Project Number:	1830-352-0501-L
Project Title:	EFFECT OF PROTEIN SOURCE ON ESTIMATES OF TAURINE REPLACEMENT AND BIOAVAILABILITY IN CALIFORNIA YELLOWTAIL AND WHITE SEABASS
Organization:	Hubbs-SeaWorld Research Institute
Principal Investigator Name:	Mark Drawbridge

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.

The goal of this project was to confirm if protein source significantly affects taurine bioavailability in White Seabass (WSB) and California Yellowtail (YT) and how it affects bioavailability. The objectives were as follows:

1. To quantify the interaction of soy protein – if any – with taurine supplementation, and its effect on taurine bioavailability and requirement estimate.
2. To investigate the same interaction effect on the histology of the gut and liver, in order to verify the presence of enteritis and other lesions as described previously in the literature.
3. To contrast two species with different taurine requirements.

Objective 1: A ten week trial with WSB was completed in late July. Fish were stocked into one of fourteen diet conditions: a poultry byproduct (PBM) based diet formulated eight different taurine levels from 0.25% to 1.6% or a soy (S) based diet formulated with 0.25%- 1.35% taurine to match the PBM taurine levels (Table 1). WSB were stocked at 15 fish per tank with an average weight of 4.9g per fish. Fish were fed daily rations via an automatic belt feeder over the course of 6 hours, and rations were adjusted to just over the satiation of the best feeding tanks. Fish in all tanks were weighed bi-weekly to assess growth, and new daily rations were calculated. At the end of the trial, all fish were euthanized and batch weighed (Table 2).

Table 1. Formulations of eight different PBM based and six different soy based diets with varying taurine levels for White Seabass.

Diet number	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Diet name	PBM								Soy					
Menhaden fishmeal														
Poultry by product meal	54.90	54.90	54.90	54.90	54.90	54.90	54.90	54.90	20.00	20.00	20.00	20.00	20.00	20.00
Meat & bone meal														
Soybean meal									24.30	24.30	24.30	24.30	24.30	24.30
SPC - Soycomil									6.10	6.10	6.10	6.10	6.10	6.10
CPC - Empareal 75									10.00	10.00	10.00	10.00	10.00	10.00
Menhaden fish oil	2.89	2.89	2.89	2.89	2.89	2.89	2.89	2.89	7.35	7.35	7.35	7.35	7.35	7.35
Lecithin (type?)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Corn Starch	**18.46**	**18.46**	**18.46**	**18.46**	**18.46**	**18.46**	**18.46**	**18.46**	**5.06**	**5.06**	**5.06**	**5.06**	**5.06**	**5.06**
Whole wheat	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Mineral premix (ASA/marine	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin premix (ASA/marine	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Choline chloride	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Stay C 35% active	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Methionine	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.16	0.16	0.16	0.16	0.16	0.16
Taurine		0.20	0.40	0.66	0.76	0.86	1.12	1.37	0.13	0.52	0.79	0.89	0.99	1.25
Cellufill - non nutritive bulk	1.6	1.40	1.20	0.94	0.84	0.74	0.48	0.23	1.47	1.08	0.81	0.71	0.61	0.35
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

TABLE 2. A comparison of the growth, survival, and food conversion ratios in white seabass using PBM or Soy proteins with varying levels of taurine across a ten week trial period.

Diet ID	Protein Source	Initial WT (g)	Final WT (g)	WT Gain (%)	Survival (%)	FCR
1	PBM	4.9	31.5	546.8	97.8	1.9
2	PBM	5.0	34.1	585.5	91.1	1.8
3	PBM	5.1	35.1	591.8	100.0	1.8
4	PBM	4.9	36.7	654.7	100.0	1.7
5	PBM	5.0	35.7	618.2	97.8	1.8
6	PBM	4.8	36.2	660.5	95.6	1.7
7	PBM	5.0	37.3	640.4	95.6	1.7
8	PBM	4.9	35.6	621.0	100.0	1.7
9	Soy	5.1	28.2	462.0	73.3	1.8
10	Soy	5.0	32.5	549.5	80.0	1.6
11	Soy	5.0	37.6	658.8	73.3	1.5
12	Soy	4.8	33.3	600.4	83.3	1.6
13	Soy	5.1	37.0	632.4	96.7	1.5
14	Soy	5.1	34.9	588.6	83.3	1.5

TABLE 3. Average proximate and taurine levels in homogenized fish samples by diet type.

