United Soybean Board
Final Report Form – Technical Bulletin

Project # and Title	2463 - - Soy protein concentrate (SPC) as fishmeal replacement in diets for summer flounder: Phase 4 – Digestive histology and blood chemistry analysis of fish fed soy molasses components as immunostimulants.
Organization & Project Leader	University of Rhode Island - Bengtson
Reporting Period	1/1/2012 – 12/31/2012

Introduction: Statement on the rationale and background for the studies

Designing diets that are primarily soybean meal (SBM) instead of fishmeal will improve the overall sustainability of the aquaculture industry. A diet must provide good growth to the animal to which it is fed, but it should also neutrally or positively impact the ability of the animal to survive disease outbreak. Thus, a soybean-based diet for summer flounder that optimizes both growth and survival during bacterial challenge, would lead to enhanced profitability and sustainability of summer flounder aquaculture. It is well known that excess SBM in diets for fish can lead to intestinal pathologies like enteritis. However, in our previous work, we have demonstrated that SBM actually appears to stimulate the immune system of summer flounder, whereas soy protein concentrate (SPC) does not do so. Thus, it appears that something that is extracted from SBM to produce SPC (the fraction that is commonly referred to as soy molasses) contains one or more compounds that stimulate the immune system in these fish. We have funding from Rhode Island Sea Grant to identify the fractions of soy molasses that stimulate immune function, but it does not include sufficient funding to examine histopathology of the liver and pancreas or the blood chemistry for certain enzymes. USB funding is enabling us to do that work as part of the Sea Grant project.
Although this work is directed toward the summer flounder industry, identification of soy molasses components that stimulate fish immune systems could be broadly applicable to other fish species in aquaculture as well. The concept is to use SPC as the main soy component of fish diets as the protein source, but then to add just the right amount of the appropriate soy molasses factor to boost the immune system as well.

Studies completed - brief summary of the number and type of studies conducted, including general study design and approach on how and where the studies were conducted, but without details of the materials and methods

Three growth trials were performed at the URI Blount Aquaculture Laboratory.

In the first growth trial, 800 summer flounder were separated into five groups and fed five specific diets for 8 weeks. The diets used in this study were a fishmeal control and 4 experimental diets that each had 60% of the protein replaced with soy protein concentrate rather than fish meal (diets derived from our previous USB research). The diets also had a fraction of soybean molasses added back to the diet in order to determine at which level soybean molasses stimulates immune response (Sea Grant research) and/or causes morphological damage (USB research). Samples were taken for histological examination during weeks 1,2,4, and 8. These fish were too small for blood to be drawn.

In the second growth trial, 500 summer flounder were separated into five treatments and fed five specific diets for 6 weeks. The diets in this study were a fishmeal control, an SPC control (60% of the fish meal replaced with SPC, but no soy molasses fractions were added), and 3 experimental diets (60% of the fish meal replaced with SPC, plus different fractions of soybean molasses added to the diet). Samples were taken for histological examination during weeks 2 and 6. These fish were also too small for blood to be properly drawn.

The histological samples were evaluated for specific morphology change using a light microscope in the Gomez-Chiarri lab. Fish from these experiments were subjected to a bacterial challenge (as in our previous USB research) at the end of each feeding trial.

In the third trial, 300 summer flounder were separated into five treatments and fed five different experimental diets for 4 weeks. All diets utilized 60% replacement of fishmeal with commercial SPC. The five diets were as follows: 1) SPC control (commercial SPC replaced 60% of the fish meal), 2) SPC control diet with the water phase of butanol extracted soybean molasses added back in, 3) SPC control diet with 2% oligosaccharide content added (80:20 ratio of stachyose to raffinose), 4) SPC control diet with 4% oligosaccharide content added, and 5) SPC control diet with 6% oligosaccharide content added. Following the 4 week feeding trial, the fish were split into 3 groups. Group 1 were euthanized, and then histological samples were taken, identical to feed trials 1 and 2. Group 2 were subjected to a typical bacterial challenge as with trials 1 and 2. Group 3 were injected with a sub-lethal dose of bacteria, and then 48 hours afterward, blood was drawn for blood chemistry and blood composition analysis.

Results - sequential summary of results, ending with recommendations on soy diet formulations, feeding protocols, economics and other related recommendations

Trial 1:

Growth
At the conclusion of the 8 week feed trial, fish fed the fish meal control had the best growth. The fish fed the highest level of soybean molasses (36%) had the worst overall growth. There appeared to be a dose dependent effect by which the SPC control fish had the best growth (other than fish meal), than the fish fed 12% soybean molasses, followed by the fish fed 24% soybean molasses.

