cause massive mortalities, reduce growth rates and increase susceptibilities towards other pathogens. The systemic hyperacute Lactococcosis usually starts with anorexia, lethargy, erratic swimming and melanosis, followed by exophthalmia and visceral/external haemorrhages. Currently it is important to know the inflammatory and immune responses of this host species, to combat or prevent the disease, The modulation of serological non-specific immune parameters and of natural and specific antibodies synthesis need to be studied in the course of infection. In addition, expression of various immune genes, for example, pro-inflammatory and anti-inflammatory cytokines, acute phase proteins, antimicrobial peptides, caspases and complement factors is important to understand and better describe the pathogenesis of the disease. Due to the lack of studies on gene expression and non-specific immune parameters focusing on selected genetic lines, this study investigated these aspects after an experimental intracelomatic infection trial of Resistant (R) and Susceptible (S) individuals by *L. garvieae*. At selected time points post-infection, we evaluated and described the profile of non-specific immune parameters in serum, such as lysozyme, antiprotease, total protein and immunoglobulins and bactericidal activity, and the expression of several immune related genes in head kidney, spleen, liver and adipose tissue.

Methodology



Results

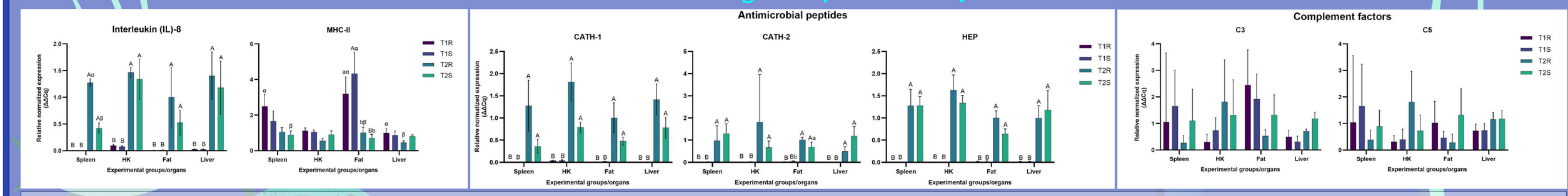
Non-specific immune parameters



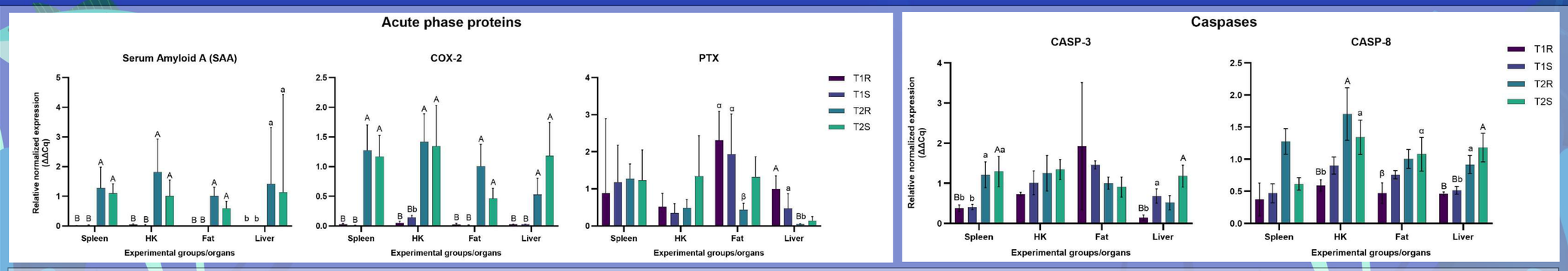
Non-specific immune parameters of resistant (R) and susceptible (S) rainbow trout on day 1 (T1) and day 3 (T2) post-infection with *L. garvieae*. Data are expressed as mean ± SEM (n = 5 for all assays, except for serum total proteins and immunoglobulins, where n = 3 for both groups). Different letters indicate significant differences among groups ($p < 0.05$).

Results

Immune gene expression analysis



Relative mRNA expression of Interleukin 8 (IL-8) and Major Histocompatibility Complex (MHC-II) and antimicrobial peptides (CATH-1, 2 and Hepcidin) and complement factors (C3 and C5) in spleen, head kidney, abdominal fat, and liver of resistant (R) and susceptible (S) fish, at first day (T1) and third day (T2) post-infection. Data are expressed as mean ± SEM (n = 5 resistant fish; n = 5 susceptible fish). Letters indicate significant differences among groups (A, B = $p < 0.01$; a, b = $p < 0.05$; α, β = $p < 0.1$).



Relative mRNA expression of acute phase protein Serum amyloid A (SAA), Cyclooxygenase-2 (COX-2) and Pentraxin (PTX) and Caspases (3 and 8) in spleen, head kidney, abdominal fat, and liver of resistant (R) and susceptible (S) fish, at first day (T1) and third day (T2) post-infection. Data are expressed as mean ± SEM (n = 5 resistant fish; n = 5 susceptible fish). Letters indicate significant differences among groups (A, B = $p < 0.01$; a, b = $p < 0.05$; α, β = $p < 0.1$).

Conclusion

The differential immune responses observed between the resistant and susceptible lines of rainbow trout challenged with *L. garvieae* revealed potential insights into the role of genetic selection in enhancing disease resistance. Regarding non-specific immune parameters, total protein and IgM showed significantly different results between 1-dpi (T1) and 3-dpi (T2) and also between R and S individuals. Similarly, gene expression results indicated that almost all the genes considered showed a significant difference in expression between 1-dpi and 3-dpi given the acute development of the infection, thus showing their involvement in the mechanisms of response to the infection and fish immunity.

Our preliminary findings on immune responses suggest that selective breeding potentially represent a valuable strategy for improving disease resilience in aquaculture. These results not only provide a deeper understanding of the immunological distinctions among rainbow trout lines but also emphasize the future use of targeted parameters as biomarkers for selecting fish with enhanced resistance to *L. garvieae*. Future work should explore these immune indicators, most of all in combination with the infection outcome (survival to the pathogens), to optimize breeding programs aimed at increasing the health and productivity of rainbow trout and hence other farmed species.

UNIVERSITÀ DEGLI STUDI DI UDINE
hic sunt futura

SUPERTROUT

*This scientific work is carried out within the **SUPERTROUT - PRIMA project** and as part of the doctoral thesis of **Tofael Ahmed Sumon**. Authors wish to thank the colleagues of IZS Torino for the application of the in vivo challenge and Dr. Lucio Fariano (Az. Agr. Canali Cavour) for the S and R rainbow trout selection and provision.*

PRIMA IN THE MEDITERRANEAN AREA

AQUAFARM