Application of Schillinger Cultivars in the Aquaculture of California Yellowtail, *Seriola lalandi*

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INTRODUCTION

The primary goal of this project was to determine the effectiveness of meal from two cultivars of Schillinger Genetics soybeans, the 3010 and Trifecta, as a substitute for fish meal in diets California yellowtail (YT; *Seriola lalandi*). YT is a highly valued commercial and sport fish in southern California and is considered an excellent food fish. HSWRI is currently culturing YT on an experimental scale but great potential exists to expand commercial culture in both northern Baja California, Mexico and Southern California. YT are in the family Carangidae or jacks – a group that typically requires high levels of high quality fish-based protein in the diet. The use of alternate ingredients such as soy protein could greatly improve profitability, while simultaneously addressing issues associated with the long term sustainability of fish meal and fish oil resources. A good opportunity exists to demonstrate the effectiveness of diets based on soy protein in the rapidly developing offshore aquaculture industry in this region.

TASK-SPECIFIC METHODS AND RESULTS

Task 1 - Feeding Trial

This portion of report details experimental results from a feeding trial involving two cultivars of Schillinger Genetics (SG) soybeans – the 3010 and Trifecta. Defatted meals from these two varieties of soybeans were investigated in a feeding trial with California yellowtail (YT - *Seriola lalandi*).

Diets

A total of 8 diets were tested in the trial (Table 1). Three diets (3010 50%, 60% and 70%) were formulated with 50,60 and 70% replacement of the total protein with defatted SG 3010 meal and contained 34.7, 41.9 and 50.9% of this meal respectively. Three diets (Trifecta 40%, 50% and 60%) were formulated with 40, 50 and 60% replacement of the total protein with defatted SG Trifecta meal and contained 31.9, 39.6 and 47.8% of this meal respectively. A fish meal (FM) and a commercial (Comm) control (Skretting Marine Grower) were also tested. The six diets containing SG meals and the fish meal control were isocaloric and isonitrogenous and were formulated to provide 45% crude protein and 13% lipid. The commercial control had a guaranteed analysis of 50% crude protein and 14% lipid.

The experimental diets were formulated by Dr. Rick Barrows at the USDA-ARS Fish Technology Center in Bozeman, Montana, extruded at the same facility and shipped to HSWRI in San Diego for the feeding trial.

Table 1. Composition of experimental diets (g/100g as is).

Ingredient	FM	3010 50%	3010 60%	3010 70%	Trifecta 40%	Trifecta 50%	Trifecta 60%
Menhaden Meal	45.0	22.5	18.0	13.5	27.0	22.5	18.0
Poultry meal	7.5	3.8	3.0	2.3	4.5	3.8	3.0
Soy PC	7.5	3.8	3.0	2.3	4.5	3.8	3.0
Wheat flour	16.0	15.0	14.0	10.0	15.0	15.0	14.0
SG-3010	--	34.7	41.9	50.9	--	--	--
SG-Trifecta	--	--	--	--	31.8	39.6	47.8
Menhaden Oil	6.9	9.7	10.3	10.9	7.5	7.7	7.8
Vit./Min. Premix	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Taurine	0.3	0.5	1.0	1.3	0.8	1.0	1.3
Lysine	--	0.1	0.1	0.3	0.1	0.1	0.1
Methionine	--	0.8	0.9	1.1	0.5	0.5	0.6
Celufil	12.8	5.2	3.8	3.6	4.3	2.0	0.4
Protein	44.6	44.6	44.6	44.6	44.6	44.6	44.6
Fat	13.0	13.0	13.0	13.0	13.0	13.0	13.0
Analyzed[1]							
Moisture (%)	3.4	4.9	3.6	5.9	2.7	2.9	3.5
Dry Matter (%)	96.6	95.1	96.4	94.1	97.3	97.1	96.5
Crude Protein (%)	46.3	44.4	44.7	44.4	45.4	45.2	45.2
Acid Hydrolysis Fat (%)	15.3	14.7	14.7	15.4	14.4	14.6	14.0
Ash (%)	10.9	8.1	7.6	6.9	8.7	8.4	7.8

[1]Texas A&M University, College Station, TX, USA.

