Selectively bred, high protein soybean meals for commercial production of California yellowtail (*Seriola dorsalis*).

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Introduction

This study investigated the inclusion of three different soybean meal (SBM) products in existing formulations for California yellowtail (*Seriola dorsalis*) using up to 34% SBM either solely or in combination. Among the SBM products was high protein (58%) variety produced by Benson Hill. Unfortunately, at the time of this study, they did not have their high protein, ultra-low oligosaccharide variety available as originally proposed. The test species for this study, California yellowtail, represents a high value species with an existing market for fresh sushi-grade product and successful rearing methods in hatchery and growout.

Methods

The 8-week feeding study was conducted in a twenty-four tank recirculating system at HSWRI's research laboratory in San Diego, CA. Juvenile yellowtail were fed various formulations using three different SBMs for a total of five treatments combinations and a fishmeal control (Table 1). Dietary formulations and feed were provided by Dr. Allen Davis' laboratory at Auburn University. Each treatment had four replicates for statistical validity. Biochemical composition of the feed and whole fish was determined at the end of the study, along with growth and survival metrics, and health assessments histopathology and gene expression.

Initially this study began with fish that averaged 5 g individual weight. However the fish did not accept the diets, including the fishmeal (FM) control (Table 2). We tried several methods to wean the fish onto the experimental diets, including mixing the experimental feeds with a commercial diet at 50:50, and top-coating the experimental diets with fish oil or krill oil. None of these approaches yielded adequate feeding responses. Dr. Davis reformulated the diets to include 10% FM and we tried again – this time successfully (Table 1). By this time, the fish were 16 g each on average. Out of curiosity, we tested the old diets on subgroups of fish held separately from the main experiment. These fish were presented two of the original diets (#4 and #6) shown in Table 2 and they consumed them readily. This suggests that the size of fish may play an important role in diet acceptability, which is an important consideration for future research.

To analyze immune genes of interest, samples of distal intestine were collected and stored in DNA/RNA Shield (Zymo Inc.) and RNA was extracted using a Zymo RNeasy kit. Extracted RNA was quantified using a Nanodrop OneC (ThermoFisher Scientific) and then converted to cDNA using a High-Capacity cDNA Reverse Transcriptase kit (ThermoFisher Scientific). The cDNA was then used in 10uL qPCR reactions to assess the expression of *apn* (alanine aminopeptidase), *atpase* (ATPase), *gpx1* (glutathione peroxidase 1), *mga* (maltase-glucoamylase), and *il1b* (interleukin 1β)in with a Quantstudio 5 (Applied Biosystems, Inc.). The primers used were previously published by Viana et al. (2019). A housekeeping gene, *actb* (β-actin), were used to normalize the samples and all samples were compared to the control diet (D1) using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). The final data were log-transformed and analyzed in GraphPad 9.

Samples of the distal intestine were processed for histology and stained with H&E for analysis. Slide images were randomized without labels (blinded) and were qualitatively assessed by three independent reviewers using a grading system by Barnes et al. (2014). The parameters evaluated were the thickness of the lamina propria, the amount of connective tissue beneath the folds adjacent to the stratum compactum, and the relative amount of large vacuoles present within the folds. Three fish per tank were scored independently and averaged by tank, followed by an average of reviewer scores. Data were then analyzed using a nonparametric Kruskal-Wallis Test in GraphPad Prism 10.

Results

The trial was successfully run for eight weeks during which time the fish grew to a maximum average of 141 g or 755% gain (Table 3). Survival was high among all treatments (>96%) and FCRs were low (1.11-1.16). There were no statistical differences among treatments for any performance measurement.

Proximate composition and fatty acid analysis of the diets are shown in Table 4. The proximate composition and mineral analysis for the fish at the end of the trial showed no differences between the diets (Table 5). We also did not see any significant differences in gene expression of intestinal tissue at the end of the trial among five genes evaluated (Figure 1). Qualitative analysis of the intestinal tissues yielded no significant differences across the dietary treatments (Figure 3).

Conclusions

This study showed that fish performance (growth, survival, FCR) was not impacted by different SBMs or different inclusion rates used in this study. In fact, performance from those diets was similar to the FM control, which bodes well for future commercial application. Further refinements will need to be made to include more SBM in the diets. Our study suggested that it is possible that larger fish would have performed well on the zero FM formulations listed in Table 2. Larger fish would be the target for growout trials where most of the feed biomass is consumed, so this area should be explored in future research.

