COMPLETED PROJECT CASE STUDY

SIMULATING IN VITRO ATLANTIC SALMON INTESTINAL MICROBIOME SYSTEM

PARTNERS

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BACKGROUND

The mucosal layer on the skin of Atlantic salmon is an important barrier that helps protect the fish against pathogens as a fundamental component of a healthy immune system. This project was aimed at enhancing the robustness and immunity of farmed salmon through innovative dietary supplements designed to mitigate the effects of repetitive stress from health interventions (e.g. hydrogen peroxide baths and freshwater or thermal exposure) that are used for managing naturally occurring parasites, such as lice and protozoa.

The research was a collaboration between Lallemand UK Ltd, a leading supplier of microbial solutions for animal nutrition, and the Institute of Aquaculture (IoA) at the University of Stirling, an international centre with a strong reputation for world-class research and innovation. The IoA oversaw field sampling and laboratory analysis and hosted the experiments at its Marine Environmental Research Laboratory (MERL) facilities. Partners at Lallemand were responsible for providing field support, performing specific assays, supporting data analysis, and coordinating resources from commercial partners, including from Lallemand's AquaPharm Biodiscovery Ltd research facility. BioMar – a leading aquafeed manufacturer – also contributed, providing valuable scientific insights and industry perspectives from trial design to feed preparation and analysis.

AIMS

Traditional research has focused on the impacts of single stressors on fish health, performance, and immune function, often without examining the cumulative effects of multiple successive interventions. This project aimed to address this gap by developing and validating a new experimental model to assess the impact of repetitive interventions on fish health, and to test novel in-feed ingredients designed to mitigate these effects.

The primary objectives of the project were to:

1. Develop and benchmark a commercially relevant repetitive stress challenge model.
2. Demonstrate the possibility of mitigating the impact of repetitive stress through dietary supplementation.
3. Identify the most informative biomarkers of repetitive stress exposure and impacts, including non-destructive biomarkers for future studies and industry practitioners.

> *"Findings from this research underscore the importance of integrated pest management strategies prioritising fish welfare and robustness."*

REPETITIVE STRESS CHALLENGE MODEL AND INNOVATIVE HEALTH INGREDIENTS

The experiment was conducted over 62 days with post-smolt salmon in indoor circular tanks at the Marine Environmental Research Laboratory at the University of Stirling's Institute of Aquaculture. Salmon were separated into four groups, each assigned different combinations of diet and stressor profiles.

The diets offered were:

- Basal diet: standard feed without additional supplements.
- Supplemented diet: feed supplemented with antioxidants, nucleotides, and yeast.

The stressor profiles included:

- Single stressor: a hydrogen peroxide bath.
- Multiple stressors: two freshwater treatments followed by hydrogen peroxide exposure.

The team sought to create a realistic repetitive stress scenario mimicking farming conditions, and monitored the stress response progression from primary cortisol responses to secondary metabolic changes and tertiary impacts on mucosal health and growth. To validate the model, successive freshwater treatments with a final hydrogen peroxide treatment were performed. The partners assessed the severity of the induced stress responses and the efficacy of the dietary supplements through various biomarkers across seven categories: stress axis, antioxidant status, skin mucus quantity and biochemistry, mucosal histology, gene expression in mucosal tissues, plasma biochemistry, as well as growth, feed and performance indicators.

The dietary strategy tested novel in-feed health ingredients, focusing on those promoting mucosal health and immunity. Single-strain yeast-cell-wall (YCW) products – such as β-glucans and mannan-oligo-saccharides – are well-documented preventive health promoters in aqua-feed. The tested product was a new-generation, multi-species yeast formulation designed to interact with a broader set of immune receptors, promoting balanced immune modulation and reducing the risk of immune fatigue. The package also included a primary antioxidant enzyme, superoxide dismutase (SOD), known to stimulate endogenous antioxidant defences in salmon, potentially mitigating the adverse effects of hydrogen peroxide treatments.

RESULTS

The repetitive stress challenge had a significant impact on the primary stress response (cortisol) and oxidative balance, inducing chronic activation and exhaustion of cortisol response, behavioural habituation, and depletion of antioxidant defences. These indicators matched the hypothesis and were partially mitigated by the supplemented diet. The project provided the first evidence of induced chronic stress under commercially relevant, controlled laboratory conditions.

The supplemented diet also showed weaker but notable impacts on mucosal tissues and internal organs, indicating a potential for dietary mitigation at the mucosal and overall health levels. No dietary or stress effect was observed on growth and performance.

The project successfully narrowed down a range of biomarkers for chronic stress activation and disruption, including plasma cortisol, serum and skin SOD levels, and skin mucus quantity and protein levels. These biomarkers are crucial for future research and practical application in monitoring fish welfare.

IMPACT

The model was successful in that a difference between stresses was observed, demonstrating the potential for dietary supplementation to mitigate the impacts of repetitive stress, and identifying vital biomarkers for stress exposure. Findings underscore the importance of integrated pest management strategies prioritising fish welfare and robustness.

The success of this project paves the way for further research and commercial implementation of innovative dietary supplements to enhance mucosal health and immunity in the farmed salmon sector. By reducing the need for medicinal interventions and improving fish resilience to stress, these strategies can help secure the economic contribution and sustainability of Scottish and European salmon farming, ensuring healthier stocks and better overall performance.