Project Number:	1830-352-0501-H
Project Title:	Closing the Performance Gap Between Natural and Formulated Feeds Through Targeted Supplementation to Soy-Based Diets for Juvenile Red Drum
Organization:	South Carolina Department of Natural Resources
Principal Investigator Name:	Aaron M. Watson, Ph.D.

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.

This project was designed to evaluate the potential growth enhancement or inhibitive effects of several key metabolites that were previously identified through our work. The metabolites that were hypothesized to enhance growth were ones observed at high concentrations in the liver tissue of red drum *Sciaenops ocellatus* fed the natural diet, and low concentrations or absent in fish fed the pelleted feeds high in soy protein ingredients. Conversely, the metabolites selected to test for potential inhibitive effects on growth were observed in higher concentrations in fish fed the pelleted feeds and low or absent in those fed the natural diet. The project consists of two, 12 week feeding trials where growth and performance metrics were recorded, and liver samples were taken at the beginning and conclusion of each trial. The liver samples were processed through our NMR-metabolomics pipeline to develop metabolite profiles. This was done in an effort to track the concentration and potential absorption of the supplemented metabolites into the juvenile red drum.

We have completed the first feeding trial of this project and the feed formulations and results are attached to this report. Due to a disease outbreak, we have not begun the second feeding trial, although a healthy batch of fish is currently being raised in-house to conduct that experiment and an addendum final report will be submitted upon its completion.

Over the course of the first feeding trial, there were no significant differences in performance characteristics (weight gain, feed consumption, feed conversion ratio) indicating that all diets performed well. During the course of the NMR metabolomics analysis, we have found several interesting results so far that we are continuing to follow-up on even though the feeding trial and all proposed deliverables are complete. The supplemented cysteine appears to have been oxidized to cystine either during the extrusion process or prior to feeding, as we only detected cystine in feed extracts that were originally supplemented with cysteine, and not cysteine itself. We are currently examining potential gene expression assays to determine if there is a functional or physiological difference between these two metabolites or if they both represent usable compounds for juvenile red drum. Additionally, the supplementation with dimethylglycine appears to have been too low a concentration to transfer appropriately from the feeds into the livers of the red drum. This could be for several reasons including too low a supplementation level, inadequate ability to absorb this form of the metabolite, or possibly the metabolite was altered in form within the intestine or liver of the red drum to another compound of necessity. Further analysis of the NMR generated data should allow us more insight into these answers as we prepare manuscripts and conference presentations from this work. The most encouraging result from the first feeding trial is that all feeds had a feed conversion ratio below 1.0, which is exceptional for a twelve week feeding trial with this size class of fish.

Overall, we have evaluated eight novel feed formulations for red drum so far, all of which performed well and would represent candidates for further large-scale research. The next steps that should be taken with these formulations as well as others evaluated by our previous work would be to conduct grow-out trials to market size at our pond facility as well as conducting an economic analysis with feed cost and production data to determine the actual commercial viability of the best performing feeds.

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.

It is too early to tell if either the full formulations or specific metabolite supplementation from this project will be incorporated into commercial use. We have completed all of the analyses on the first feeding trial of the project, and although growth and performance were adequate, it does not appear that the metabolite supplementations had direct effects on production parameters over the un-supplemented reference formulation. The overall performance of all evaluated feeds however was such that they would make viable commercial feeds. We are conducting follow-up work on the feeds and tissues to determine why this may have been the case. The second feeding trial was delayed from its original start date to due issues with the initial batch of fish to be used as well as delays due to tropical systems. That trial is scheduled to start in December 2018.

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.

We have completed one of the two feeding trials for this project, and the second feeding trial will begin in December 2018 and a subsequent addendum final report will be submitted upon its completion. From the first feeding trial we have evaluated eight unique feed formulations for juvenile red drum, all of which produced viable growth and performance to translate to commercial use and/or further research efforts. We were unable to identify a specific metabolite or combination of metabolites that increased growth over the reference feed, simply because all feeds performed well and statistically equivalent to one another.

We anticipate at least one manuscript to be developed from this project for submission to a peer-reviewed journal. We will be presenting one talk from this project at the 2019 World Aquaculture Triennial meeting in New Orleans, LA and anticipate another to be developed after the completion of the second feeding trial.