Table 1. Diet formulations for six treatments fed to replicate groups of yellowtail starting at 16g.

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Menhaden fishmeal [1]	100.0	100.0	100.0	100.0	100.0	300.0
Poultry meal [2]	200.0	200.0	200.0	200.0	200.0	238.0
SE Soybean meal [3]	344.0	172.0	172.0			
SBM Bright Day [4]		144.0		289.0		
SBM HP 300 [5]			139.0		279.0	
Corn protein concentrate [6]	80.0	80.0	80.0	80.0	80.0	80.0
Menhaden fish oil [7]	60.7	61.1	59.3	61.6	57.8	38.7
Corn Starch [8]	0.4	26.0	32.8	52.5	66.3	152.8
Whole wheat [9]	173.0	175.0	175.0	175.0	175.0	175.0
Mineral premix [10]	2.5	2.5	2.5	2.5	2.5	2.5
Vitamin premix [11]	5.0	5.0	5.0	5.0	5.0	5.0
Choline chloride [8]	2.0	2.0	2.0	2.0	2.0	2.0
Rovimix Stay-C 35% [12]	1.0	1.0	1.0	1.0	1.0	1.0
CaP-dibasic [8]	25.0	25.0	25.0	25.0	25.0	0.0
Methionine [13]	1.4	1.4	1.4	1.4	1.4	0.0
Taurine [13]	5.0	5.0	5.0	5.0	5.0	5.0

[1] Special Select™, Omega Protein Inc., Houston, Texas, USA.
[2] River Valley Ingredients., 1170 Country Road 508. PO. Box 429 Hanceville, AL.
[3] Solvent Extracted Soybean Meal, De-hulled solvent-extracted soybean meal, Bunge Limited, Decatur, AL, USA.
[4] Benson Hill, St. Louis, MO 55.1% protein.
[5] Hamlet Protein Inc., Findlay, OH 56.0% protein.
[6] Empyreal 75™, Cargill Corn Milling, Cargill Inc., Blair, Nebraska, USA.
[7] Omega Protein Inc., Reedville, Virginia, USA.
[8] MP Biomedicals Inc., Solon, OH, USA.
[9] Bobs Red Mill Natural Foods, Milwaukie, OR, USA.
[10] Trace mineral premix (g/100g premix): cobalt chloride 0.004, cupric sulphate pentahydrate 0.250, ferrous sulphate 4.0, magnesium sulphate anhydrous 13.862, monohydrate 0.650, potassium iodide 0.067, sodium selenite 0.010, zinc sulphate heptahydrate 13.193, filler 67.964.
[11] Vitamin premix (g/kg premix): Thiamin HCl0.751, riboflavin4.505, pyridoxineHCl1.502, D-Pantothenic acid hemicalcium salt7.508, nicotinic acid 7.508, biotin 0.075, folic acid 0.270, vitamin B12 0.003, inositol 7.508, menadione 3.003, vitamin A acetate (500,000 IU/g) 0.300, vitamin D3 (1,000,000 U/g) 0.60, DL-α-tocopheryl acetate (250/ IU g-) 12.012, α-cellulose 804.847.
[12] Stay C®, (L-ascorbyl-2-polyphosphate 35% Active C), Roche Vitamins Inc., Parsippany, New Jersey, USA.
[13] TCI (Tokyo Chemical Industry), Portland, OR, USA.

Table 2. Diet formulations for laboratory feeding trial using 5 g California yellowtail.

