

Replacement of Fishmeal and Fish Oil in the Diets of *Seriola rivoliana* Using Soy-based Proteins and Oils
Final Report

Summary

Since 2007 Kampachi Farms (formerly Kona Blue Water Farms) and the University of Nebraska Lincoln have been working to reduce the use of fishmeal and fish oil in the diets of *Seriola rivoliana*, by substituting soy-based proteins and oils. These efforts are intended to reduce the need for wild-caught fishmeal (FM) and fish oil (FO). Results of previous years' trials have shown that fish consuming a diet containing 40% soy protein concentrate (SPC) and a high omega-3 soy oil (STA), maintain growth rates comparable to fish eating the standard commercially prepared diet used to raise Kona Kampachi® (*Seriola rivoliana*) as long as supplementary taurine (a non-essential amino acid) is included in the SPC based diets. In 2011 two trials took place in order to continue this research and expand upon results.

The first of the two trials (designated as the “growout” trial) expanded upon a short-term study performed in 2010, when commercially viable growth rates and food conversion ratios (FCR) were achieved with a SPC/STA based diet. The 2011 growout trial used the same feed formulation for an eight month period, such that *S. rivoliana* could be reared to a marketable size ($\geq 1.5\text{kg}$) in land-based tanks. The growout trial then culminated in a consumer taste test. The consumer panel participated in a sensory evaluation to note detectable differences (if any) between the two types of fillets and to assess the palatability of SPC fed fish versus fish fed the standard control diet. In addition a questionnaire was included to garner consumer perceptions of aquaculture and soy-fed fish.

The second trial (referred to as the “fish oil reduction” trial) focused on further decreasing the amount of fish oil in Kampachi diets. All experimental feeds in this trial contained 40% SPC, with ascending levels of stearidonic acid (STA)-rich soybean oil. The results of this trial will indicate the changes of the fatty acid profiles of the fish (and thus marketability of health aspects of marine fish) and also if there may be any unforeseen fish health issues with the reduction in fish oil.

Results from both trials are outlined within this report. Overall health, growth, feed conversion ratios and final weights were used to compare the effectiveness of the soy-derived feed with that of the standard diet currently used by Kona Blue. The consumer panel taste test was conducted by Oregon State University's Food Innovation Center, whose responsible parties provided all taste test related results and statistical analyses, presented herein. Tissue analyses of fish fed all diets used throughout the year are also included.

Growout Trial

Introduction

Since 2007 Kampachi Farms (formerly Kona Blue Water Farms) and the University of Nebraska Lincoln have been working to reduce the use of fishmeal and fish oil in the diets of *Seriola rivoliana*, by substituting soy-based proteins and oils. Initial work examined the two ingredients of interest separately, first by replacing fish oil with modified soybean oil which contains high levels of the omega three fatty acid stearidonic acid (18:4, n-3, STA). If *S. rivoliana* could elongate STA into the highly desired EPA and/ or DHA the oil could be substituted into the fish's feed and still offer the nutritional value to the fish and the final consumer. It was determined that *S. rivoliana* could not elongate STA into EPA (or DHA), and the fatty acid profile of the fish closely correlated what is given in the diet.

To decrease fishmeal inclusion the fish's ability to utilize Soy Protein Concentrate (SPC, ~69% protein) as a primary protein source was determined. A step-wise reduction of fishmeal inclusion, and consequent increase in SPC inclusion was performed. Initial work indicated that no more than 20% of the diet could come from SPC without negatively impacting the growth rate and fish health, if taurine was not included as a dietary supplement. As work progressed, taurine was included in the SPC based diets (at a level of 5%) and this allowed for SPC inclusion rates of 40% of the weight of the diet without negatively impacting growth rates or food conversion ratios, when compared to commercially available feeds.

