

<b>Project Number:</b>	USB Project #1830-352-0501f
<b>Project Title:</b>	Exploring the Use of Trimethylamine Oxide as a Feed Additive to Combat Soy-Induced Enteritis in Farmed Rainbow Trout
<b>Organization:</b>	University of Idaho
<b>Principal Investigator Name:</b>	Madison S. Powell
<p><b>Project Status - What key activities were undertaken and what were the key accomplishments during the life of this project? Please use this field to clearly and concisely report on project progress. The information included should reflect quantifiable results (expand upon the KPIs) that can be used to evaluate and measure project success. Technical reports, no longer than 4 pages, may be included in this section.</b></p> <p>Partial or complete replacement of fishmeal with soybean meal (SBM) as a sustainable and economic alternative has been explored in several commercially targeted aquaculture species, including freshwater rainbow trout (<i>Oncorhynchus mykiss</i>). However, SBM decreases feed efficiency and impairs growth in a dose dependent manner. Substitutions above 20% in rainbow trout cause subacute enteritis of the distal intestine; a condition characterized by increased intestinal permeability, inflammation and morphological abnormalities. These physiological limitations place a threshold on the acceptable inclusion levels of soy in a standard aquaculture diet. Previous research has addressed a number of mechanisms to combat soy-induced enteritis, including manipulation of the gut microbiome, ingredient processing to remove anti-nutrients, and antioxidant and probiotic additives. However, this is the first study to examine the efficacy of the feed additive trimethylamine oxide (TMAO). TMAO is a small intracellular molecule and universal cytoprotectant, accumulated in numerous species to stabilize protein and membrane structure in response to various perturbates. Here, we examine whether addition of TMAO to a soy-based diet can combat enteritis of the distal intestine allowing for higher inclusion rates and increased feed efficiency. Further, we determine whether marine fish solubles, a rich source of TMAO, can be used as an economically practical means of administering this additive, enabling its application on the commercial scale. Research proposed in this project specifically addresses SAA Program Area Priority "Dietary Formulations and Nutrition" and provides a mechanistic solution to enable higher utilization of U.S. soy products in salmonid aquafeeds.</p> <p>A 12 week full factorial feeding trial was undertaken examining varying levels of TMAO and varying levels of SBM in the diet of rainbow trout. Fish were sampled several times and histologically examined for signs of distal enteritis. Other immunological and stress-related proteins and the concomitant gene expression related to distal enteritis was also examined. Throughout the study, fish were also weighed and various performance metrics calculated (FCR, SGR, etc.).</p> <p>Fish consuming diets with 40% SBM showed clinical, histological signs of distal enteritis. TMAO inclusion appears to ameliorate histological signs somewhat and does show significant differences in serum proteins associated with stress and gene expression associated with stress. Diets containing fish solubles (i.e. containing less TMAO) were less effective at diminishing the development of distal enteritis.</p>	
<p><b>Did this project meet the intended Key Performance Indicators (KPIs)? List each KPI and describe progress made (or not made) toward addressing it, including metrics where appropriate.</b></p>	
<p>Yes, all KPIs were met or exceeded by the collaborating groups:  A 12-week feeding study was performed at the Bozeman Fish Technology Center (BFTC) with multiple diets and multiple levels of TMAO and SBM. Histological analyses were performed at BFTC. Performance metrics and proximate composition analyses were analyzed at BFTC. TMAO levels in diets and fish samples was measured at the Louisiana Universities Marine Consortium (LUMCON). Analyses of stress related genes and proteins in serum and intestines were performed at the University of Idaho. Originally only HSP70 was to be</p>	

examined. The project leveraged funding from ongoing analyses and examined 16 additional indicators of stress and immunological function - allowing for a second publication from this research and additional insight into the use of TMAO to enhance the use of SBM in rainbow trout diets.

All performance outcomes of this project were met or exceeded. Quantifiable data and additional insight into the use of TMAO to reduce distal enteritis and enhance the use of SBM in fish diets was accomplished.

**Expected Outputs/Deliverables** - List each deliverable identified in the project, indicate whether or not it was supplied and if not supplied, please provide an explanation as to why.

Abstract and Presentation at the triennial meeting of the World Aquaculture Society (New Orleans, LA, Feb. 2019): **EXPLORING THE USE OF TRIMETHYLAMINE OXIDE AS A FEED ADDITIVE TO COMBAT SOY-INDUCED ENTERITIS IN FARMED RAINBOW TROUT** by Abigail B. Bockus\*, Madison S. Powell, Wendy M. Sealey, and Gibson T. Gaylord

A manuscript of the same title is in preparation for submission to Aquaculture Research.

A second manuscript entitled: **Trimethylamine oxide effects on immunological and stress-related pathways in rainbow trout (*Oncorhynchus mykiss*)** by Madison S. Powell, Abigail B. Bockus, Wendy M. Sealey, and Gibson T. Gaylord is in preparation for submission to the Journal of Fish and Shellfish Immunology.

**Describe any unforeseen events or circumstances that may have affected project timeline, costs, or deliverables (if applicable.)**

None. All deliverables were met within the budget and additional analyses of proteins and gene expression associated with stress and immunological function were examined by leveraging ongoing laboratory activities at no additional cost to USB. This will result in another manuscript as well as provide additional insight into the use of TMAO as a potential feed additive to reduce the incidence of distal enteritis caused by high levels of SBM in the diet.

**What, if any, follow-up steps are required to capture benefits for all US soybean farmers?**  
Describe in a few sentences how the results of this project will be or should be used.

TMAO is naturally present in fish hydrolysates and it has been previously shown to reduce the expression of HSP70, a key gene involved in the stress response. We examined the use of TMAO as part of a rainbow trout diet which contained levels of SBM known to cause distal enteritis. The results show that TMAO does have a positive effect on reducing stress related to SBM ingestion in rainbow trout. To fully capture the benefits of this line of inquiry, additional studies examining species (those that are highly sensitive to SBM such as Atlantic salmon) should be undertaken. Inclusion of TMAO in a diet containing SBM may improve performance and allow for increased use of SBM in fish diets.

**List any relevant performance metrics not captured in KPI's.**

None.