TRANSCRIPTOMIC ANALYSIS OF SKIN RESPONSE TO Tenacibaculum maritimum INFECTION IN EUROPEAN SEABASS FED DIETARY METHIONINE SUPPLEMENTATION

Carvalho I^{1,2}, Peixoto D^{1,2}, Ferreira I^{1,2}, Robledo D^{3,4}, Gonçalves JF², Machado M^{1,2*}, Tafalla C⁵, Costas B^{1,2}









CHMENT

GENE







INTRODUCTION

The essential amino acid **methionine** plays pivotal roles in several physiological processes, from growth to immune function

Dietary methionine supplementation has been shown to improve cell immunity and disease resistance in fish

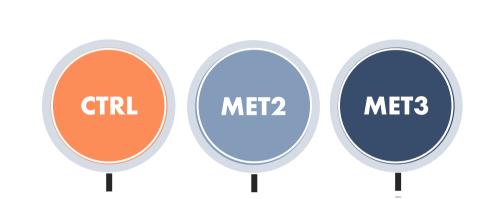
Its impact on mucosal tissues, particularly the skin, remains poorly explored

To investigate the effects of graded levels of methionine surplus on the skin tissue transcriptomes of European seabass (*Dicentrarchus labrax*) upon infection with *Tenacibaculum maritimum*

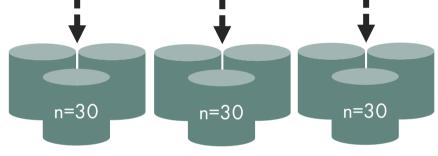
MATERIAL & METHODS

European seabass juveniles (5.19 ± 0.65 g) were fed:

- Commercial diet (CTRL)
- CTRL supplemented with 1.9% methionine (MET2)
- CTRL supplemented with 2.9% methionine (MET3)



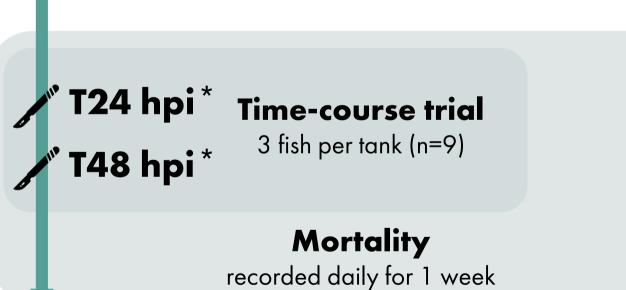
x2 day at an average ratio of 3% biomass per day for 4 weeks





TO h (after the 4 weeks feeding trial)

T. Maritimum (strain ACC13.1) bath infection
(2 hours, 24 °C, strong aeration)

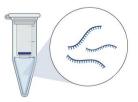


*hpi hours post-infection

Skin tissue samples were collected from 3 fish per tank and pooled within each tank



1 RNA extraction



n=3

2 RNA sequencing



Illumina PE150

3 Differential expression analysis

Infected dietary groups were compared with the CTRL group before infection using R/DESeq2 package v1.28.1

(p-value < 0.01, $|Log 2 Fold Change | <math>\geq 2$)



Gene Ontology (GO) enrichment analysis was performed on g:Profiler

(Benjamini-Hochberg False Discovery Rate (FDR)-corrected *p*-value < 0.05)