		Diet	Fish Composition					
		Taurine	Crude Protein	Moisture	Crude Fat	Crude Fiber	Ash	Taurine
1	PBM	0.25%	17.01	76.12	3.20	0.11	3.47	0.11
2	PBM	0.45%	16.82	75.96	3.43	0.10	3.33	0.25
3	PBM	0.64%	16.86	76.12	3.31	0.12	3.34	0.34
4	PBM	0.90%	16.94	75.89	3.46	0.15	3.26	0.41
5	PBM	1.00%	17.18	75.79	3.38	0.11	3.41	0.41
6	PBM	1.10%	16.93	76.16	3.36	0.11	3.28	0.41
7	PBM	1.35%	16.93	76.04	3.27	0.16	3.31	0.43
8	PBM	1.60%	16.70	76.48	3.31	0.08	3.21	0.44
9	Soy	0.25%	16.10	77.23	2.71	0.22	3.43	0.08
10	Soy	0.64%	16.17	77.07	2.99	0.14	3.29	0.34
11	Soy	0.90%	16.40	76.64	3.29	0.13	3.25	0.46
12	Soy	1.00%	16.09	77.27	2.98	0.18	3.18	0.47
13	Soy	1.10%	16.18	77.06	3.08	0.18	3.05	0.48
14	Soy	1.35%	16.27	77.00	3.03	0.11	3.15	0.49

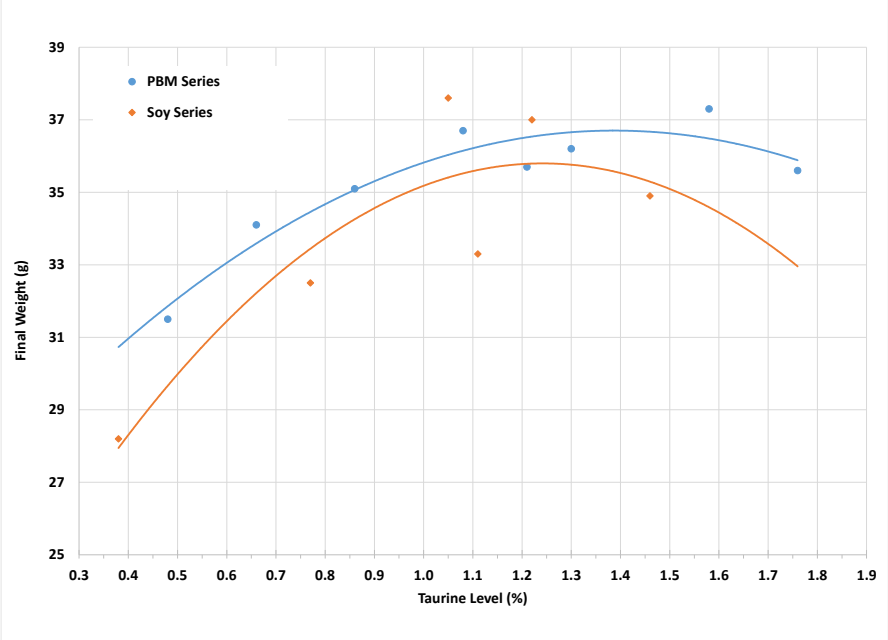

Figure 1. Relationship between taurine level and final weight among groups of seabass fed increasing dietary levels of taurine.

We found no statistically significant differences (at P <0.05) in WSB growth between the two treatment series when analyzed by 2-Way ANOVA or Analyses of Covariance. As shown in Figure 1, final weights reached an asymptote at approximately 1.2-1.4% in each diet series. Fish fed the PBM did grow slightly larger at the optimized level of taurine. The data shown in Table 3 as well, as taurine retention efficiencies, indicate that there is no negative effects for the inclusion of high levels of soy on taurine

retentions. In fact, the data suggest that Soy protein diets allow greater deposition of taurine in the fish and comparable dietary inclusion levels. These data will add to the discussion that soy-based diets are the causative agent for a taurine deficiency and that they induce a higher requirement for taurine. Based on all data that we have been able to develop; we do not see where soy based diets are the causative agent for a taurine deficiency. Hence, data created by this project are critical to the continued expansion of the use of soy in marine fish feeds.

Objective 2.

Three WSB from each treatment group (at least one fish per replicate tank) were euthanized and 1.0 cm sections of liver and distal intestine, and whole heart were preserved in Bouin's solution for 20 hours. Samples were transferred to 70% ethanol for final fixation prior to submission to an outside laboratory for histopathologic analysis. Liver and intestinal sections were ranked according to criteria as described in Novriadi et al. (2018 and 2019). In general, fish consuming the protein byproduct meal as a primary protein source had more uniform findings in terms of liver glycogen accumulation, hepatocellular nuclear size, and intestinal goblet cells. With the exception of one outlier, there was minimal liver inflammation noted in all samples observed regardless of treatment. No cardiac lesions were noted. When statistically comparing rank scores by histology category for fish on a primary poultry byproduct meal protein source and those in combination with soy, fish in the soy treatment groups were more likely to rank higher in liver glycogen storage and the presence of intestinal goblet cells. When evaluating associations between taurine content and histology rank by diet protein type, fish in soy diets demonstrated decreasing intestinal inflammation ranks as the taurine levels increased (Figure 2).