Using information from these baseline studies a 50% protein; 17% lipid formulation was developed. The diet contained 40% SPC and 12% fishmeal (dry weight) as the primary protein components. The lipid portion of the diet was composed of STA oil and fish oil in equal proportions. Taurine was included at 5% (dry weight). Fish fed this diet during a three month trial had faster growth rates than animals raised on a commercial feed. With this positive information the next step was to do a growout trial to examine a) if growth rate and food conversion ratios could be maintained at levels that would be acceptable commercially; b) if fish health could be maintained on a SPC based diets and c) what the economics of such a diet would be. The details of the methodology and results of this growout trial are presented here.

Methods

The grow-out trial took place during March 8-November 3, 2011 (a total of 238 days) at the Kona Blue Water Farms research facility. Forty-five juvenile *Seriola rivoliana* were randomly distributed to each of 6, 4-MT high-density polyethylene (HDPE) tanks, for a total of 270 fish. Average fish weight was determined for each tank during stocking, by weighing each group of fish and dividing the total biomass by the number of fish in that tank (weighing between 41.8-46.6 g each). Averages were then determined for each diet. All fish were 80 days old, coming from a single cohort.

Diet Formulation

The experimental diet was 50% protein and 17% lipid, formulated to contain 40% SPC and 12% fishmeal. The lipid portion of the diet was composed of equal parts fish oil and STA oil, and was added to the pellets after extrusion. Taurine was supplemented at a rate of 5%. The reference,

diet which had been used in offshore culture of *Seriola rivoliana* has varying protein and lipid levels based on pellet size. The ranges of the feed used in this study are shown in Table 1. Table 2 illustrates the ingredients of the experimental feed. Both diets were sinking pellets. During the course of the trial, the commercial feed was reformulated to contain a higher level of fishmeal, as decreased growth rates were seen in offshore cage culture. As a consequence the level of fishmeal in this trials reference diet also increased.

Table 1: Protein and lipid levels of reference diet

Commercial Reference Diet		
Size (mm)	Protein	Fat
3.0/4.0	48	24
6	47	26
9	45	26

Table 2: Diet formulation (lipid not included)

Dry Blend Recipes		
Ingredients	40% SPC w/ taurine	Dry blend %
Procon 2000 (SPC) 68.9/0.8	40.00	48.37%
Fish Meal, Anchovy 71.6/7.8	11.89	14.38%
Potato Starch	7.42	8.97%
Fish, HFPC 74.6/8	3.44	4.16%
Squid Meal 85.2/3.6	4.40	5.32%
Blood Meal SD 92/0.3	6.07	7.34%
Taurine	4.60	5.56%
Cellulose	0.00	0.00%
Soy Lecithin	1.50	1.81%
Vitamin Premix-F2	0.50	0.60%
Stay C - 35%	0.06	0.07%
Choline Chloride 60%	0.29	0.35%
Mineral Premix F-1	0.25	0.30%
Calcium Phosphate monobasic (21%P)	1.50	1.81%
Calcium Carbonate	0.01	0.01%
L-Lysine 95%	0.35	0.42%
MHA (methionine) 84%	0.38	0.46%
Ethoxyquin, SQ mixture 6	0.02	0.02%
Mold Inhibitor	0.02	0.02%
	82.70	100%
	82.88	0

System

All tanks were supplied with flow-through, ambient temperature filtered seawater (FSW) with flow rates of at least 45 L/minute. In order to prevent parasite infections, water was filtered to

5 μ , using a series of bag, sand and cartridge filters. Tanks were cleaned daily, and mortalities were removed, weighed and examined when found. After examination, mortalities were bagged, labeled and frozen, for later reconciliation during final data analysis.

Fish Feeding Protocol

Fish were allowed to acclimate in tanks for 2 days before the trial began. Diets were randomly assigned to each tank using a random number generator. Fish were fed by hand to visible satiation each day. The number of feeds per day changed as the fish grew, and began eating larger pellets. Feeds per day went as follows: 3 times per day while eating 2mm and 3mm pellets, 2 times per day while eating 6mm pellets, and once per day while eating 9mm pellets. Any uneaten pellets were collected and counted within 30 minutes of feeding. The amount of feed consumed each day was recorded (total amount consumed minus dry weight of any uneaten pellets). The weight of a dry pellet was determined by counting out 100 pellets, weighing those 100 pellets and then dividing the total weight by 100. This sampling was done three times, and the mean of those three samples was used to determine final average pellet weight.