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Menhaden fishmeal [1]						212.0
Chicken by product meal [2]	200.0	200.0	200.0	200.0	200.0	0.0
SE Soybean meal [3]	380.0					380.0
SBM Bright Day [4]		320.0		412.0		0.0
SPC Soycomil PE [5]	80.0	80.0	80.0			80.0
SBM Hamlet HP 300 [6]			308.0		398.0	
CPC - Empareal 75 [7]	85.0	85.0	85.0	85.0	85.0	85.0
Menhaden fish oil [8]	67.9	68.9	64.7	68.9	63.5	76.2
Corn Starch [9]	15.2	74.2	90.4	62.2	81.6	0.5
Whole wheat [10]	130.0	130.0	130.0	130.0	130.0	125.0
Mineral premix [11]	2.5	2.5	2.5	2.5	2.5	2.5
Vitamin premix [12]	5.0	5.0	5.0	5.0	5.0	5.0
Choline chloride (0.2% all diets) [9]	2.0	2.0	2.0	2.0	2.0	2.0
Rovimix Stay-C 35% [12]	1.0	1.0	1.0	1.0	1.0	1.0
CaP-dibasic [9]	25.0	25.0	25.0	25.0	25.0	25.0
Methionine [13]	1.4	1.4	1.4	1.4	1.4	0.8
Taurine [13]	5.0	5.0	5.0	5.0	5.0	5.0

[1] Special Select™, Omega Protein Inc., Houston, Texas.
[2] Darling Ingredients Inc., 5601 N MacArthur Blvd, Irving, TX.
[3] Solvent Extracted Soybean Meal, De-hulled solvent-extracted soybean meal, Bunge Limited, Decatur, AL.
[4] Benson Hill, St. Louis, MO 55.1% protein.
[5]ADM Animal Nutrition, Quincy, I. 62.00% protein.
[6] Hamlet Protein Inc., Findlay, OH 56.0% protein.
[7] Empyreal 75™, Cargill Corn Milling, Cargill Inc., Blair, Nebraska, USA.
[8] Omega Protein Inc., Reedville, Virginia, USA.
[9] MP Biomedicals Inc., Solon, OH, USA.
[10] Bobs Red Mill Natural Foods, Milwaukie, OR, USA.
[11] Trace mineral premix (g/100g premix): cobalt chloride 0.004, cupric sulphate pentahydrate 0.250, ferrous sulphate 4.0, magnesium sulphate anhydrous 13.862, monohydrate 0.650, potassium iodide 0.067, sodium selenite 0.010, zinc sulphate heptahydrate 13.193, filler 67.964.
[12] Vitamin premix (g/kg premix): Thiamin HCl0.751, riboflavin4.505, pyridoxineHCl1.502, D-Pantothenic acid hemicalcium salt7.508, nicotinic acid 7.508, biotin 0.075, folic acid 0.270, vitamin B12 0.003, inositol 7.508, menadione 3.003, vitamin A acetate (500,000 IU/g) 0.300, vitamin D3 (1,000,000 U/g) 0.60, DL-α-tocopheryl acetate (250/ IU g-) 12.012, α-cellulose 804.847.
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[13] TCI (Tokyo Chemical Industry), Portland, OR, USA.

Table 3. Summary statistics for final growth, FCR and survival among California yellowtail fed six dietary treatments for 8 weeks.

Treatment Diet	Final Weight (g ± SD)	Weight Gain (% ± SD)	FCR (mean ± SD)	Survival (mean ± SD)
1	135.45 ± 7.14	722.62 ± 43.33	1.16 ± 0.02	100
2	141.31 ± 5.11	755.61 ± 32.09	1.13 ± 0.03	96.7± 6.7
3	139.00 ± 6.01	742.42 ± 36.13	1.14 ± 0.05	100
4	137.56 ± 3.77	731.18 ± 20.91	1.16 ± 0.11	100
5	133.50 ± 3.05	705.84 ± 18.66	1.13 ± 0.03	100
6	141.08 ± 4.52	753.41 ± 30.38	1.11 ± 0.03	100

Table 4. Proximate composition and fatty acid analysis of six experimental diets offered to California yellowtail (*Seriola dorsalis*) for an eight week trial.