Fish and Feeding

Fish utilized in the trial were grown from eggs spawned by captive broodstock at Hubbs-SeaWorld Research Institute (HSWRI). Twenty juvenile YT (initial weight 4.0g) were stocked into 24 individual 330L round tanks. These tanks were connected to a semi-closed recirculating system consisting of a water pump, supplemental aeration and mechanical and biological filtration. A small amount (< 2L/min) of seawater was continuously added to the system for water exchange. Tanks were siphoned daily to remove solids. Water quality parameters were maintained within reasonable limits for this species (TAN 0.02 $\pm$ 0.03, temp. 19.3 $\pm$ 0.6 °C, salinity 33.5 $\pm$ 0.2 ppt, pH 7.7 $\pm$ 0.1, D.O. 7.5 $\pm$ 0.2).

Fish were fed by hand twice a day to apparent satiation 5 days per week. Daily averages for each tank were calculated for the week and this amount was automatically fed to

fish with belt feeders the remaining two days of the week. Fish were group weighed every two weeks.

Analyses

At the end of the trial, fish were group-weighed for final determination of weight gain. Feed usage was totaled and used to calculate feed conversion ratio (FCR). Individual lengths and weights were taken from six fish per tank. Intestines were removed from three of the six fish, measured for length and fixed with Davidson's fixative for histological analyses at Texas A&M University. Three fish per tank were frozen and analyzed for proximate composition at Texas A&M University.

Results

Fish that were fed the diets with Trifecta performed the best with regards to final weight (68.6-78.4g) and weight gain (1611-1853%) with the fish fed the Trifecta 50% diet performing the best overall (Table 2). Weight gain decreased with increasing inclusion of 3010 (1611-1276%). Survival was not significantly affected by diet except with the commercial control. FCR was highest in fish fed the 3010 60% and 70% (1.21 and 1.32 respectively) and significantly higher in those fed 3010 70% than any other group (Table 3). Specific growth rate (3.5-4.0), condition factor (1.0-1.2), protein efficiency ratio (0.43-0.50) and protein retention (24.2-31.0) were not significantly affected by diet. Fish whole body proximate composition was not affected by diet except for protein by dry weight which was significantly highest in fish fed the commercial control (64.3 %). Fish being fed the 3010 50% had the lowest protein content (56.3 %) by dry weight (Table 4). As reported by TAMU, intestinal morphology showed that fold height in the middle and posterior intestine was significantly affected by diet although fold height did not seem to be related to inclusion rate of meal from either SG cultivar (Table 5). Fold height values from fish fed diets with meal from SG cultivars were both above and below values from fish fed the FM diet.

Table 2. Initial weight, final weight, percent weight gain, and survival of YT fed diets with graded replacement of fish meal with two SG cultivars, a fish meal and a commercial control diet.

Diet	Initial wt. (g)	Final wt. (g)	Wt. Gain (%)	Survival (%)
FM	4.1	66.8^{b}	1542^{b}	100^{a}
3010 50%	4.0	68.7^{b}	1611^{b}	100^{a}
3010 60%	4.1	64.9^{b}	1501^{bc}	100^{a}
3010 70%	4.0	55.2^{c}	1276^{d}	95^{a}
Trifecta 40%	4.0	68.6^{b}	1611^{b}	98^{a}
Trifecta 50%	4.0	78.4^{a}	1853^{a}	98^{a}
Trifecta 60%	4.0	75.2^{a}	1776^{a}	97^{a}
Commercial	4.0	59.8^{c}	1389^{cd}	63^{b}
PSE	0.015	1.676	42.164	1.863
P>F	0.520	0.898	0.867	0.615

Table 3. Feed conversion ratio (FCR), specific growth rate (SGR), condition factor (CF), protein efficiency rate (PER) and protein retention (PR) of YT fed diets with graded replacement of fish meal with two SG cultivars, a fish meal and a commercial control diet.