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

The major unforeseen event that has delayed the start of our second feeding trial for this project was a disease outbreak with the batch of fish that was to be utilized. The decision was made that it would be better to delay the experiment and raise another batch of fish as opposed to carry out the trial with a batch of fish that was not optimal. This will not cause any increases in cost of the project to SAA/USB and the feeding trial will be completed in 2019. An addendum to this final report will be submitted upon completion of that trial.

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.

While we await the completion of the second feeding trial to complete the original objectives of this project, we have begun follow-up analyses of the first trial's results to better understand the fate of metabolites that are supplemented to feeds in crystalline form. We are interested in determining if some of these metabolites did not survive the extrusion and pelleting process (which could cause significant changes to feed formulation needs), if there may be issues with the form of the metabolites supplemented that prevented their absorption by the fish, or if due to a combination of these potential factors, if the targeted supplementation level was simply not high enough. We are looking at potential gene expression patterns that may have affected at least one metabolite's processing. The next major step that should be undertaken is an economic analysis of the high soy protein feeds evaluated here to determine the long-term overall cost of these feeds and their potential viability at the commercial production scale.

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

Table 1. Feed Formulations for experimental feeds evaluated in the first feeding trial of the project.

Ingredient (g/100 g dry weight basis)								
Diet	**Reference**	**Cysteine**	**DMG**	**Glucuronate**	**CYS+DMG**	**CYS+GLCRTE**	**DMG+GLCRTE**	**CYS+DMG+GLCRTE**
Soybean Meal 48%CP	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00
Chicken 42 - ADF	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Squid - CSF	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
SC Blood 13	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Menhaden fish oil	12.29	12.30	12.29	12.29	12.30	12.30	12.30	12.30
Mayflower pastry flour	12.43	12.17	12.43	12.41	12.17	12.15	12.40	12.15
Lecithin	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Stay-C 35	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Vitamin premix ARS 702	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
TM ARS 1440	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
NaCl	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28
Magnesium Oxide	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Potassium chloride	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56
Monocalcium Phosphate	4.20	4.20	4.20	4.20	4.20	4.20	4.20	4.20
Choline Cl 50%	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60
DL-Methionine	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82
Lysine HCl	2.32	2.32	2.32	2.32	2.32	2.32	2.32	2.32
Threonine	0.64	0.64	0.64	0.64	0.64	0.64	0.64	0.64
Taurine	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Cysteine	0.00	0.25	0.00	0.00	0.25	0.25	0.00	0.25
Dimethylglycine	0.00	0.00	0.0017	0.0000	0.0017	0.0000	0.0017	0.0017
Glucuronate	0.00	0.00	0.00	0.0221	0.0000	0.0221	0.0221	0.0221
	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Table 2. Performance characteristics from the first feeding trial of the project. No significant differences between feeds.

Diet	Specific Growth Rate (SGR)	Weight Gain (% initial)	Feed Consumed (g/fish)	Feed Conversion Ratio (FCR)
Reference	2.1 ± 0.03	463.16 ± 13.70	142.78 ± 3.35	0.95 ± 0.02
Cysteine (CYS)	2.1 ± 0.06	476.56 ± 26.99	143.82 ± 6.13	0.93 ± 0.03
Dimethylglycine (DMG)	2.1 ± 0.04	471.05 ± 18.93	146.19 ± 6.91	0.96 ± 0.01
Glucuronate (GLC)	2.1 ± 0.08	469.47 ± 36.19	141.27 ± 9.02	0.93 ± 0.02
CYS+DMG	2.1 ± 0.06	500.51 ± 28.15	146.03 ± 7.49	0.90 ± 0.01
CYS+GLC	2.1 ± 0.10	466.82 ± 45.06	141.14 ± 10.69	0.94 ± 0.03
DMG+GLC	2.0 ± 0.08	457.62 ± 37.07	141.01 ± 8.56	0.95 ± 0.02
CYS+DMG+GLC	2.1 ± 0.10	483.33 ± 49.91	147.94 ± 15.33	0.94 ± 0.07
P (significant if <0.05)	0.816	0.845	0.96	0.468