# Final Report

United Soybean Board Project # 1463 –

Soy protein concentrate as fishmeal replacement in diets for summer flounder (*Paralichthys dentatus*) – phase 3

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## Introduction

During phase I of this project, in 2009, we identified an optimal diet for the replacement of fish meal (FM) with soy protein concentrate (SPC) and/or soybean meal (SBM) for summer flounder, based on a feeding trial in which six diets were tested. The diets included a FM control, one diet based on a mixture of FM and SBM, one diet based on a mixture of FM and SPC, and three diets based on a mixture of FM, SBM and SPC. Our work for phase 2 in 2010 was to compare the "best" diet (diet 6, all SPC replacement of FM, with no SBM) from that trial with a "standard" commercial diet in a six-month study using a quasi-commercial-scale rearing environment.

While that work was going on, we also had funding from the National Oceanic and Atmospheric Administration National Marine Aquaculture Initiative (NOAA-NMAI) to investigate different levels of FM replacement with SBM, and especially to examine if those levels affected the performance of the fish (summer flounder) in a bacterial challenge, which tests their resistance to disease. To our surprise, the fish survived best in the bacterial challenge after they had been fed the diet with the highest level of SBM (70% replacement of FM with SBM), even though their growth on that diet was significantly worse than that of fish grown on diets with lesser amounts of SBM. This unanticipated result suggested to us that something in SBM (but perhaps lacking in SPC) may serve as an immunostimulant to boost the immune system of fish.

Based on the results of the NOAA-NMAI work, we proposed to USB that we would examine the relationship between levels of FM, SBM and SPC during 2011. The graduate student involved in the project also had some separate funding for another experiment along these lines in early 2011. Our goal in these studies was to try to quantify the relationship between SBM and SPC levels in the diet and the survival of fish in a bacterial challenge. Fish were grown in our standard feeding trial prior to their use in the bacterial challenge, so we were also able to obtain data on survival, growth, and food conversion before they were challenged.

## Methods

Fish used in the experiment were obtained from the GreatBay Aquaculture hatchery in Portsmouth, NH, as were fish that we had used in previous phases of this study. Fish were moved to our facility and acclimated for approximately one month prior to the start of the experiment.

The experiment was designed with seven treatments with varying mixtures of FM, SBM and SPC. The control diet used only FM, no SBM or SPC. All non-control diets (Diets 1-6) had 60% of the FM replaced with SBM, SPC, or some combination of the two, as shown in Table 1. Full composition of the diets is given in Table 2. All diets were made at URI. Each diet treatment contained five replicates and each replicate contained 20 fish.

*Table 1. Percentages of fish meal (FM) replaced by soybean meal (SBM) or soy protein concentrate (SPC) in diets 1-6 used in the experiment.*

| % FM replaced by | Diet 1 | Diet 2 | Diet 3 | Diet 4 | Diet 5 | Diet 6 |
|---|---|---|---|---|---|---|
| SBM | 60 | 48 | 36 | 24 | 12 | 0 |
| SPC | 0 | 12 | 24 | 36 | 48 | 60 |

*Table 2. Composition of the seven diets used in the experiment.*

| **Ingredients** | **Contr FM Diet** | **Diet 1 SBM60** | **Diet 2 SBM48 -SPC12** | **Diet 3 SBM36 -SPC24** | **Diet 4 SBM24 -SPC36** | **Diet 5 SBM12 -SPC48** | **Diet 6 SPC60** |
|---|---|---|---|---|---|---|---|
| Fish Meal | 670 | 268 | 268 | 268 | 268 | 268 | 268 |
| Soybean Meal | 0 | 402 | 321.6 | 241.2 | 160.8 | 80.4 | 0 |
| SPC | 0 | 0 | 80.4 | 160.8 | 241.2 | 321.6 | 402 |
| Fish Oil | 32 | 87.2 | 69.17 | 69.17 | 69.17 | 69.17 | 65.2 |
| Wheat flour | 239 | 26.1 | 67 | 87.81 | 93.34 | 110 | 121 |
| Corn gluten | 25 | 123 | 101.7 | 83.73 | 79.76 | 63.43 | 49.2 |
| Starch | 4.5 | 2.5 | 4.5 | 4.5 | 4.5 | 6.5 | 14.5 |
| Mineral Premix | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Ca-Phosphate | 0 | 30 | 30 | 30 | 30 | 30 | 30 |
| Vitamin Premix | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Arginine | 0 | 2.01 | 0.49 | 0 | 0 | 0 | 0 |
| L-Lys 95% | 0 | 5.83 | 4.57 | 3.3 | 2.03 | 0 | 0 |
| DL-Met 99% | 0 | 2.89 | 2.64 | 2.38 | 2.12 | 1.86 | 1.61 |
| Thr 100% | 0 | 1.69 | 0.78 | 0 | 0 | 0 | 0 |
| Taurine 95% | 0 | 14 | 14 | 14 | 14 | 14 | 14 |
| Phytase | 0 | 0.2 | 0.16 | 0.12 | 0.08 | 0.04 | 0 |
| Gly 100% | 10 | 15 | 15 | 15 | 15 | 15 | 15 |
| Total wt (g) | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |
| Protein | 50.02 | 50.05 | 50.23 | 50.54 | 51.72 | 52.15 | 52.87 |
| Lipid | 9.23 | 11.33 | 9.53 | 9.53 | 9.53 | 9.53 | 9.13 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Energy (cal) | 1173.9 | 1067.8 | 1029.04 | 1088.6 | 1108.76 | 1165.1 | 1193.6 |

