1. SUB/CONTRACTOR INFORMATION

1.1. Principal Investigator Name:	Bridget Owen
1.2. Organization:	Soy Aquaculture Alliance
1.3. Address:	8425 Keystone Crossing, Suite 200 Indianapolis, IN 46278
1.4. Phone:	317-495-4361
1.5. Email:	bowen@soyaquaalliance.com

2. PROJECT INFORMATION

2.1. Project Number:	1540-512-5289
2.2. Project Title:	Genomic and physiological assessment to identify changes allowing high-soy use in genetically-improved lines of rainbow trout, Phase III
2.3. Primary Contractor:	SmithBucklin
2.4. Project Manager's Name:	Philip Lobo
2.5. Start Date:	04/01/2015
2.6. Completion Date	03/31/2016
2.7. Final Project Report Date:	04/30/2016
2.8. Action Team:	Meal
2.9. Long Range Strategic Plan Objective:	Increase the value of U.S. soy meal to the entire value chain.
2.10. Long Range Strategic Measurement:	Changes in volume and value of U.S. soy meal.
2.11. Target Area:	Domestic Opportunities
2.12. Target Area Goal:	Feed
2.13. Program:	Soy in Aquaculture
2.14. Constraint or Opportunity Statement:	Soybean farmers can increase value of U.S. soybean meal by establishing soybean meal as a preferred protein ingredient to replace fish meal in global aquaculture feeds.

3. PROJECT DESCRIPTION

3.1. Project Summary:

In a few bullet points formulate (a) the project objectives, (b) the activities that will be undertaken to meet the objective, and (c) the targets.

- In year 1 of the SAA-funded project, we focused on mechanisms by which growth of fish fed high-soy diets is reduced. Results revealed that plasma amino acid levels and amino acid transporters in rainbow trout fed high soy blends with amino acid supplementation were strikingly different than in fish fed blends without amino acids. More significantly, the plasma amino acid pattern of our selected strain of trout was consistent with feeding trial results and contrasted with that of non-selected fish. These findings shifted the focus of research to intestinal epithelial cell physiology and the intestinal microbial community, the two factors likely responsible for differences in plasma amino acid patterns.
- Year 2 research utilized high-throughput genome-wide sequencing technology, new bioinformatics and statistical tools, and selected lines of rainbow trout that flourish when fed high-soy feeds to characterize the intestinal microbiome and gene expression in selected tissues, including the intestine.
- Year 3 extended extended research completed in years 1 and 2 into two areas, meta-transcriptomics and shotgun proteomics to complete the picture concerning the factors that limit soy protein levels in fish feeds and resolve metabolic mysteries concerning the effects of soy antigens in fish. The two technologies were combined in a novel way to (a) understand the roles the intestinal microbiota play in the function of the digestive tract and in diet-induced inflammation by screening bacterial gene expression (meta-transcriptomics) and (b) determining the host-microbiota cross-talk influence at the intestinal level by screening the host protein response (shotgun proteomics). These two analyses were conducted on individual fish from each strain and dietary treatment group, a unique approach that has not been done before in fish or other animal. This approach greatly increased the power of the analyses to identify factors that cause enteritis in fish.
- Rainbow trout from non-selected (commercial) strain and the ARS/UI selected rainbow trout strain were fed either a fishmeal-based or soy protein-based feed for seven months, with an initial weight of 5g and final weight of 750g, the typical harvest weight of rainbow trout in the USA. This trial began in January 2015 and ended in August. Tissues from 15 fish per treatment group were collected along with intestinal contents from the distal region, collected aseptically. Samples were stored in RNAlater, then mixed with TRIzol and homogenized to isolate RNA. Trout intestinal tissue samples were flash-frozen in liquid nitrogen and later shipped to Dr. Kueltz's laboratory at the University of California, Davis, for proteomic analysis by mass spectrophotometry. Proteomic data was analyzed using bioinformatics tools and returned to the UI on May 2, 2016. This data will be matched with RNA sequencing data that is expected to be returned to the UI in late May. While the sequencing is being completed, we established a biostatistical analysis pipeline to facilitate rapid analysis of sequencing data. The two data sets will be then compared to finalize the analysis, plus matched on an individual fish basis with histological results being finalized by the Washington State University College of Veterinary Medicine pathology laboratory, where intestinal samples will be examined to score the degree of distal enteritis in the fish as described in previous final reports to SAA.