	Treatment					
	1	2	3	4	5	6
Crude protein	45.79	46.54	46.84	44.88	43.85	44.43
Moisture	7.17	6.34	5.1	9.38	10.49	9.39
Crude Fat	9.62	9.56	9.64	9.21	8.81	8.73
Crude Fiber	2.25	2.02	2.21	1.94	2.42	1.24
Ash	8.59	8.57	8.62	8.09	8.03	8.64
Phosphorus	1.46	1.49	1.59	1.38	1.38	1.41
Taurine §	0.8	0.81	0.79	0.79	0.77	0.97
Hydroxyproline	0.45	0.48	0.52	0.44	0.46	0.69
Aspartic Acid	3.91	3.91	3.88	3.79	3.78	3.32
Threonine	1.65	1.65	1.65	1.59	1.61	1.57
Serine	1.79	1.78	1.81	1.74	1.74	1.58
Glutamic Acid	7.68	7.73	7.76	7.55	7.32	6.5
Proline	3.02	2.99	3.01	2.88	2.82	2.75
Lanthionine §	0.05	0.06	0.06	0.06	0.06	0.04
Glycine	2.52	2.59	2.62	2.46	2.48	2.94
Alanine	2.59	2.61	2.63	2.5	2.46	2.7
Cysteine	0.63	0.61	0.6	0.57	0.58	0.5
Valine	2.16	2.15	2.17	2.07	2.06	1.98
Methionine	1.01	1	1.03	0.96	0.97	0.98
Isoleucine	1.99	1.97	1.99	1.91	1.89	1.73
Leucine	3.95	3.95	4	3.84	3.74	3.55
Tyrosine	1.64	1.64	1.64	1.59	1.57	1.39
Phenylalanine	2.17	2.16	2.2	2.12	2.08	1.85
Hydroxylysine	0.06	0.06	0.06	0.06	0.06	0.09
Ornithine §	0.03	0.04	0.04	0.03	0.04	0.04
Lysine	2.47	2.45	2.43	2.37	2.3	2.49
Histidine	1.05	1.05	1.05	1.02	0.99	0.97
Arginine	2.67	2.71	2.71	2.63	2.61	2.39
Tryptophan	0.45	0.44	0.44	0.42	0.42	0.38
Total	44.74	44.84	45.09	43.39	42.81	41.4

Table 5. Proximate composition and mineral composition of whole fish at the completion of the 8 week feeding trial.

		Treatment					
	Initial	**1**	**2**	**3**	**4**	**5**	**6**
Moisture (%)	76.6	72.7 ± 0.7	71.3 ± 0.7	72.3 ± 0.7	71.8 ± 0.8	72.7 ± 0.3	72.7 ± 1.2
Dry (%)	23.4	27.3 ± 0.7	28.7 ± 0.7	27.7 ± 0.7	28.2 ± 0.8	27.3 ± 0.3	27.3 ± 1.2
Protein (%)	15.2	19.7 ± 0.7	19.4 ± 0.6	20.3 ± 0.3	19.5 ± 0.2	19.2 ± 0.3	19.7 ± 0.7
Fat (%)	3.48	4.8 ± 0.4	4.7 ± 1.1	5.6 ± 0.4	5.1 ± 0.6	4.9 ± 0.5	5.0 ± 1.0
Ash (%)	3.15	2.9 ± 0.7	3.4 ± 0.5	2.9 ± 0.4	3.1 ± 0.2	2.7 ± 0.4	2.8 ± 0.5
PRE (%)		0.34 ± 0.01	0.33 ± 0.01	0.34 ± 0.01	0.35 ± 0.01	0.35 ± 0.02	0.35 ± 0.02
Sulfur (%)	0.24	0.23	0.23	0.23	0.23	0.23	0.24
Phosphorus (%)	0.58	0.57 ± 0.04	0.62 ± 0.11	0.54 ± 0.03	0.57 ± 0.03	0.57 ± 0.04	0.53 ± 0.05
PHRE (%)		0.29 ± 0.02	0.33 ± 0.07	0.26 ± 0.02	0.32 ± 0.03	0.32 ± 0.02	0.30 ± 0.04
Potassium (%)	0.36	0.35 ± 0.01	0.35	0.35 ± 0.01	0.35	0.35 ± 0.01	0.36
Magnesium (%)	0.05	0.04	0.04	0.04	0.04	0.04	0.04
Calcium (%)	0.8	0.63 ± 0.08	0.75 ± 0.21	0.58 ± 0.07	0.64 ± 0.06	0.64 ± 0.09	0.55 ± 0.11
Sodium (%)	0.25	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.12 ± 0.01	0.13 ± 0.01	0.13 ± 0.01
Iron (ppm)	13.4	13.97 ± 0.73	14.05 ± 0.56	14.05 ± 0.75	14.07 ± 0.79	15.97 ± 2.29	16.15 ± 2.36
Manganese (ppm)	1.6	0.95 ± 0.67	1.05 ± 0.71	0.55 ± 0.64	1.10 ± 0.08	0.88 ± 0.59	0.60 ± 0.69
Copper (ppm)	0	0.50 ± 0.58	0	0.25 ± 0.50	1	0.25 ± 0.50	0.25 ± 0.50
Zinc (ppm)	16.4	12.45 ± 1.04	12.88 ± 1.30	14.30 ± 0.67	13.30 ± 1.27	13.45 ± 0.25	12.60 ± 1.85