Growth Performance

Fish were weighed approximately once per month during the length of the trial, for a total of 9 weighing events. Fish were not fed on the morning of weighing days. Average individual fish weight per tank was calculated by weighing the entire population of a tank and dividing the total by the number of fish present.

The following equations were used to determine average weight gain, average percent weight gain, average daily growth (in grams) and feed conversion ratios (FCRs):

Average weight gain = Final average fish weight (g) – Initial average fish weight (g)

Average % weight gain = [(Final average fish weight – Initial average fish weight) / Initial average fish weight] x 100.

Average daily growth (g) = (Final average fish weight (g) – Initial average fish weight (g)) / Number of days in trial

FCR = average individual feed intake (dry weight) (g) / average individual live weight gain (g).

Fish Removal

The lengthiness of the growout trial made it necessary to periodically cull fish from tanks during weighing events as their total biomass increased, in order to keep densities at a manageable level. Because supplemental oxygen (O₂) was not available, stocking protocol designates that no more than 5 kg of fish are housed per metric ton of water. Trial tanks had a total volume of 4 MT each, thus the maximum allowable biomass per tank at any time was 20 kg. Fish that were removed were weighed and euthanized, and feed adjustments were made according to the procedure below.

Feed Adjustments

When calculating the final FCR the amount of feed consumed by fish that had died (or were culled as stated above) was removed, such that the FCR would not be erroneously high. This was done by determining individual feed intake; totaling the amount of feed eaten by a tank from the start of the trial, to the day before the mortality was found (assuming that the fish did not consume food on the day it died). Dividing the total feed eaten by the number of animals in the tank to determine average feed intake/ fish. The result of this calculation was referred to as “feed removed,” and was subtracted from total feed intake.

Statistical Analysis

Differences in growth performance (average individual fish weights, total weight gain, percent weight gain, average growth rate and final FCRs) among groups were tested using 2-sample t-tests (MiniTab Statistical Software).

Tissue Analysis

Of primary interest is the change in fish fatty acid profile due to the lipid component of the diet. Fish were sampled twice during the course of this study; first after 78 days of feeding and again at the end of the trial 238 days of feeding. Three fish from each replicate were used for sampling, this provided a pooled sample of 9 fish per treatment.