3.2. Other Cooperators/Funding Sources $45,000

3.3. Expected Outputs/Deliverables:

Findings from the proposed studies will provide significant insights regarding the mechanisms responsible for prevention of distal enteritis and improved digestion/protein retention of the USDA/UI selected strain of rainbow trout when they are fed high-soy diets. New knowledge will establish a foundation for new directions in fish nutrition research in a range of commercially important species farmed worldwide. The project will also provide new knowledge on the metabolic role of the intestinal microbiome in fish and incorporate this information with host protein production providing the most complete picture of nutrient

(soybean), microbiome and host intestine interactions, something never before explored in fish as well as livestock or laboratory animals. Combining the results of this project with results of our previous two years of research, supported by the Soy Aquaculture Alliance, will likely lead to the development of new approaches to increase the efficiency of high-soy diets in aquaculture, improving the sustainability of aquaculture as the leading source of animal protein for the growing human population. New knowledge on the role of the intestinal microbiome in digestion and the intestinal immune response will show, for the first time, the importance of the microbiome in the development of enteritis in fish and provide an explanation of why dietary antinutrients in soy products do not always cause metabolic and physiological disturbances. Further, a critical outcome of the research will be to clarify whether genetic selection is the main factor responsible for adaptation to high-soy diets in trout, or whether increased performance results from interaction between the fish and the microbiome**. A final outcome will be information to suggest ways to modify the microbiome in commercial (non-selected) strains of fish by using feed additives, such as acidifiers or probiotics, to allow higher levels of soy proteins to be used in feeds for carnivorous species of farmed fish to prevent enteritis and maintain high growth performance.**

3.4. Key Performance Indicators (KPIs):

*Develop KPIs specific to this project. The KPIs must focus on outcomes (impact), **not** outputs (actions, activities). They must be measurable and record meaningful progress. A good KPI also measures a change in behavior.*

*Hint: **Outputs** indicate what has been done (e.g. organized a workshop, conducted experiments, published a newsletter). **Outcomes** indicate the quantified impact of the action that progresses US soy farmer interests (e.g. Percentage/number of participants that changed their attitude/behavior as a result of what they learned at the workshop; impact of experiment results on research hypothesis; use of research results in commercial applications, percentage/number of newsletter readership that changed attitudes/ behavior as a result of the information shared in the publication).*

1. Identify at least one strain of bacteria that can be supplemented to increase soy inclusion in fish diets by 2017.
2. Delineate the physiological processes that lead to the development of enteritis in non-selected carnivorous species of fish when reared on a high soy diet. This information will be used by nutritionists and aquaculture producers to improve feeds and management practices to alleviate the activation of these processes, thereby increasing the opportunity for higher utilization of soy protein in aquaculture feeds.
3. Determine the mechanisms responsible for enteritis resistance found in selected fish. This information will then be used by geneticists and aquaculture producers to generate commercially important stocks of fish that can utilize feeds containing higher levels of soy protein.
4. Determine the level of interaction between host and microbiota populations in nutrient digestion and retention. This information will be useful in both the development of probiotic treatments that can change the population of microbiota to either alleviate the development of enteritis through preferential colonization or to alter the metabolic processing of the intestinal microbiota to enhance nutrient uptake. Either or both situations will favor a higher incorporation of soy protein in future aquaculture feeds for carnivorous fish.

3.5. Approved Budget:	$132,164
3.6. Billed to Date:	$

4. PROJECT PROGRESS ASSESSMENT

4.1. LRSP Accomplishments: How does this project address the relevant long range plan strategic objective?

This project addresses the Soy LSRP of increasing the value of soybeans to aquaculture markets. The knowledge generated from this and the proceeding projects should not only assist in maintaining domestic aquaculture markets for soybean meal but increase the market by enhancing dietary incorporation in existing species and developing means to utilize higher levels of soy in new species.