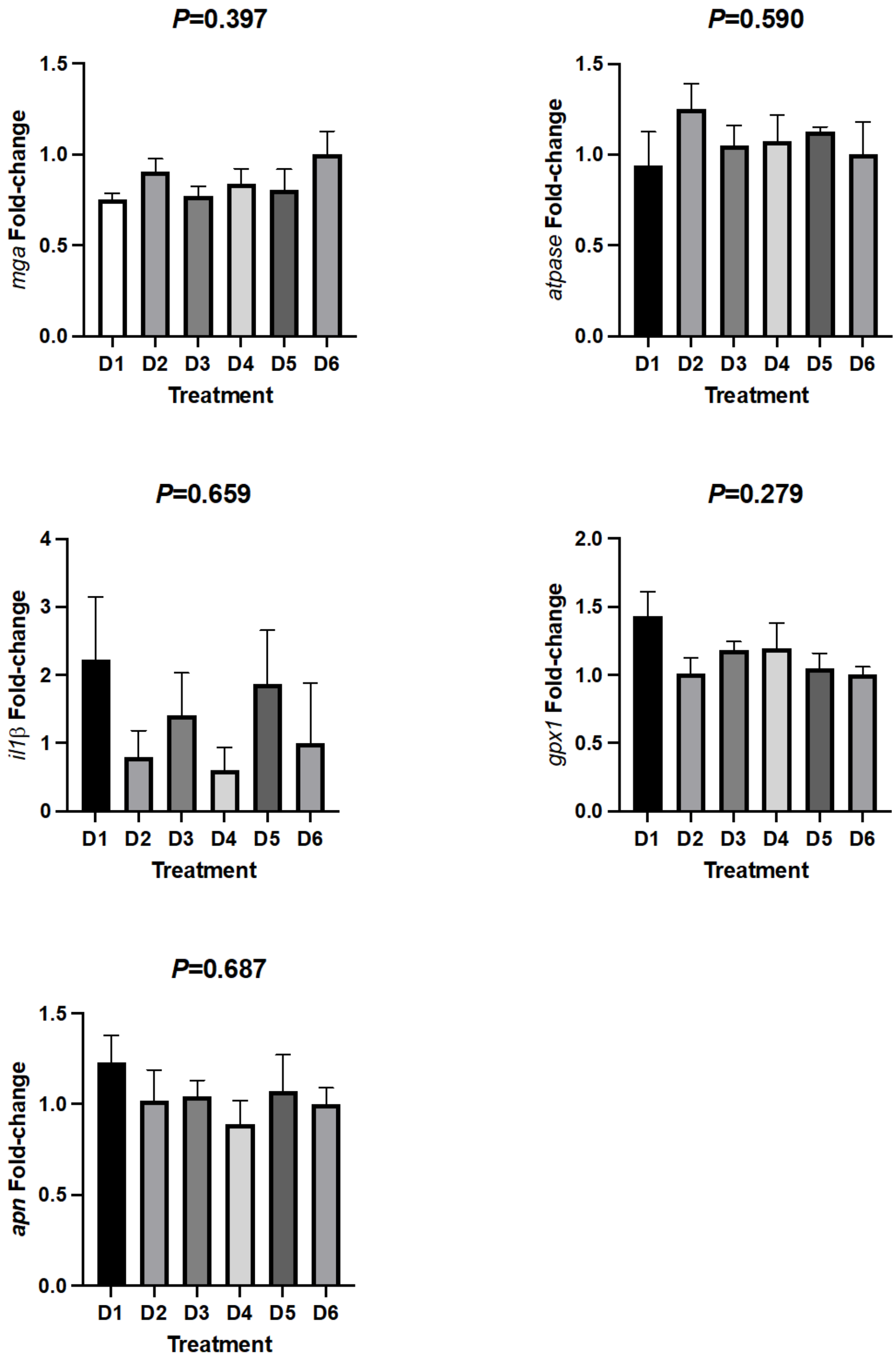

Figure 1. Gene expression from the gut of California yellowtail (*Seriola dorsalis*) fed six experimental diets.