Figure 1: Comparison of fatty profiles after 78 days of feeding

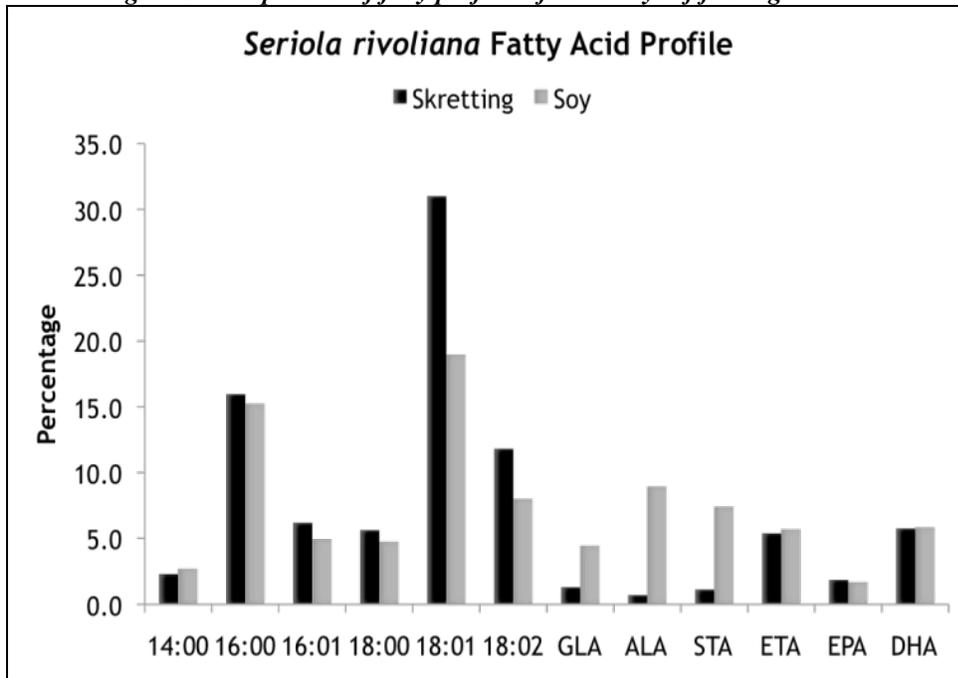
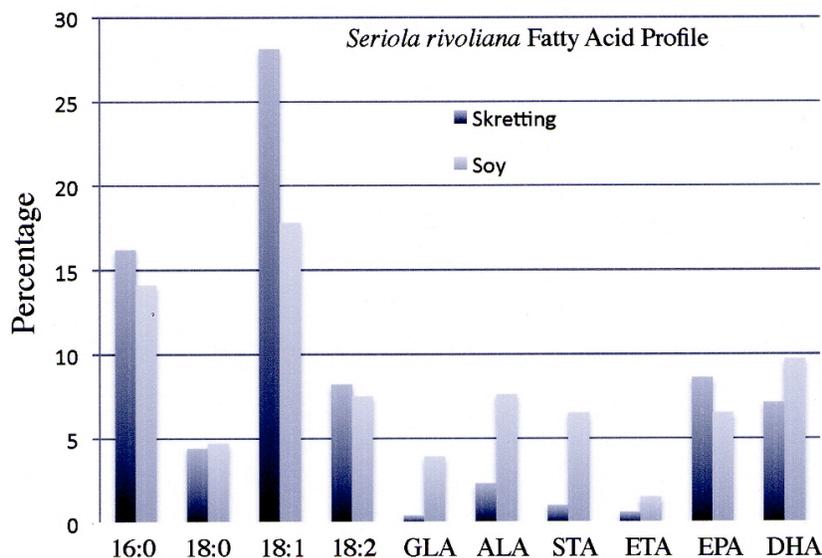


Figure 2: Comparison of fatty acid profiles after 238 days



Histological Analysis

As previously mentioned fish were removed from culture tanks periodically to avoid overcrowding. During the third weighing event (approximately 90 days into the trial) the fish that were culled were used for histological examination of the fore, middle and hind gut to learn if there were differences in gut morphology based on diet type. Three fish from each treatment were sampled and three sections of the gut (fore, middle and hind) were taken from each fish. 10% Buffered formalin was used as a fixative. Pathologists at the University of California Davis noted mild to moderate enteritis in all fish examined. There was no clear difference between treatment groups.

Results

Growth performance

There was no significant difference in final FCRs among diets; however, there was a significant difference in final average fish weight, weight gain, percent weight gain, and daily growth rate (all p-values <0.05) by diet. Overall, fish fed the soy-based diet were larger and had gained more weight and consumed more feed. This data is summarized in Table 3. The growth curve for the entirety of the trial is shown in Figure 2. The final FCR and total weight gain (with standard error bars) are displayed graphically in Figure 3. The soy-fed fish had higher levels in percent weight gain, and average daily growth rate as well

Table 3: Compilation of final data illustrating changes in weight, weight gain and FCR

Diet	Mean Start Weight (g) (±SD)	Mean Final Weight (g) (±SD.)	Mean Weight Gain (g) (±SD.)	% Weight Gain	Mean daily growth (g/day)	FCR
Soy	46.6 (±1.85)	1710.2 (±119.73)	1663.6 (±120.42)	3569.29	6.99	1.45
Control	42.1 (±0.69)	1175.8 (±12.87)	1134.0 (±11.82)	2712.22	4.76	1.31