Bacterial Challenge
The fishmeal group had the worst survival during bacterial challenge. The SPC control, and the fish fed 12% soybean molasses had a higher level of survival than any other group. This suggests that there are properties within the soybean molasses that are enhancing the survival of fish that are subjected to bacterial challenge. The fish fed the 24% and 36% soybean molasses diet had worse survival than the former. These diets may not give the same benefit in survival because there was too much morphological change caused by anti-nutritional factors within the soybean molasses.

Histology

Fish that were fed the fishmeal control diet had normal morphology when their tissues were analyzed using histology. Fish fed the 36% soybean molasses diet had extensive pathological changes in the spleen, intestine, and liver. These pathological changes occurred as early as the Week 2 time point, and became more extensive at both the Week 4 and Week 8 time point. Fish fed the 12% soybean molasses diet had slight pathological changes in some of the organs, most noticeably in the liver and the anterior intestine. Changes were not apparent at Week 2 time point; however, they did become noticeable at both Week 4 and Week 8. These results suggest that a 12% soybean molasses diet may increase the ability of summer flounder to survive during bacterial challenge; however there appears to be some agent that is causing a reduction in growth. Therefore, 12% soybean molasses diets should be used in further studies.

Trial 2

Growth

Fish that were fed the fishmeal control diet had the best growth, followed by fish fed the SPC control diet. Fish fed the experimental diet where a fraction containing mostly saponins was added to the diet had the worst growth, but only slightly. The fish fed a fraction containing mostly oligosaccharides and a fraction where both oligosaccharides and saponins were mixed had slightly better growth than the strictly saponin diet.

Bacterial Challenge

Fish fed the fishmeal control diet had the worst survival. The diet that contained mostly saponins had the highest survival rate. This suggests that saponins may have a role in enhancing the survival of summer flounder to bacterial challenge. The diets containing oligosaccharides and the saponin-oligosaccharide fractions had enhanced survival over fishmeal control fish. This suggests that oligosaccharides may also play a role in allowing immunostimulation.

Histology

Fishmeal control fish had normal morphology. Fish feed the SPC control diet had an abnormally high number of melanomacrophage centers within the liver. Usually high melanomacrophage center numbers indicate that a fish is responding to an antigen or toxic substance. However, these fish had good growth, so the correlation should be further explored. Fish fed the oligosaccharide fraction diet and the saponin fraction diet had elevated melanomacrophage numbers in the spleen (30-50 vs. around 20). Both the oligosaccharide and saponin fraction fed fish had slight necrosis of the anterior intestine. Usually this was accompanied by the loss of vacuoles and the proliferation of lamina propria.

Trial 3

Growth

The group of fish fed the 60% SPC control diet grew the most overall (46.8±1.9g final weight), whereas the group fed the diet with the water phase of the soybean molasses grew the least (41.5±4.9 final weight). However, there were no significant differences between any of the groups for final weight or length (ANOVA with Tukey's post-test, α=0.05).

Bacterial Challenge

The group fed the SPC control diet had the lowest survival following the bacterial challenge (11.1±10.2%), whereas the group fed the additional 4% oligosaccharide content had 57.8±19.2% survival, and the group fed the additional 6% oligosaccharide content had survival of 44.4±7.7% following the seven day bacterial challenge. All groups had higher survival than the SPC control, demonstrating that there is measurable protective benefit to soybean oligosaccharide addition to any SPC replacement diet for summer flounder.

Histology and Blood Work

Histology samples and complete blood chemistry and blood composition samples have been sent out for processing, and results will be forthcoming.

Conclusions - summarize overall value of research results and application opportunities by industry

Oligosaccharides and saponins appear to enhance the ability of summer flounder to survive bacterial challenge. These soy specific products may become of economic benefit if further studies can demonstrate their effectiveness as a prebiotic. This information could lead to the production of effective prebiotics that farmers across the world may use. However, these substances may also cause a decrease in growth over a 6 or 8 week period. Therefore, it is important to perform further trials and determine if specific saponins or specific oligosaccharides provide more benefit than others. Diets containing either SBM (containing soybean oligosaccharides), butanol extracted soybean molasses (continaing soybean oligosaccharides), or purified stachyose:raffinose additions to SPC replacement diets have demonstrated a reduction in mortality to bacterial challenge in repeated feed trials. The mechanism of action has yet to be fully investigated, which will lead to optimization of levels to incorporate into diets. However, it has been shown that there are protective benefits to using specific soy products for summer flounder diets, which may lead to a reduction in mortality in commercial grow out systems, thereby increasing profitability and economic viability for summer flounder operations.