4.2. Program constraint/opportunity accomplishments: How does this project address the specific program constraint or opportunity?

This project addresses the constraint of increasing soybean incorporation in aquaculture feeds. The understanding of the physiological, genetic, and microbiota interactions that occur in fish that causes them to develop enteritis when fed high soy based feeds long-term and the use of a selected fish model to understand what can be done to prevent the development of enteritis is powerful information that should lead to increased diet formulation, development of probiotics and management practices, and genetic improvement of fish that stimulate the industry to formulate and utilize feeds containing higher levels of soy protein.

4.3. Target Area accomplishments: How does this project impact the relevant target areas and target area goals?

The research from this project impacts the relevant target goal of increasing the value and usage of soybean meal in domestic feeds by showing that fish can be selected to utilize feeds containing higher levels of soybean meal and by identifying mechanisms responsible for enhanced utilization. This information will be used to develop new approaches to enhance soybean utilization in other species, thereby increasing soybean meal use in aquaculture in the USA and globally.

4.4. KPI Accomplishments: On reflection, did the project KPIs	Check Box	
(Respond to this question for both midterm and final project reports)	YES	NO
a) Address the relevant program constraint(s)?	x	
b) Address the project objective(s)?	x	
c) Prove measurable?	x	

4.5. How were the KPIs measured?

(Summarize in a few sentences)

The experiments to generate the data that will be utilized in evaluating the KPIs have all been performed. However because of the short funding cycle and because of the timeframe involved in rearing fish to the correct size and harvesting samples, data analysis has not been completed at this time. We

know our experiments generated the correct data set for addressing the KPIs named in this project. This information will be provided after complete analysis of both the proteomic and metatranscriptomic data sets are complete.

4.6. Did this project meet the intended KPIs measured?

For final project reports [For each KPI check one]		KPI not met - little or no progress	KPI not met - significant progress	KPI met	KPI exceeded
For midterm project reports give your best forecast		*KPI will probably not be met*	*KPI is on track*	*KPI will probably be exceeded*	
1.	Identify at least one strain of bacteria that can be supplemented to increase soy inclusion in fish diets by 2017.		KPI is on track for successful conclusion		

4.7. Elaborate on the circumstances that played a role in (a) achieving, or (b) <u>not</u> achieving the KPI(s):

The short funding cycle has made it difficult to grow fish for the experiment, take and prepare samples, analyze samples and then run a complete bioinformatics and statistical analysis of substantial amount of data generated from this project within the timeframe. All fish rearing trials have been completed, and all samples taken from experimental fish have been analyzed via RNA sequencing and proteomics. Analysis of the host intestinal proteome data is complete and has been returned to us (on May 2, 2016) but we are still waiting for the metatranscriptome sequencing data to be returned. We will then evaluate changes between experimental treatments and then correlate these with proteomic changes.

4.8. Were all project deliverables supplied?	[Check one]	<u>No deliverables due yet</u>	<u>YES</u>	<u>NO</u>
				X

If NO, then why:

We were unable to rear trout to harvest size, extract and purify RNA, prepare and process the libraries and have the sequencing of the samples completed before the end of the grant.

4.9. Are all project deliverables on schedule?	[Check Yes or No]	<u>YES</u>	<u>NO</u>
(For midterm reports, provide an assessment whether the project and its deliverables are on track)		X	

If No, then why

For the following question, select ONE response that applies best to the impact of specific type of project you are reporting.