Figure 3: Graph showing growth curves at each weighing event, for each diet over the course of the trial. The star indicates when the formulation changes to include more fishmeal

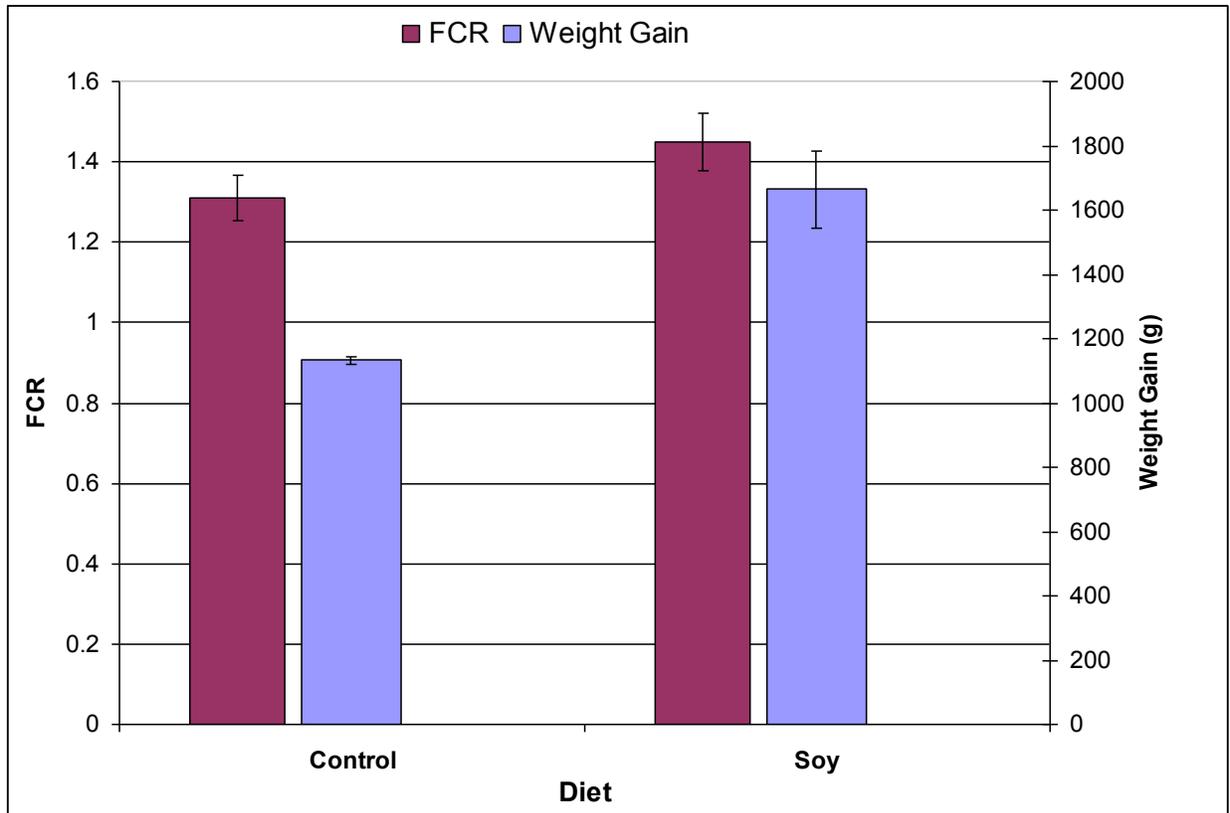


Figure 4: Graph showing final average weight gain by diet as well as final FCRs by diet (including feed adjustments for mortalities).

Consumer Panel Sensory Test

The consumer taste test and all related analyses were conducted by Oregon State University’s Food Innovation Center in Portland, Oregon. The test took place on November 9, 2011 in two parts: the first part (i.e. the “difference” test) was designed to assess the consumer’s ability to detect an overall difference among samples. The second part of the test (designated as the “preference test”) was structured to better understand consumer perception of aquacultured fish, and to get product feedback in the attributes of appearance, color, aroma, flavor, texture, moisture, oil content and purchase intent. This was done using a series of 28 hedonically- and demographically-based questions. A 2oz serving of sashimi was provided for each sample. In addition a questionnaire was included to learn the consumers’ opinion about aquaculture and using soy-based products in fish feed.