Diet	FCR[1]	SGR[2]	CF[3]	PER[4]	PR[5]
FM	1.11^{c}	3.7^{b}	1.1	0.49	28.7
3010 50%	1.17^{bc}	3.8^{b}	1.1	0.49	27.2
3010 60%	1.21^{b}	3.7^{bc}	1.0	0.50	30.0
3010 70%	1.32^{a}	3.5^{d}	1.0	0.43	24.2
Trifecta 40%	1.15^{bc}	3.8^{b}	1.1	0.49	28.3
Trifecta 50%	1.15^{bc}	4.0^{a}	1.1	0.49	31.0
Trifecta 60%	1.19^{bc}	3.9^{a}	1.1	0.46	26.9
Commercial	1.11^{c}	3.6^{cd}	1.2	0.46	29.4
PSE	0.025	0.035	0.050	0.025	1.766
P>F	<0.001	<0.001	0.309	0.540	0.254

[1] FCR = dry feed intake/weight gain
[2] SGR = ln final wt. – ln initial wt. / 75 days x 100
[3] CF = weight x 100/length^3
[4] PER = weight gain/protein fed
[5] PR = (final body protein-initial body protein) x 100/total protein fed

Table 4. Proximate composition (g/100g dry matter)** of fed diets with graded replacement of fish meal with two SG cultivars, a fish meal and a commercial control diet.

Diet	Dry matter	Protein	Fat	Ash
FM	25.0	59.2^{abc}	28.0	12.0
3010 50%	25.3	56.3^{c}	28.4	11.0
3010 60%	26.9	60.3^{abc}	24.2	12.2
3010 70%	25.3	56.7^{c}	26.3	12.4
Trifecta 40%	25.5	58.1^{c}	26.0	12.3
Trifecta 50%	25.4	63.6^{ab}	22.1	12.2
Trifecta 60%	24.4	59.0^{bc}	29.1	11.7
Commercial	25.4	64.3^{a}	22.1	12.1
PSE	1.223	1.583	2.378	0.326
P>F	0.929	0.019	0.301	0.141

**Analyses conducted by Texas A&M University, College Station, TX, USA.

Table 5. Intestinal morphology** of juvenile YT fed diets with graded replacement of fish meal with two SG cultivars, a fish meal and a commercial control diet.

		3010			Trifecta					
Description	FM	50%	60%	70%	40%	50%	60%	Comm	PSE	P>F
Anterior intestine										
Fold height	497.7	657.7	521.1	520.2	433.4	533.9	468.3	587.6	60.658	0.304
Enterocyte height	34.8	35.5	32.8	36.3	34.4	31.9	37.5	36.2	2.033	0.564
Microvilli height	4.3	4.3	4.2	4.2	4.2	4.2	4.6	4.2	0.270	0.927
Middle intestine										
Fold height	444.1^{bc}	464.2^{bc}	427.1^{bc}	446.7^{bc}	447.7^{bc}	581.4^{a}	405.4^{c}	545.7^{ab}	36.462	0.043
Enterocyte height	30.1	27.9	29.8	31.1	27.8	29.7	28.1	30.5	1.860	0.843
Microvilli height	4.3	4.1	4.0	4.3	4.0	4.2	4.4	4.3	0.215	0.809
Posterior intestine										
Fold height	486.7^{bc}	529.1^{ab}	402.5^{c}	501.5^{bc}	461.8^{bc}	434.5^{bc}	526.9^{ab}	623.1^{a}	36.720	0.020
Enterocyte height	28.4	32.3	31.3	35.1	32.7	30.9	32.8	33.6	1.392	0.116
Microvilli height	4.0	4.2	4.4	4.1	4.3	4.3	4.3	4.4	0.129	0.414

**Histology conducted by Texas A&M University, College Station, TX, USA.

Task 2 – Digestibility Study

In order to determine the digestibility of defatted meals from SG cultivars 3010 and Trifecta, via forced physical extraction, approximately 200, 500g YT were held over from the 2011 production season. Beginning in June 2012, YT were fed one of five diets containing chromic oxide as an inert marker. These diets included diets with 3010 meal, Trifecta meal, soybean meal, soy protein concentrate and a reference diet. Feeding and extraction methodologies were extensively tested to determine the most efficient way to harvest feces. Parameters that were tested and adjusted included feeding time, feeding method (multiple vs. single feeding) and the delay in time after feeding that extraction of fecal matter was attempted. In addition, several techniques for physical extraction as well as multiple dose levels of the anesthetic MS222 were investigated. None of the methods tested produced useable amounts of feces.

