Progress Report

Project Proposal – How are methionine absorption and gut barrier function affected by soybean meal-induced enteritis in salmonids, and what are the roles of specific anti-nutritional factors?

Proposal Information

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Organization: University of Idaho – Hagerman Fish Culture Experiment Station

Start Date: May 1, 2024

Completion Date: April 30, 2026

Species: Atlantic Salmon (*Salmo salar*) and Rainbow Trout (*Oncorhynchus mykiss*)

Project Goal

This proposal will focus on project priority number II: understand and reduce anti-nutritional factors of soy in aquaculture. Specifically, we will be investigating the intestinal absorption of methionine and gut barrier function in response to soybean meal-induced enteritis (SBMIE) from soybean meal-based (SBM) diets compared with fishmeal-based diets and assess the effects of specific anti-nutritional factors in Atlantic salmon and rainbow trout.

Progress Summary

Year 1 – Objective 1Approximately 100 mg of tissue (intestine/liver) was put in a screwcap cryotube (#16466-058, VWR® Micro Centrifuge Tube) containing 1 ml of RNA isolation reagent (#TR118, TRI Reagent®, Molecular Research Center, Inc.) and zirconium oxide beads (#E-3396, 1.0 mm diameter, Next Advance, Inc.) used for total RNA isolation following standard protocol.

We have started with the baseline characterization of methionine transport along the intestinal segments (proximal, mid-, and distal intestines) in both rainbow trout and Atlantic salmon. Specifically, we are using *ex vivo* intestinal segments and adding specific concentrations of methionine to the apical side in the Ussing chamber to establish a concentration gradient. This gradient will allow us to calculate a saturation curve using enzyme kinetic models (i.e.: Michaelis-Menten or Sigmoidal) to determine methionine transport saturation along specific intestinal segments. We can correlate these results with RT-qPCR to identify specific transporters involved in methionine transport along these segments. Additionally, to characterize possible ANF inhibition of methionine transport, we need to establish methionine transport at saturation to ensure complete inhibition. Therefore, it is essential we complete the baseline characterization before commencing with the ANF study and the feeding trial.

The resulting methionine samples from the Ussing chamber need to be measured on a liquid chromatography-mass spectrometer (LC-MS). Therefore, these samples are sent to a collaborator at the National Cold Water Marine Aquaculture Center (Orono, Maine) where they are measured. Currently, we have generated preliminary data for methionine transport in both species in the Ussing chamber, and the LC-MS results are pending.

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<tr><td>Baseline characterization</td><td colspan="3">12-week feeding trial</td><td colspan="3">Ussing chamber characterization of methionine transport and gut barrier function with diets and ANFs</td><td>Gene expression analysis</td></tr>
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