Consumers were recruited as follows:

- Male/Female as it falls
- Ages 21 and older
- Must eat sashimi frequently, preferably hamachi or yellowtail
- Allergy, diet, participation and security screen
- High school graduate

– No affiliation with food, advertising or research

The taste test took place at the Food Innovation Center on November 9th, 2011, from 9am to 5pm. Data was collected using touch screen monitors with the Compusense® Data Acquisition System. A total of 114 consumers were tested. Sample presentation was randomized and tested in multiple sample presentation.

Kampachi fillets were received one day prior to testing and were stored in a 36 degree refrigerator. The fillets were cut on the bias sashimi style and two pieces each were served on six inch plates on a tray with optional soy sauce and wasabi on the side. Samples were coded with 3-digit numbers and served side by side. Sample presentation was randomized over the entire test to avoid order effects.

Part 1: Difference Test, “Triangle” Testing

The difference test utilized triangle testing, in which tasters were presented with 3 pieces of raw fillet; two of which were the same, and one of which was different. After sampling each fillet, participants were then asked to determine which of the 3 pieces was dissimilar.

Part 2: Preference Test

Fillets were sampled by participants and responses to questions were collected via multiple choice and range scales. These questions evaluated sensory attributes such as fillet appearance, color, aroma and texture; as well as factors affecting consumer purchasing choices and product appeal.

Figure 5: Photo of preference test presentation



Results

Difference test

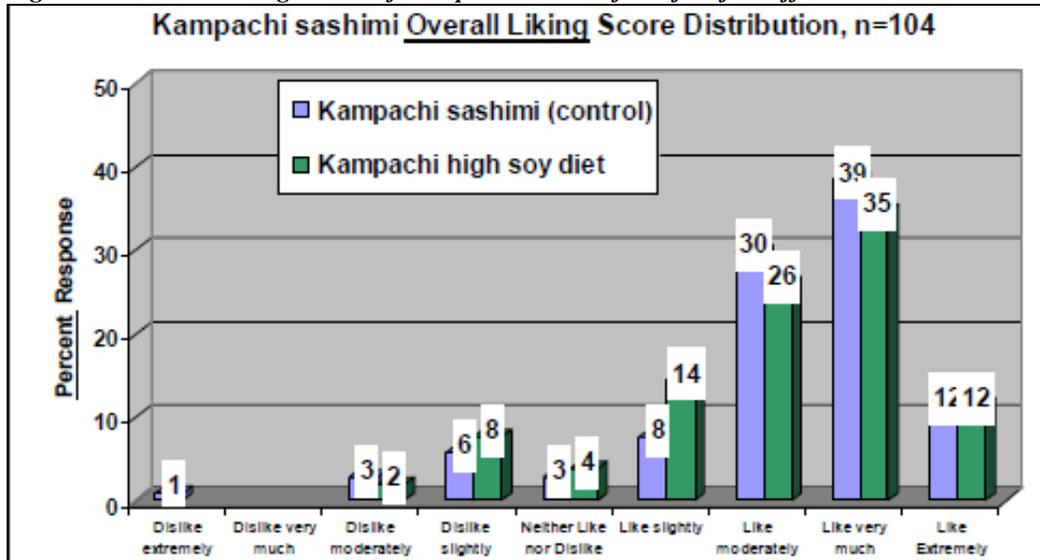
Statistically, consumers could not tell a difference between the Kampachi fed the control fishmeal diet vs. the Kampachi fed the SPC diet. Triangle testing was used to determine whether

a difference existed. Three consumers of the 10 tested chose the correct sample, which is the same number as could have guessed by chance alone (probability=1/3).