As part of an expanded investigation, we dissected several fish after feeding and determined that Seriola lalandi has a unique gut morphology that prevents most of the feces from being physically extracted from the fish. Specifically, there is a dramatic U-bend in the posterior intestine of YT just before the rectum that coincides with the point

that feces are blocked from coming out (Figure 1). It is not clear if the bend itself is the bottleneck or some contracted muscles associated with that section of intestine. Regardless, the middle intestine retained the majority of feces even after aggressive abdominal massage. We subsequently compared the gut morphology of YT with striped bass (SB) of similar size and found that the SB do not have the U-bend. In this regard, we have successfully extracted feces from SB for settling rate studies by squeezing the abdomen.

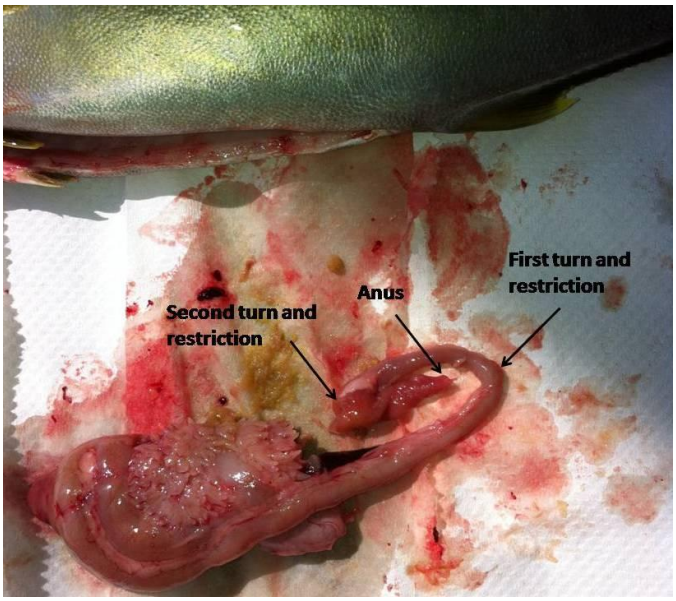

Figure 1. Gut morphology of YT showing the restriction at the posterior end.

The digestibility portion of the study was concluded after we determined that proposed methods would not work for this species and practical alternatives were not readily available to be incorporated into the scope of this project.

Task 3 – Provision of Fish for Commercial Feeding Trial in Mexico

Approximately 3500, 65g YT were successfully delivered to Pacifico Aquafarm's transport vessel in Oceanside harbor in October of 2012. The fish were arrived in good health at the cage site off of Ensenada, MX, thus fulfilling our requirements for this objective.

Additional Work

Although not part of HSWRI's subcontract agreement, we shipped approximately 1,100 juvenile YT to Austin, TX for TAMU researchers on August 28, 2012. These fish were

intended to be used for a disease challenge trial at TAMU testing disease resistance in YT when fed diets containing defatted meals from SG 3010 and Trifecta cultivars.

DISCUSSION AND CONCLUSIONS

The results of the feeding trial showed that FM can be partially replaced with meals from both the SG 3010 and Trifecta cultivars. Growth performance decreased in fish fed diets with increasing inclusion of 3010 meal above 50% of the total protein although the fish fed the diet with 60% of the total protein coming from 3010 meal still performed better than the FM diet. Fish fed all three diets containing Trifecta meal performed at least as well as those fed the FM diet, with fish fed the diets with 50 and 60% of the total protein coming from Trifecta performing significantly better overall. Based on the data collected from these trials, the 3010 and Trifecta defatted meals are prime candidates for incorporation into production diets for California yellowtail.

Digestibility of meals from the two cultivars was not determined because we were unable to collect feces using the traditional method of abdominal massage. We determined that the unique gut morphology of YT prevented collection of feces in this manner. More research will be necessary to determine a practical methodology for collecting feces from YT.

The commercial growout trial was successfully launched. The results of that trial and the disease challenge research are outside of the scope of the research contract reported on here.