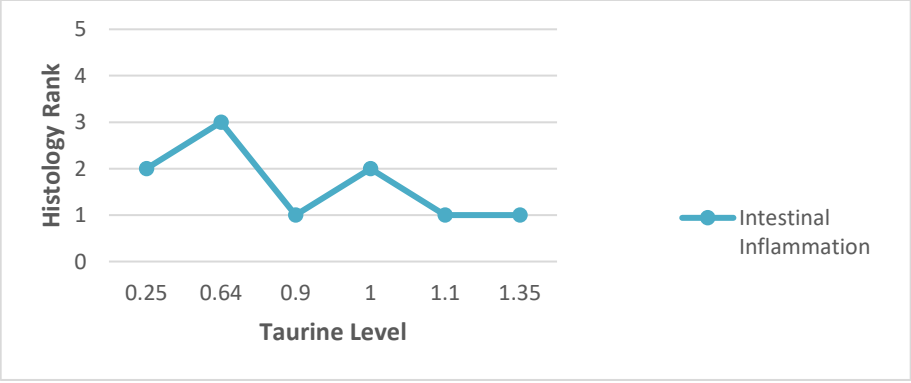

Figure 2. Intestinal inflammation median histology rank for fish fed diets with soy protein by taurine level.

Objective 3.

We initiated a similar trial with California Yellowtail (YT) shortly after the termination of the WSB trial. Diets for these fish utilized either poultry by-product meal (PBM) or soy. Diet types included seven PBM variants (a baseline diet formulated with 0.15% taurine and seven addition diets formulated with 0.19% to 0.54% taurine) fed in triplicate and six soy diets (12% PBM and 35.8% soy) with additional taurine to match the taurine range in the high PBM diets were fed in duplicate for a total of 33 tanks. YT were stocked at 11 fish per tank with an average weight of 8.8g per fish. Fish were fed daily rations via an automatic belt

feeder over the course of 6 hours, and rations were adjusted to just over the satiation of the best feeding tanks. Fish in all tanks were weighed bi-weekly to assess growth, and new daily rations were recalculated.

Approximately three weeks into the new trial, we determined that many fish in both poultry and soy treatments were not eating. The trial was immediately reset with a new crop of YT that received either the high taurine PBM food formulated or high taurine soy for a period of two weeks before stocking. Only fish that appeared to be eating the acclimation diets were stocked into the trial. YT consuming the PBM acceptance diets were stocked at 15 fish per tank and weighed 12.3g on average. YT consuming the soy acceptance diet were stocked at 15 fish per tank and weighed 15.3g on average. Approximately three weeks into the trial, we determined that most fish in both treatment series were not eating their rations again, and the trial was suspended. Water quality was within acceptable ranges for this species during both trial attempts. In addition to evaluations of water quality, diets were also remade with different sources of poultry meal to ensure that the consumption issues were not related to low quality ingredients or other ingredient issues. No definitive cause was identified. Hence, we were not able to determine why the both diet series were mostly not palatable to the YT. Because we could not complete this trial with YT, we were unable to answer the species comparison as stated in Objective 3.

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.

The primary Key Performance Indicators (KPIs) for this research will be the use of the results in the future by nutritionists and feed formulators. Our ability to demonstrate that fish performance was not significantly affected by the interaction of protein source and taurine level under the conditions of this study design will be useful in future feed formulations for marine fish. Additionally, we anticipate that more studies like this will be conducted in order to cover a broader range of ingredients and species.

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 confirmed that protein source does not significantly affect taurine bioavailability in white seabass and we reconfirmed what level of taurine in the diet maximizes fish growth performance. Primary deliverables from this project will be novel feed formulations for seabass; a written report for lay-audiences; and a peer-reviewed publication reporting our findings.

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

The YT trial to assess different PBM and Soy based diets with varying taurine levels had to be suspended due to a lack of feeding response among all treatments. The trial was immediately reset with a new crop of YT but these fish did not response well to the diets either, so this trial had to be discontinued. The reason for the lack of feeding response was not determined – water quality and fish health were both good. These are diet formulation that have been run in the past without major issues and the diets we made twice with new sources of ingredients to eliminate one possibly being of low quality.

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.
The results of this project further illustrate how soy based feeds can be effective in rearing commercially important fish species. Demand for sustainable, species-specific soy based diets will increase the demand for US soybeans both domestically and internationally as the popularity of aquaculture continues to grow. In the scientific community, the results here should stimulate additional research to better understand potential interactive effects between ingredients.
List any relevant performance metrics not captured in KPI's.