The experiment was conducted using the same set of 75-L glass aquaria that we used in our 2009 experiment for USB, with flow-through Narragansett Bay seawater (sand- and UV-filtered) supplied at 18 $\pm$ 1 °C and 30 $\pm$ 2 o/oo salinity. Twenty fish were stocked into each aquarium on a randomized basis. Determinations of initial fish total lengths and wet weights were conducted on May 31, which is considered the start date for the experiment. The feeding trial was terminated on August 3, so that the bacterial challenge could begin. Daily procedures during the feeding trial were identical to those that we used in 2009 on our initial experiment for USB. That is, each tank was siphoned clean daily in the morning and fish were fed to apparent satiation twice a day (in the morning and afternoon). Any mortalities were removed daily. Feed for each aquarium was weighed out on a weekly basis and weight of any uneaten feed was determined so that we could calculate the total weight of feed that the fish in each aquarium consumed.

At the end of the feeding trial (9 weeks), after fish had been measured and weighed, they were subjected to a bacterial challenge with cultured bacteria, *Vibrio harveyi*, the causative agent for Flounder Infectious Necrotizing Enteritis (FINE), according to methods described by Gauger et al. (2006). Specifically, fish in three replicates of each treatment received injections with bacterial cultures and fish in two replicates of each treatment received sham injections with saline water as a control (except Diet 2, which had one replicate only for the sham injections). Fish remained in their respective aquaria for seven days following injection and any fish that died were counted and removed from the aquaria as soon as possible after death, but at least on a daily basis.

For the feeding trial, we collected data on survival, growth and food conversion ratio (FCR), all of which were statistically tested by analysis of variance after appropriate transformations to normalize proportionate data. For the bacterial challenge, mortality data were collected and statistically tested by log-rank analysis.

**Results**

Survival of fish in all treatments of the feeding trial was excellent and did not differ significantly among treatments (Table 3); however, one replicate of the Diet 2 treatment was lost due to a system malfunction and was not included in the analysis.

Final lengths and weights of the fish did differ significantly among treatments (Figs. 1 and 2). Fish in the FM control and the SPC 60 diet were statistically indistinguishable, but both grew significantly more than did fish in the SBM 60, SBM 48 – SPC 12, SBM 36 – SPC 24, SBM 24 – SPC 36, and SBM 12 – SPC 48 diet treatments in both length and weight. In addition, fish in the SBM 12 – SPC 48 treatment grew significantly more than did fish in the SBM 60 and the SBM 48 – SPC 12 treatments. Food conversion ratio also differed significantly among treatments (Fig. 3). Specifically, fish in both the SBM 60 and SBM 48 – SPC 12 treatments had significantly greater FCR's than did fish in both the SPC 60 and the FM control treatments; all other treatments were not significantly different from each other.

*Table 3. Survival percentage (mean ± SE) of fish in the experimental treatments. Data are based on five replicates per treatment, except for Diet 2, which lost one replicate due to a system malfunction.*

| Diet | Survival (%) (mean ± SE) |
|---|---|
| Control | 96 ± 2 |
| Diet 1 | 97 ± 4 |
| Diet 2 | 96 ± 3 |
| Diet 3 | 97 ± 2 |
| Diet 4 | 100 ± 0 |
| Diet 5 | 98 ± 1 |
| Diet 6 | 94 ± 2 |

*Fig. 1. Final total lengths (mean ± SE) of fish in the seven diet treatments used in the experiment.*

