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| Project Number: | USB #1730-352-0506 |
| Project Title: | **Comparing selected vs. non-selected rainbow trout to elucidate resistance to soybean meal caused distal enteritis.** |
| Organization: | University of Idaho, US Fish and Wildlife Service |
| Principal Investigator Name: | Matt Powell, Gibson Gaylord and Wendy Sealey, Ken Overturf |
| 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. | |
| Selected line, USDA/UI rainbow trout at the Hagerman Fish Culture Experiment Station were spawned in mid-October and eggs were held on station until a Montana Fish Wildlife and Parks importation permit (No. 13011-4-1016-1) was issued on October 19th. As per permit requirement, approximately 2500 eyed eggs were transferred from the Hagerman Station to the Bozeman Fish Technology Center after November 1st. Selected line eggs were received in Bozeman on November 3rd and have hatched. Fry are being fed a commercial diet prior to the experimental feeding trial. Unselected line eggs (obtained from Troutlodge in the first week of November) have also hatched are are being fed the same diet prior to treatment.  The 2x2 factorial design feeding trial (lasting 12 weeks) began May 18th once both groups of fish reached approximately 40 g in size. Fry from both groups were placed on experimental diets and initial measurements and samples were taken. Fish were also measured (weight) and tissues sampled for qPCR and histology on 4 week, 8 week and 12 week sample points. Specific genes that were examined included all Interleukin 17 variants: Il-17 alphaF1a, Il-17 alphaF2a, Il-17 alphaF2b, Il-17 alphaF3a, Il-17C Il-17D and Il-17N along with Tumor necrosis factor alpha, Interleukin 1, Interleukin 6, Interleukin 10, Interleukin 22, Forkhead box P3 and RAR-related orphan receptor gamma.  All research activities were completed by the end of September 2017. The data are scheduled to be presented at the annual Aquaculture America meeting in Las Vegas, NV in February. An abstract has been submitted for this meeting:  **growth Performance and Intestinal Health of two Different Rainbow Trout *Oncorhynchus mykiss* Strains fed a High Soy Diet**  Patrick C. Blaufuss\*, T. Gibson Gaylord, Wendy M. Sealey, Kenneth E. Overturf and Madison S. Powell  Aquaculture Research Institute  University of Idaho  Moscow, ID 83844  pblaufuss@uidaho.edu  In an effort to improve the utilization of soybean meal (SBM) in diets for rainbow trout, fish at the Hagerman Fish Culture Experiment Station (HGM) have been selected for growth on a complete plant-based diet over several generations. Utilizing fish of the F7 generation and a commercial selected strain (CSS), we sought to investigate differences in growth and intestinal health when fed a 40% SBM diet. Triplicate tanks (30 fish, initial weight 77.9 ± 1.1 g) of each strain were fed diets for 12 weeks containing either 0% or 40% SBM in a 2 X 2 factorial design. Five fish from each tank were sampled every four weeks to collect samples of liver and distal intestine for gene expression analysis. At twelve weeks, the HGM strain showed superior growth on both diets (p<0.001) when compared to the commercial strain, with no significant differences in growth within each strain in response to diet (p=0.158; Fig. 1). Analysis of genes related to gut health and inflammation show reduced inflammation in the HGM fish. These results suggest selection for growth on a plant-based diet also drive selection for increased tolerance for dietary soybean meal inclusion.  **Figure 1. Percent weight gain between selected strains of rainbow trout fed a high soy diet for 12 weeks.** | |
| 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  **A new way to select fish for soybean tolerance:**  To increase soybean use in aquafeeds, the mechanism(s) responsible for oral tolerance of soybeans in fish must be characterized. This project compared a line of rainbow trout selected for soy tolerance and an unselected line with a known mechanism for oral tolerance found in numerous species. This project found evidence that T cell regulation is at least partially responsible for oral tolerance and represents a specific mechanism by which fish can be selected for increased soybean tolerance and thereby increase the use of soybeans in aquafeeds. **Fish breeders can select fish with this type of T cell regulation in order to increase their tolerance to soybeans.**  **A new tool to assess soybean tolerance in fish:**  This project introduced a novel laboratory approach to examination of cell specific gene expression. Laser-capture microdissection was found to be unnecessary to quantify immunogenic and inflammatory gene expression in intestinal enterocytes in this project. Although the laser-capture microdissection process works, general isolation of total RNA from intestinal tissues was able to quantify immunogenic an inflammatory genes without the added steps and expense of this technique. **Thus, fish scientists can use general RNA isolation techniques to refine gene expression data from the intestinal tissue of fish being evaluated for their tolerance to soybeans.**  In summary, this project identified a new way to potentially select fish for increased oral tolerance of soybean meal and showed that straightforward qPCR techniques can be used effectively to assess selection potential (i.e. marker-assisted selection). | | |
| 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 (SBM) on fish performance are quite variable. In rainbow trout, the first adverse effects of soybean intolerance manifests in higher food conversion ratios. In figure 1, significantly higher FCRs were observed in the non-selected fish on the 40% SBM diet after 12 weeks indicating clinically identifiable enteritis which is confirmed through histology. Moreover, overall fish growth was higher in the selected strain of fish compared to the non-selected strain (see the abstract above and Figure 2). The project succeeded in producing a clinical effect (SBM caused distal enteritis) in a non-selected line of trout in order to compare gene expression for oral tolerance between it and a rainbow trout strain selected to utilize a soybean-based plant diet.  **Figure 1. Feed conversion ratios for selected (HGM) and non-selected (CSS) fish fed a 0% or 40% SBM diet for 12 weeks.**    **Figure 2. Mean fish growth for selected (HGM) and non-selected (CSS) fish fed a 0% or 40% SBM diet for 12 weeks.**  **Output 1:**  This project identified a new way to potentially select fish for increased oral tolerance of SBM by selecting for lower expression of interleukin genes associated with T17 cell regulation and increased oral tolerance. Figure 3 shows significant differences in the expression of one of the isoforms of Interleukin-17. This gene was upregulated in the non-selected fish strain (CSS) suggesting increased sensitivity to SBM. Thus for example, fish that exhibit lower expression of this IL-17 isoform may be more tolerant to SBM as evidenced in the selected strain (HGM). The data also suggest fish that are selected to utilize a plant-based diet may also result in selection for increased oral tolerance of SBM.  **Figure 3. Relative expression (fold change) in Interleukin-17 alphaF2b in non-selected (CSS) and selected (HGM) lines of rainbow trout fed diets with either 0% or 40% SBM over 12 weeks.**  **Output 2:**  In this study, we compared expression among various isolation techniques for cells and RNA including laser-capture microdissection. We found that simple tissue extraction of total RNA from intestinal enterocytes was sufficient to observe differences in gene expression among the milieu of cell types within the intestinal tissue samples. The added expense and time associated with the microscopic technique was unnecessary.  Both outputs 1 and 2 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 October 1, 2016 through September 30, 2017. The project started on time with the fish feeding trial at the USFWS Bozeman Fish Technology Center. 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. | | |
| Follow-up evaluations of Il-17alphaF2b for use in marker-assisted selection of SBM tolerance should be examined in additional species such as Atlantic salmon and others that are susceptible to distal enteritis. | | |
| **List any relevant performance metrics not captured in KPI’s.** | | |
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