RESULTS & DISCUSSION

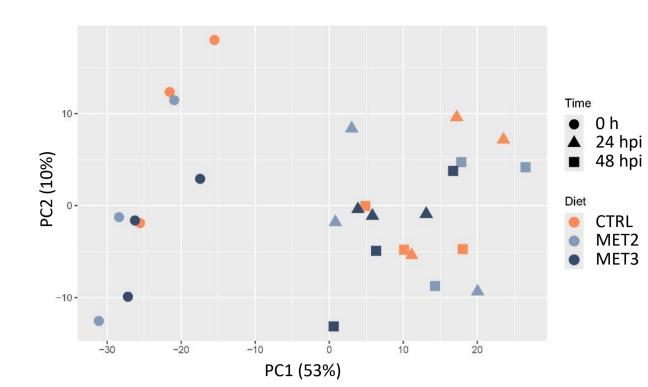


Fig. 1 Principal component analysis showing the clustering of RNA-seq data

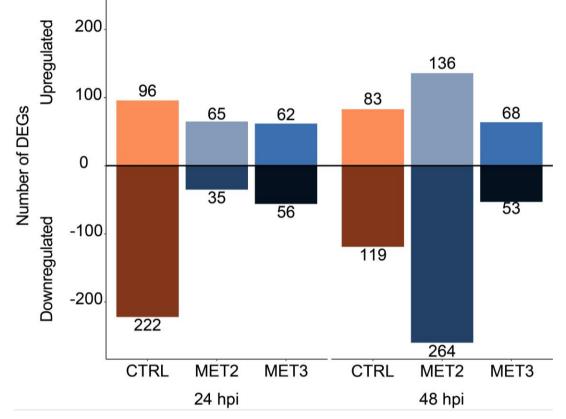


Fig.2 Diverted stacked bar chart of the number of differentially expressed genes (DEGs)

• The exposure to *T. maritimum* elicited a local response in the skin of all dietary groups

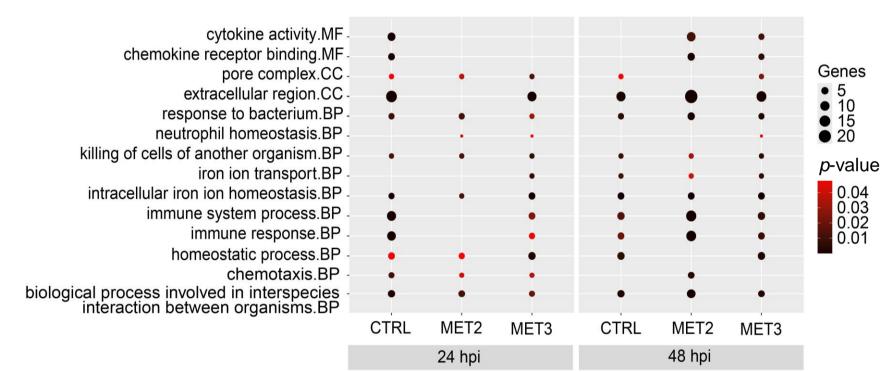


Fig.3 Bubble chart of the gene ontology enrichment analysis in up-regulated DEGs

- The CTRL group exhibited the strongest pro-inflammatory response at 24 hpi
- The initially attenuated immune response in the MET2 group was replaced by the most intense one at 48 hpi

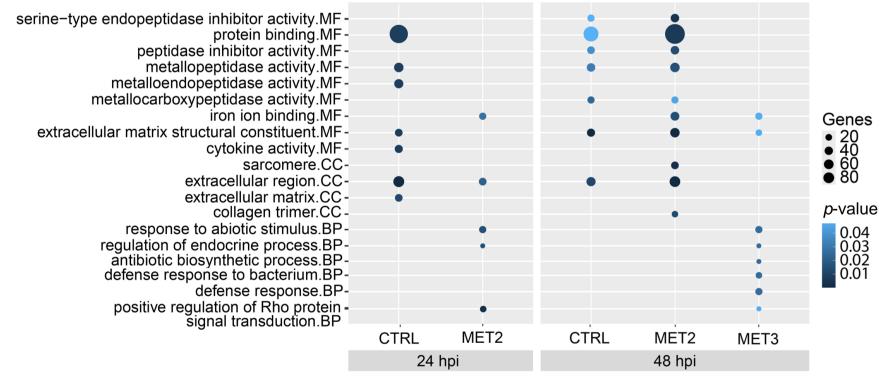


Fig.4 Bubble chart of the gene ontology enrichment analysis in down-regulated DEGs

- The down-regulation of transcripts associated with the immune response suggested an impaired response to infection in the MET3 group at 48 hpi
- Cumulative mortality was higher in the MET3 group (95%) compared to the other groups (85%) (statistically non-significant)

CONCLUSION

The delayed pro-inflammatory response of the MET2 group and the down-regulation of immune-related transcripts in the MET3 group, coupled with higher mortality, highlight the need for further investigation into potential adverse effects associated with elevated methionine intake

ACKNOWLEDGMENTS