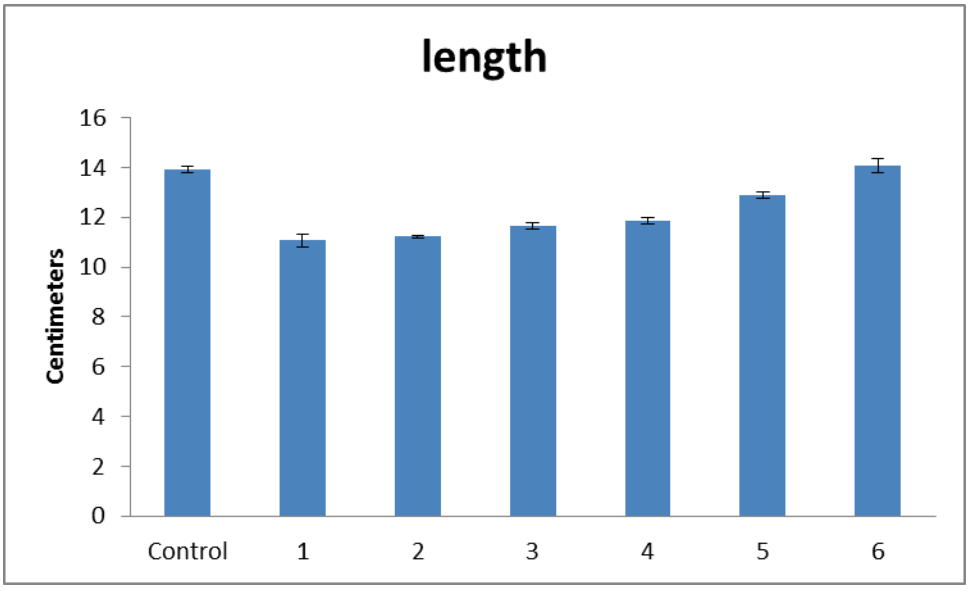


*Fig. 2. Final weights (mean ± SE) of fish in the seven diet treatments used in the experiment.*

