

Project Number:	USB 1640-512-5288
Project Title:	Modified Dietary Amino Acid Balance to Increase Soy Protein Inclusion in Trout Feeds & Validation of Calprotectin as a Biomarker of SBM Induced Intestinal Enteritis
Organization:	University of Idaho, US Fish and Wildlife Service
Principal Investigator Name:	Matt Powell, Gibson Gaylord and Wendy Sealey
<p>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.</p> <p>The project began March 1, 2016 with a rainbow trout feeding trial at the US Fish and Wildlife Service Bozeman Fish Technology Center (BFTC). A factorial design was used with 3 amino acid supplement levels and two soy inclusion levels. Rainbow trout were grown on these diets over 12 weeks in triplicate experimental units (tanks) per dietary treatment. Growth and performance data for the feeding trial were analyzed at the BFTC.</p> <p>Results of the feeding trial showed there was no effect of soybean meal level or amino acid supplementation level on final fish weights. However, FCR was higher with 40% soybean meal inclusion and there was a detrimental interaction with high soy level and high AA supplementation. Feed intake results in this study were also similar. Moreover, protein retention efficiency was depressed by high soy inclusion and further depressed with excessive AA supplementation. These results address the first objective and clearly provide three conclusions:</p> <ol style="list-style-type: none"> 1. Soybean meal is detrimental to production efficiency of rainbow trout at 40% inclusion in the diet. 2. Current ideal amino acid targets may be in excess and need to be revised. 3. There are detrimental effects to over-supplementing rainbow trout diets with amino acids. <p>All tissue samples from the feeding trial were transferred to the University of Idaho in July. Laboratory work commenced on August 19 and completed on December 29, 2016. Total RNA was extracted from samples of distal intestine, reverse transcribed to cDNA, and pooled by tank. Resulting qPCR data of different sequences for putative calcium-binding proteins from the distal intestine in rainbow trout were generated and analyzed statistically.</p>	

Total protein was extracted from samples of distal intestine and feces and quantified. Commercial rabbit polyclonal antibodies for human S100A1 and S100A10 (ab66041 and ab125438, Abcam plc.), were examined as S100A1 showed the largest degree of divergence between treatments, while S100A10 showed the highest relative expression among all S100 genes from qPCR data. The secondary antibody used was a Europium-labeled goat anti-rabbit antibody from Molecular Devices, LLC.

Results from molecular analyses of intestinal tissues and feces were able to isolate nine S100 genes from rainbow trout. Several S100 calcium-binding protein genes were expressed at significantly higher levels in diets containing 40% soybean meal. Indicating possible candidates for biomarkers for soy-induced distal enteritis. However, antibody work using commercially available antibodies were disappointing in that enzyme-linked assays produced only weak signals suggesting the antibodies are weakly recognizing the fish proteins or there is only a small amount of protein present. These results address our second objective and provide the following conclusions:

1. At least nine S100 calcium-binding proteins are expressed in rainbow trout when fed a diet with 40% soybean meal inclusion.
2. Small amounts of these proteins were detectable in feces with antibodies but were not significantly different among treatments.
3. Calcium-binding proteins associated with inflammation in the gut are similar to other vertebrates in sequence but are not strongly bound with commercially available antibodies for S100 proteins (that normally cross react among many taxa) suggesting conformational differences.

Write up of the data for peer-reviewed submission is underway. The data are part of the dissertation research of Patrick Blaufuss, the PhD student at the university of Idaho supported by this funding. Data are tentatively scheduled to be presented by Mr. Blaufuss at the American Fisheries Society Meetings in Tampa, Florida in August.

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.

Our overall goal is to increase soybean meal inclusion levels in trout feeds. We addressed the following KPIs as part of our continuing efforts to increase soybean meal utilization and evaluate the negative effects of soy caused distal enteritis. Both key performance indicators were a continuation of our previously funded SAA work. The research activities outlined above met both KPIs

KPI #1: Define amino acid absorption limitations in trout fed high levels of soybean meal. We used a factorial design to examine the effects and interaction between amino acid supplementation and soybean meal inclusion in rainbow trout diets.

KPI #2 Further develop and validate a molecular tool to assess soybean tolerance in fish. We further examined a putative biomarker for SBM induced enteritis in rainbow trout.

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.

The impacts of soybean meal on fish performance are quite variable. For some species, formulating diets based on digestible nutrients to include protein, amino acids and digestible energy can ensure a high utility of soybean meal.

Output:

In this study we examined amino acid inclusion levels and showed that current ideal amino acid targets may be in excess and that adding excess amino acids can have detrimental and interactive effects in diets with high soy inclusion levels in rainbow trout. These data allow feed formulators to further refine amino acid targets when supplementing soybean meal in rainbow trout diets.

Unfortunately, high levels of soybean meal also commonly affect gut health resulting in an inflammatory response with a complex milieu of proteins being produced and many genes being expressed.

Output:

In this study, we further examined SA100 calcium-binding proteins that are commonly expressed during inflammation for use as biomarkers for soybean meal induced enteritis in rainbow trout. We determined that as many as nine SA100 protein genes are expressed but commercially available antibodies for the resulting proteins are not sufficiently discriminatory for use as non-destructive biomarkers. Further developmental research will need to be undertaken to synthesize specific antibodies for these proteins.

Both outputs directly address the SAA Program Area Priority of “Enabling Technologies to Advance Domestic Aquaculture”.

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

The original contract period for the project was March 1, 2016 through September 30, 2016. The project started on time with the fish feeding trial at the USFWS Bozeman Fish Technology Center. However, funding for the trial was not approved by the University of Idaho and SAA until August 19, 2016. Work on samples from the feeding trial could not start until funding was in place. With only 43 days until the end of the project contract, a no-cost extension was requested and granted until December 31, 2016. Even though an extension was granted, subsequent analytical laboratory work had to proceed at a very rapid pace and employed more laboratory help than originally allocated. Even so, not all salaries were able to be expensed from the project given the extremely truncated timeline. Despite this deliverables were met.

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.

When diets are formulated for fish that are susceptible to the inflammatory effects of high soybean meal inclusion, care should be taken to address amino acid inclusion levels. Current levels may be in excess and excess amino acid levels can be detrimental to fish production in high soy diets.

Distal enteritis is a complex process with many genes and proteins involved, While it is relatively easy to use any of several inflammatory genes as a biomarker for distal enteritis, further research is warranted to examine the potential for a non-destructive biomarker such as calprotectin found in humans. A sensitive non-destructive marker such as an antibody test would allow early detection before production problems with higher FCRs and lower protein retention efficiencies become compounded.

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

--