

## COMPLETED PROJECT CASE STUDY

# NOVEL FUNCTIONAL FEEDS FOR IMPROVED PERFORMANCE IN ATLANTIC SALMON BASED ON NETTLE: WITH SPECIFIC EMPHASIS ON IMMUNE FUNCTION AND RESILIENCE

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### PROJECT LEADS

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## BACKGROUND

There is a continual search for novel feed additives for improved performance in Atlantic salmon. Feed producers are keen to increase the diversity of functional feed components, as this represents up to 60% added value in diets.

Key prebiotics are based on yeast cell wall extract, specifically indigestible mannose oligo saccharides (MOS), while other functional feeds include phospholipids and probiotics. However, there is a commitment to increase the diversity of these components.

Nettles could have potential to complement current products. The University of Aberdeen has been investigating functional feeds for some time, and has shown promising results as to how these feeds impact intestine and immune parameters.

The common nettle (*Urtica dioica*) has a long history of beneficial properties to terrestrial and aquatic farmed animals. A recent review by De Vico *et al* (2018) of use in fish repeatedly showed dose-dependent increases in immune parameters, describing nettle as an “underestimated and frequently neglected plant”. Nettle contains many bioactive natural organic compounds including alkaloids, polyphenolic compounds, flavonoids and minerals, which may contribute to anti-inflammatory, antioxidant and analgesic properties.

Several studies in Rainbow trout (*Oncorhynchus mykiss*) have shown improved growth, haematocrit and immunological parameters (Awad and Austin 2010; Awad *et al.* 2012; Bilen *et al.* 2016; Saeidi *et al.* 2017; Mehrabi and Firouzbakhsh 2020) within increases in survival when challenged with both *Yersinia ruckeri* and *Aeromonas hydrophila*. It is likely that nettle acts as a prebiotic altering the microbial community in the intestine, changing nutrient uptake and potentially the production of short chain fatty acids.

These findings are of direct relevance to Atlantic salmon (*Salmo salar*) when considering the increased occurrence of anaemia and gill health challenges.

## AIMS

The overarching goal of this project was to assess the health benefits of nettles as a novel functional feed for farmed Atlantic salmon.

To carry out this work, there were three main objectives:

- Diet design, feeding trial for growth performance and food conversion.
- Intestinal and blood parameters in fish that were fed the experimental diets.
- Immune function following pathogen challenge.



*This project was the first experimental feeding trial to be carried out using nettle in Atlantic salmon.*

## EXPERIMENTAL STUDY

The trial diets were formulated by BioMar, ensuring that nutrients were balanced and accounted for 1 and 3% addition of nettle supplement volume.

Salmon parr were supplied from Scottish Sea Farms. These fish were transported to the University of Aberdeen's aquarium in April 2021. The fish were retained at 12°C and on a 16hrL:8hrD photoperiod throughout the trial. Fish were all fed the control diet until mid-June, when it was observed that all fish were feeding well. Fish were separated into nine tanks to allow for triplicate tanks per diet. Each tank contained 40 fish, hence a total of 120 fish per diet. At the start of the feeding trial, all fish weighed a mean of 45.34g and were fed as 1.5% body weight per day. Fish were weighed approximately every four weeks to assess growth and to allow for adjustment of feed. The trial lasted from June until September for a total of 88 days.

Whole body weight was determined at each weighing and no significant differences were observed between diets. During July-August there was a technical issue with the feeders, seeing a period of decreased water quality due to excessive feed. During this time, the fish on the 1% nettle diet continued to grow better than the fish fed control and 3%. However, by the end of the trial all fish were of comparable weight (mean weight 73g). Condition factor was determined at each sampling, which was highly consistent between dietary groups. The specific growth rate for each diet was also determined for the whole trial, with no significant differences between groups. Overall, the feeding trial demonstrated that the fish fed the nettle supplemented diets all performed well and similarly to the commercial control diet.

Atlantic salmon intestinal morphology is highly sensitive to plant-derived antinutritional factors, which are unknown in the case of nettle extract. Previous studies in salmon using soybean meal have resulted in significant levels of intestinal inflammation or enteritis.

It was necessary to determine if this may also be the case with nettle. At the terminal sampling, the distal intestine was taken for histology. Histological sections were carried out for nine fish per tank, with 27 fish per diet. For each fish, five sections were scored for six well-characterised morphological parameters – sub-epithelial mucosa, lamina propria, supra-nuclear vacuoles, eosinophilic granulocytes, goblet cells, and mucosa folds – and given a score between 0 and 5, with 5 suggesting intestinal inflammation.