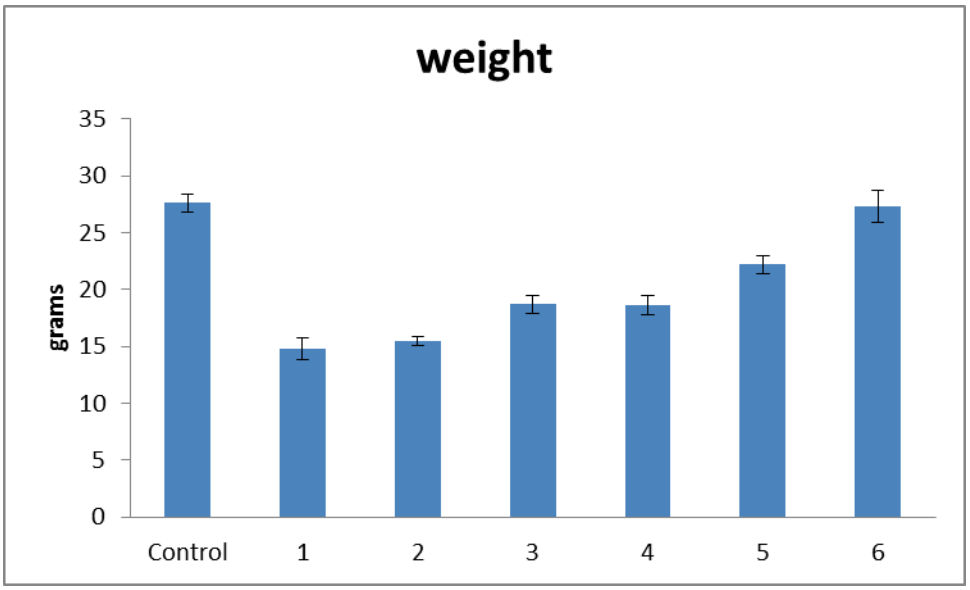
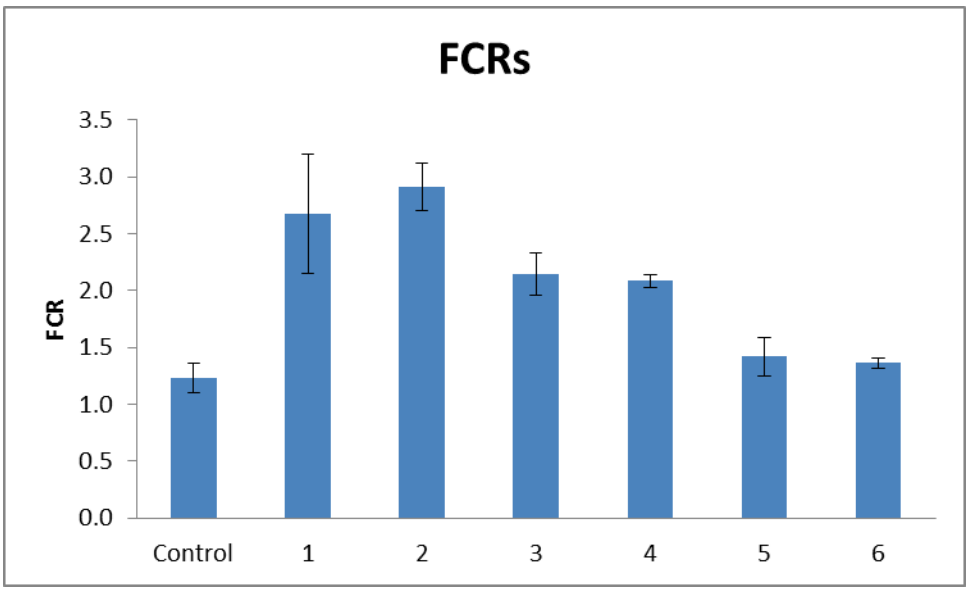
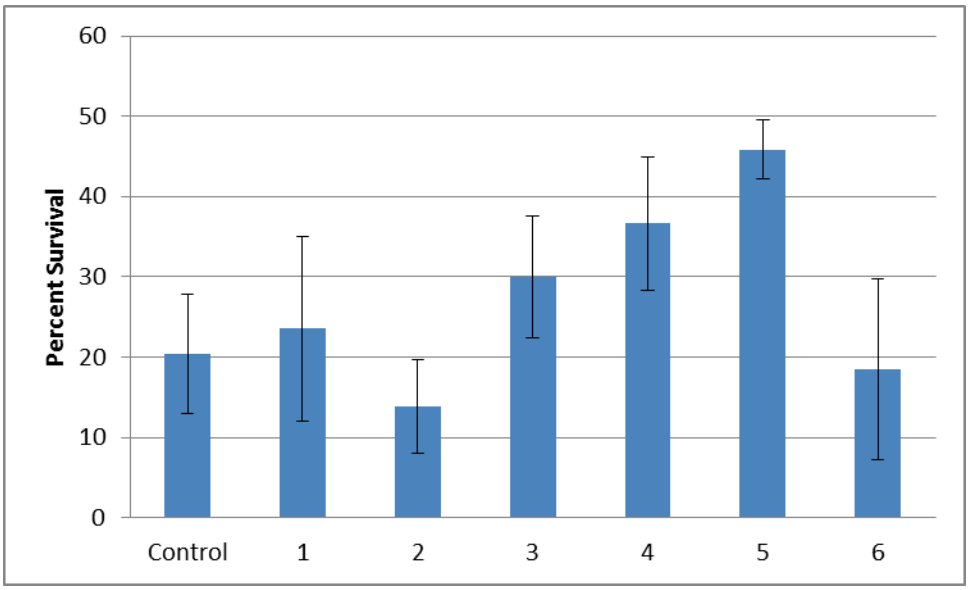



*Fig. 3. Food conversion ratios (mean ± SE) of fish in the seven diet treatments used in the experiment.*




Regarding the bacterial challenge, we observed 100% survival of fish that received sham injections of saline, so that any differences in survival of the bacteria-injected fish must have been due to the bacteria alone. Significant differences were seen in survival of the bacteria-injected fish (Fig. 4). Specifically, fish fed diet 5 during the feeding trial showed significantly greater survival than did fish fed the control FM diet and Diets 1, 2, 3, and 6. Also, fish fed Diet 4 showed significantly greater survival than did those fed Diet 2.

*Fig. 4. Survival (%) (mean $\pm$ SE) of fish in a 7-day bacterial challenge following a 9-week feeding trial during which fish were fed the diets indicated.*



Finally, we conducted an economic analysis based on the calculated cost of the diets and the amount of growth obtained by the fish in each of the treatments during the feeding trial to determine the cost per kilogram of fish produced (Table 4).