Preference test

- The two samples were rated statistically equally for the **overall liking** of the product. This is to be expected after difference testing showed that consumers could not tell a difference between the two products when tested blind. Both products were rated at “like moderately” on average.
- About 81% of the consumers tested rated the Kampachi fed the control diet at “like moderately” to “like extremely” for the attribute overall liking. 73% felt the same of the Kampachi fed the high soy diet (Figure 5)

Figure 6: "Overall Liking" score of Kampachi sashimi from fish fed different diets



The results of the testing are summarized below. A full report from the Food Innovation Center is available as an appendix to this report.

- *Statistically*, consumers could not tell a difference between the Kampachi fed the control fishmeal diet vs. the Kampachi fed the high soy diet. The two samples were rated statistically equally for overall liking of the product at “like moderately” on average.
- 70% of the consumers tested found both products to be “somewhat” to “very appealing” in the attribute appearance liking. The Kampachi fed the high soy diet was rated as having a fishier aroma than the Kampachi fed the control fishmeal diet.
- About 80% of the consumers tested rated both Kampachi products between “like moderately” to “like extremely” for the attribute flavor. The high soy diet Kampachi was rated statistically more firm in texture than the control diet Kampachi.
- 86% of the consumers tested rated the two samples as “just about right” for the amount of moisture and over 75% felt the same about the oil content.

- Purchase intent was rated statistically equally among the two samples and was rated closest to “would probably buy” on average. About 60% of the consumers tested “would probably buy” or “would definitely buy” both the Kampachi fed the control diet as well as the Kampachi fed the high soy diet.
- A significant majority (71%) of consumers felt that the sustainability of a seafood factors into their purchase decision and they are aware that some species are endangered. Only 21% of these consumers like the idea of purchasing aquacultured fish. 41% dislike the idea and 38% are neither for or against it.
- Almost half of the participants (46%) were unsure, while 44% were certain that aquaculture can be a solution to the threat of overfishing that endangers ocean ecosystems. Only 10% felt that aquaculture could not be a solution.
- Almost 90% of these participants felt that the diet of farmed fish is of concern to them. Quality, safety and sustainability continue to be the most important characteristics of aquacultured fish, while origin and price are less so.
- 50% of these consumers get their information about aquaculture from the media, 41% from environmental organizations, 38% don't notice this type of information and 22% from their seafood retailer.

Fish Oil Reduction Trial

Methods

The grow-out trial took place during August 6-October 11, 2011 (a total of 66 days) at the Kona Blue Water Farms research facility. Thirty juvenile Kona Kampachi® (weighing between 222.6-228.4 g each) were randomly distributed to each of 9, 4-MT high-density polyethylene (HDPE) tanks, for a total of 270 fish. Average fish weight was determined for each tank during stocking, by weighing each group of fish and dividing the total biomass by the number of fish in that tank. Averages were then determined for each diet.

Diet Formulation

The 3 experimental diets were based on the same dry matter formulation as the growout trial (40% SPC and 12% FM). While the total amount of lipid in each diet was the same, the lipid portions were made up of varying combinations of stearidonic acid (STA)-rich soybean oil and fish oil (FO), at percentages of 50/50 STA:FO, 75/25 STA:FO and 90/10 STA:FO.

System

All tanks were supplied with flow-through, ambient temperature filtered seawater (FSW) with flow rates of at least 45 L/minute. In order to prevent parasite infections of trial fish, water filtration was to 5 μ , and used a series of bag, sand and cartridge filters. Tanks were cleaned daily, and mortalities were removed, weighed and examined when found. After examination, mortalities were bagged, labeled and frozen, for later reconciliation during final data analysis.

Fish Feeding Protocol

Fish were allowed to acclimate in tanks for one day before the trial began. Diets were randomly assigned to each tank using a random number generator. Fish were fed by hand to visible satiation with 6mm pellets two times per day during the course of the trial. Any uneaten pellets were collected and counted within 30 minutes of feeding. The amount of feed consumed each day was recorded (total amount consumed minus dry weight of any uneaten pellets). The weight of a dry pellet was determined by counting out 100 pellets, weighing those 100 pellets and then dividing the total weight by 100. This sampling was done three times, and the mean of those three samples was used to determine final average pellet weight.