The projects are classified as follows:

- **Research projects** - all fundamental and applied research projects related to soybean yield and composition improvement, production practices, industrial uses, aquaculture, and human nutrition;
- **Market development/marketing/advertising/PR projects** - all projects that aim to change targets' attitudes and behavior.
    - These can be domestic or international projects with a wide range of targets spanning the whole value chain and key influencers of the value chain.
    - They can include research projects aiming to identify advantageous attributes of US soy and soy products when the results will be used to differentiate and create preference for US soy products vs. competitor origins or alternative products.
    - Communication with US soy farmers about best farming practices should be included here.
- **Farmer** check**-off communication projects** - Check-off dollars are invested to benefit US soybean farmers. A small number of projects aim to keep the broader soy farmer community informed about how check-off dollars are spent, the key priorities and the work being done to maintain/increase farmers' wellbeing so that they continue to support the check-off legislation.
- **Special projects**:
    - Information **gathering** for planning and for use by USB and the industry - these projects collect relevant market or other information, or represent on-going market and industry sector monitoring. In most cases, the information/data provided is used internally.
    - **Other** - these cover Director travel, various subscriptions, program administration, etc. They frequently support other types of projects.

4.10. How is the impact of this project best characterized?
(Respond to this question for final project reports only; leave blank for midterm reports)

Research projects	CHECK ONE
a) This project has resulted in an identified product/technology/research outcome that is commercialized and will lead to benefit to soybean farmers.	
b) This project has resulted in specific prospects for commercialization; the potential benefits are clear but an industrial partner needs to be identified in order to realize benefits	X
c) This project has contributed to building a foundation and knowledge base that may lead to benefits for future generations of soybean farmers	
d) The results of this project are unlikely to lead to commercially viable benefits or to changes in attitudes and behavior in favor of soy farmer and industry interests	
Market development/marketing/advertising/PR projects	CHECK ONE
a) This project has resulted in positive changes in the targets' behavior	
b) This project has resulted in changes in the targets' awareness and attitudes that will potentially lead to positive changes in behavior	X
c) The results of this project are unlikely to lead to commercially viable benefits or to changes in attitudes and behavior in favor of soy farmer and industry interests	
Farmer communication projects	CHECK ONE
a) US soy farmers understand how check-off funds are being invested and how this work will benefit them; they continue to support the check-off	X
b) This project is unlikely to contribute to farmers' understanding and support of the check-off	
Special projects	CHECK ONE
a) This project has met its objectives and the outcomes and will contribute to meeting USB's strategic objectives	X
b) This project has not developed according to plan and expectations	

4.11. Is any further investment required for this project to realize its intended benefits for US soybean farmers? *(Respond to this question for final project reports only; leave blank for midterm reports)*	CHECK ONE
a) No further investment is required benefits, have been realized	X
b) Smaller (75% or less) than the current level of investment is required to realize benefits.	
c) Maintain approximately the current level of investment.	
d) Larger (25% or more) than the current level of investment in further work is required.	
e) There should be no further investment in this project because the results are unlikely to lead to contribute to soybean farmers and industry well-being.	

4.12. Over what period of time will the expected benefits to US farmers be realized? *(Respond to this question for final project reports only; leave blank for midterm reports)*	CHECK ONE
a) Immediate to near future	
b) 3 - 5 years	X
c) More than 5 years	
d) N/A, project is not showing potential to realize benefits or this has been a one-off Special Project	

5. LEARNING AND NEXT STEPS

5.1. Have similar projects been funded in the past? [Check Yes or No]	YES	NO
(Answer for both final and midterm project reports)		X

If yes, how do they relate to the current one?

5.2. Is this project funded for FY15? [Check Yes or No]	YES	NO
		X

5.3. What, if any, follow-on steps are required to capture benefits for all US soybean farmers?

Understanding the physiological, genetic, and microbiota interactions that occur in salmonids and other carnivorous farmed fish that results in development of intestinal enteritis when fish are fed high soy based feeds has eluded the fish research community for decades. Unique to this project has been the use of a selected fish strain that does not develop enteritis when fed high-soy feeds. Such a tool is not available to any other fish research laboratories in the world. The use of a selected fish model to understand what can be done to prevent the development of enteritis is powerful information that should lead to increased diet formulation, development of probiotics and management practices, and genetic improvement of fish that stimulate the industry to formulate and utilize feeds containing higher levels of soy protein.