*Table 4. Economic analysis of the seven diets used in the 9-week feeding trial, indicating for each diet the cost of the feed per kg, the food conversion ratio, and the cost per kg of fish produced.*

| Diet | Cost per kg (feed) | FCR | Cost per kg (fish) |
|---|---|---|---|
| FM Control | $1.43 | 1.23 | $1.76 |
| 1 – SBM 60 | $1.16 | 2.67 | $3.10 |
| 2 – SBM48-SPC12 | $1.21 | 2.91 | $3.51 |
| 3 – SBM36-SPC24 | $1.28 | 2.15 | $2.74 |
| 4 – SBM24-SPC36 | $1.36 | 2.08 | $2.83 |
| 5 – SBM12-SPC48 | $1.44 | 1.42 | $2.04 |
| 6 – SPC 60 | $1.52 | 1.36 | $2.07 |

**Discussion**

Results of the feeding trial essentially confirmed the results of the feeding trial that we conducted in 2009, indicating that excellent growth of summer flounder is obtained on diets in which SPC replaces 60% or more of FM. Similarly, very acceptable growth is obtained when fish are fed diets in which FM is replaced a combination that is mostly SPC with a small amount of SBM. The real objective of this study was not the feeding trial, but the bacterial challenge for which the feeding trial prepared the fish. Thus, the new finding is that the diet in which 60% of FM was replaced by a combination of 48% SPC and 12% SBM yielded significantly greater survival of summer flounder in the bacterial challenge to test the fishes' resistance to a pathogen that is a real problem to the summer flounder industry.

We have conducted previous bacterial challenges with summer flounder fed soy diets. A graduate student found that fish fed a diet in which 70% of FM had been replaced by SBM survived significantly better than did those fed a diet in which 40% of FM had been replaced, as well as those fed an FM control diet, even though the 70% replacement-fish had grown significantly less in the preceding feeding trial (Lightbourne, 2011). At the end of the commercial-scale feeding trial that we conducted for USB in 2010, a small-scale bacterial challenge showed that fish fed the commercial diet had 100% survival, whereas those fed the test diet in which 65% of FM had been replaced by SPC had only 50% survival. Finally, our current graduate student obtained supplemental funding from the USDA Northeast Sustainable Research and Extension Program to conduct a feeding trial that consisted of an FM control, a diet in which 60% of FM was replaced by SBM, and a diet in which 60% of FM was replaced by

a 50:50 mixture of SBM and SPC, followed by a bacterial challenge as was conducted here. That study, conducted in late winter – early spring of 2011, showed that the SBM/SPC combination diet yielded significantly greater growth of the fish, but there were no significant differences in survival during the subsequent bacterial challenge. Thus, there are a number of pieces of evidence that suggest that inclusion of SBM in diets may provide some immunological stimulus to the fish, along with other evidence (not completely unequivocal) that fish fed diets containing only FM or a combination of FM and SPC may not provide that same stimulus. Although we standardize the bacterial challenges as much as possible, we recognize that there are differences in size and age of fish tested, as well as potential differences in the actual concentration and/or virulence of the bacteria injected, in the various experiments that we have conducted.

We have presented the economic analysis using our standard method of calculating cost/kg of fish produced at the end of the feeding trial. It indicates that fish fed the FM diet were cheaper to produce than any of the other fish fed the SBM/SPC diets. It should be noted that these numbers are for direct comparison in this experiment only and may not represent realistic production costs, since the fish were grown under experimental conditions in small aquaria. Furthermore, as we go forward, we will need to develop a method to incorporate results of the bacterial challenges into the economic analyses. A simple look at the current data indicates that the diet 5 fish, which cost $2.04/kg to grow, had about 45% survival in the bacterial challenge, whereas the FM fish, which cost $1.76/kg to grow, only had about 20% survival in the challenge. While it is not realistic to use those survival percentages directly for economic analysis (fish in aquaculture facilities are not injected with bacteria), the data suggest that greater resistance to the pathogen could lower production costs over time for fish fed some combination of SBM and SPC compared to fish fed FM diets.

Based on our previous work, we have been able to obtain funding from Rhode Island Sea Grant (RISG) for 2012-2014 to investigate whether SBM contains some immunostimulant(s) that is/are extracted during the production of SPC and that might explain the increased resistance to the pathogen that we have seen in our bacterial challenges. We thank USB for their letter to RISG in support of our proposal and for providing additional funds that will be used to further examine the histology of the fish used in the RISG studies.

**References**

Gauger, E., R. Smolowitz, K. Uhlinger, J. Casey, and M. Gomez-Chiarri. 2006. *Vibrio harveyi* and other bacterial pathogens in cultured summer flounder. Aquaculture 260: 10-20.

Lightbourne, C. 2011. Soybean meal diets for summer flounder. M.S. Thesis, University of Rhode Island.