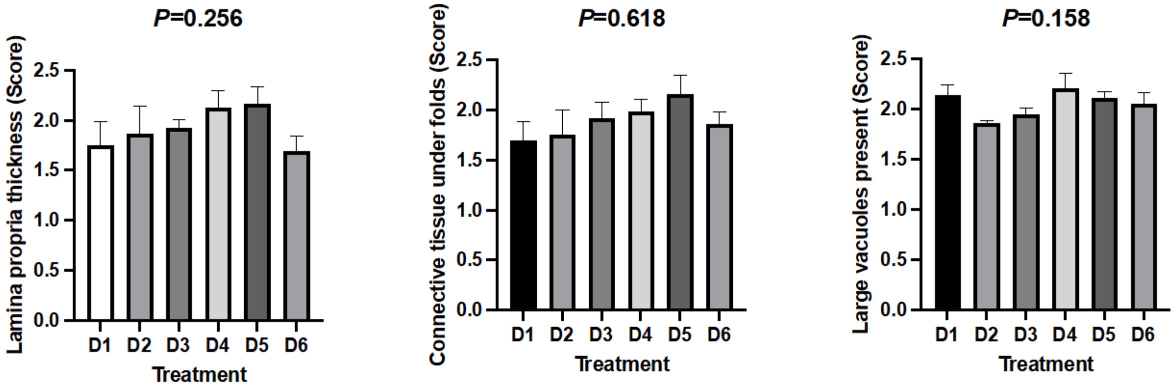

Figure 2. Qualitative histological assessment of distal intestine slides, reviewed by three independent reviewers.

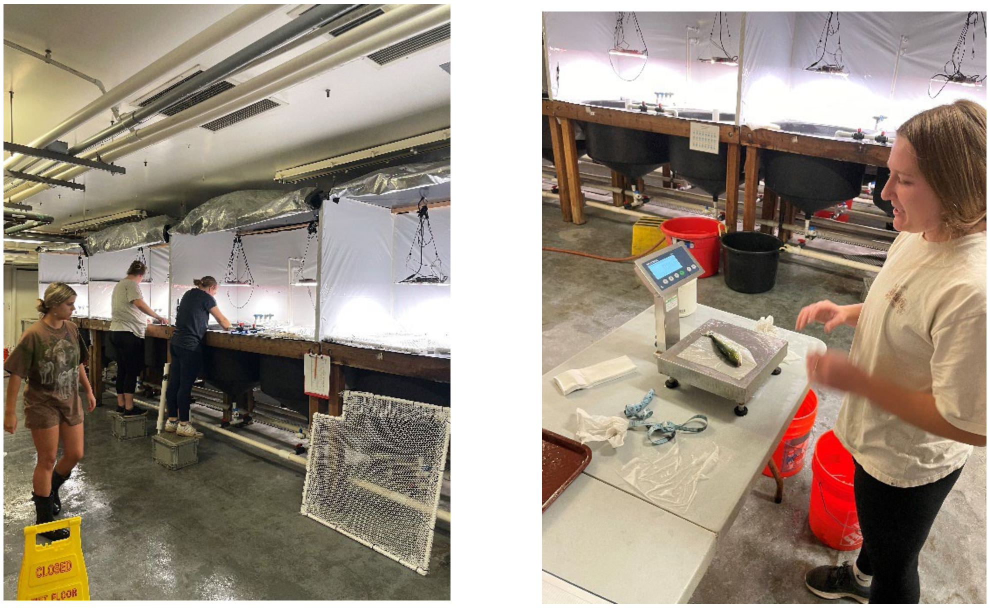

Figure 3. Photographs from the completion of the 8 week feeding trial as well as the experimental system. (left) Four of the twenty-four 300L tanks used in the trial. (right) HSWRI staff collecting weight and length information on individual fish.