On examination of the histology, it was found that the subepithelial mucosa had significantly higher scores in the fish fed the 3% diet than the fish fed the control and 1%. For the subepithelial mucosa parameter, the fish fed 1% nettle were slightly lower (but not significantly) than the control fish. A similar pattern was observed for the supra-nuclear vacuoles, where the 3% fish had significantly higher scores than the control and 1% fed fish. For the mucosal folds, lamina propria and eosinophilic granulocytes, no significant differences were observed between diets.

These results suggest all the fish had healthy intestines. The fact that two parameters showed increased scores does not alone suggest any major damage. It is of

interest to note that the fish fed 1% had marginally lower scores for eosinophilic granulocytes and lamina propria compared to the control fish, however this was not significant.

As such, it was concluded from the histology that the level of nettle supplement should not exceed 3%, but that levels below this are well tolerated.

To further examine the fish at the time of the terminal sampling, clinical blood chemistry was performed at the University of the West of Scotland using protocols generated by Wellfish Tech. A panel of 18 parameters were performed including assays for gill, liver, heart, kidney and muscle function.

In terms of fish fed with 1% and 3% inclusion, levels measured of chloride, sodium, lactate, calcium and ammonia did not differ from control fish, and therefore gill health was not impaired. Liver biomarkers (albumin, total protein) and oxidative stress indicator (zinc) were all indicating that fish fed with 3% nettle showed some early signs of oxidative stress. This should be confirmed with further oxidative stress biomarker analysis and immune response biomarkers. This effect was not observed at 1% and, therefore, 1% nettle is a preferable inclusion level in feed, with a potential consideration of increasing to 2%.

All parameters measured were determined to be within the normal expected range, as per the current dataset maintained by Wellfish Tech. A number of the parameters could not be relied upon with confidence, as some samples may have undergone haemolysis due to technical and transport factors.

Overall, based on clinical chemistry analysis and considering all possible pre-analytical changes in biomarkers, it was concluded that diets with 1% and 3% nettle caused no detrimental effects on fish health. A number of the parameters would suggest a marginal increase in healthy markers, but further work would need to be carried out in an independent trial to confirm these results.

Furthermore, to assess the capacity of the Atlantic salmon fed the different nettle-supplemented diets to mount an efficient immune response, a bacterial pathogen challenge was performed with *Aeromonas salmonicida*. The salmon in this trial had not been vaccinated, so would be susceptible. For each diet, 30 fish were inoculated with the bacteria.

For this challenge, 30 fish from each diet were split between three tanks: 10 fish per diet per challenge tank, resulting in a total number of 90 fish. All fish were marked at time of infection, and so could be identified for the diet fed. A further nine fish per diet were separately retained as control. The fish were closely monitored and when they showed clinical signs, they were removed and humanely euthanised.

It was found that the highest survival was the group fed the 3% diet at 43.3%, with fish fed 1% showing a 36.7% survival. The control group had the lowest rate at 33.3% survival. However, these results did not reach significance.

## OUTCOMES

This project was the first experimental feeding trial to be carried out using nettle in Atlantic salmon. Based on these observations, it is suggested to keep supplementation below 3% as the maximum. It was shown that growth was completely comparable with BioMar's commercial diet.

There was an indication that fish fed 1% showed more resilience during the feeding trial, as well as some improvements in gut morphology on the fish fed 1% nettle, but further work would be needed to examine this in more detail. Likewise, there were some suggestions that the fish fed the nettle showed improved performance, albeit not reaching significance. These included several blood chemistry measurements, several distal intestine histology scores, and survival against bacterial infection.

The conclusion from the blood chemistry was that an optimum level of nettle inclusion could be between 1 and 2% without negative impact, but a significant improvement has yet to be proved.

The research has been useful for BioMar as there is a continual search for new functional feed components. As it currently stands, the 'raw' nettle product does not significantly improve any parameter of fish health. The nettle is locally sourced and has potential, possibly with further refinement of the product. This would require identification and extraction of the relevant bioactive components. Also, the possible trends seen in this project would have to be confirmed and amplified to significant improvements, on a scale that would result in material gains for fish producers to justify the additional product cost.

From an academic point of view, there is strong interest to further these studies with additional stressors. There are indications of improved performance, but this was a relatively small trial to establish some baseline information. The 1% inclusion during stress events appears to improve specific growth rate along with improved histology, and certain blood parameters and further research is required to identify how and why.

For Urtica Plus, this has been a good opportunity to demonstrate the use of their products in feed and show the potential to the salmon sector. Urtica Plus would look to further explore the positive trends identified in this project, as there were no negatives, and to assist overcoming stress events during the production cycle.

## REFERENCES

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