Growth Performance

Fish were weighed approximately once per month during the length of the trial, for a total of 3 weighing events. Fish were not fed on the morning of weighing days. Average individual fish weight per tank was calculated by weighing the entire population of a tank and dividing the total by the number of fish present.

The following equations were used to determine average weight gain, average percent weight gain, average daily growth (in grams) and feed conversion ratios (FCRs):

Average weight gain = Final average fish weight (g) – Initial average fish weight (g)

Average % weight gain = [(Final average fish weight – Initial average fish weight) / Initial average fish weight] x 100.

Average daily growth (g) = (Final average fish weight (g) – Initial average fish weight (g)) / Number of days in trial

FCR = average individual feed intake (dry weight) (g) / average individual live weight gain (g).

Feed Adjustments

When calculating the final FCR the amount of feed consumed by fish that had died was removed, such that the FCR would not be erroneously high. This was done by determining individual feed intake; totaling the amount of feed eaten by a tank from the start of the trial, to the day before the mortality was found (assuming that the fish did not consume food on the day it died). This feed total was then divided by the total number of fish in the tank (to obtain average individual feed consumption), and multiplied by the number of mortalities that day. The result of this calculation was referred to as “feed removed,” and was subtracted from total feed intake.

Replicate Removal: Diet 90/10

During the trial, one of the three replicates from the 90/10 diet was found to have contracted *Cryptocaryon*. Due to the structure of the feed trial, treatment for the parasite was not administered, because fish would not be fed during that time and final growth data would be skewed. Therefore, fish in this tank were euthanized on October 5, 2011 and data for that replicate was not included in final analyses. The entire trial was ended on October 11, 2011.

Statistical Analysis

Differences in growth performance (average individual fish weights, total weight gain, percent weight gain, average growth rate and final FCRs) among groups were tested using one-way analysis of variance (MiniTab Statistical Software).

Results

Fatty acid profiles (%) of the fish sampled across treatments is shown in **Table 4**. The data revealed that further displacement of fish oil below 50% led to a reduction in both EPA and DHA. Although the 50/50 diet had the highest overall growth rates, there was no significant difference ($p>0.05$) among diets for any of the variables measured: final individual weight, total weight gain, percent weight gain, daily growth rate and FCRs. Means for these variables (with standard deviation) can be found in **Table 5**. Growth curves by diet are shown in **Figure 7**, and means for FCR and total weight gain are shown graphically in **Figure 8** and **Figure 9**.

Table 4: Fatty acid profile of flesh across fish oil reduction levels

Treatment	GLA	ALA	STA	ETA	EPA	DHA
90:10	7.4±0.5	12.7±0.7	10.2±0.8	1.1±0.8	2.8±0.1	3.2±0.3
75:25	6.4±0.3	11.2±0.4	9.0±0.4	1.0±0.1	3.7±0.1	3.9±0.5
50:50	4.4±0.2	8.5±0.3	6.9±0.2	1.2±0.0	6.0±0.1	6.3±0.5

Table 5: Mean final weight data (with standard deviation) and final FCRs (adjusted for mortality) are shown for all experimental diets in the trial.

Diet	Mean Start Weight (g)	Mean End Weight (g)	Mean Total Weight Gain (g)	Mean % Weight Gain (g)	Daily growth rate	FCR
50/50	225.3 (±4.7)	843.94 (±13.5)	618.60 (±16.9)	274.69 (±12.4)	9.37 (±0.26)	1.12
75/25	222.6 (±3.9)	815.09 (±11.8)	592.53 (±15.4)	266.37 (±11.3)	8.98 (±0.23)	1.20
90/10	228.4 (±6.2)	831.48 (±2.6)	602.20 (±11.2)	262.92 (±14.7)	9.12 (±0.17)	1.15

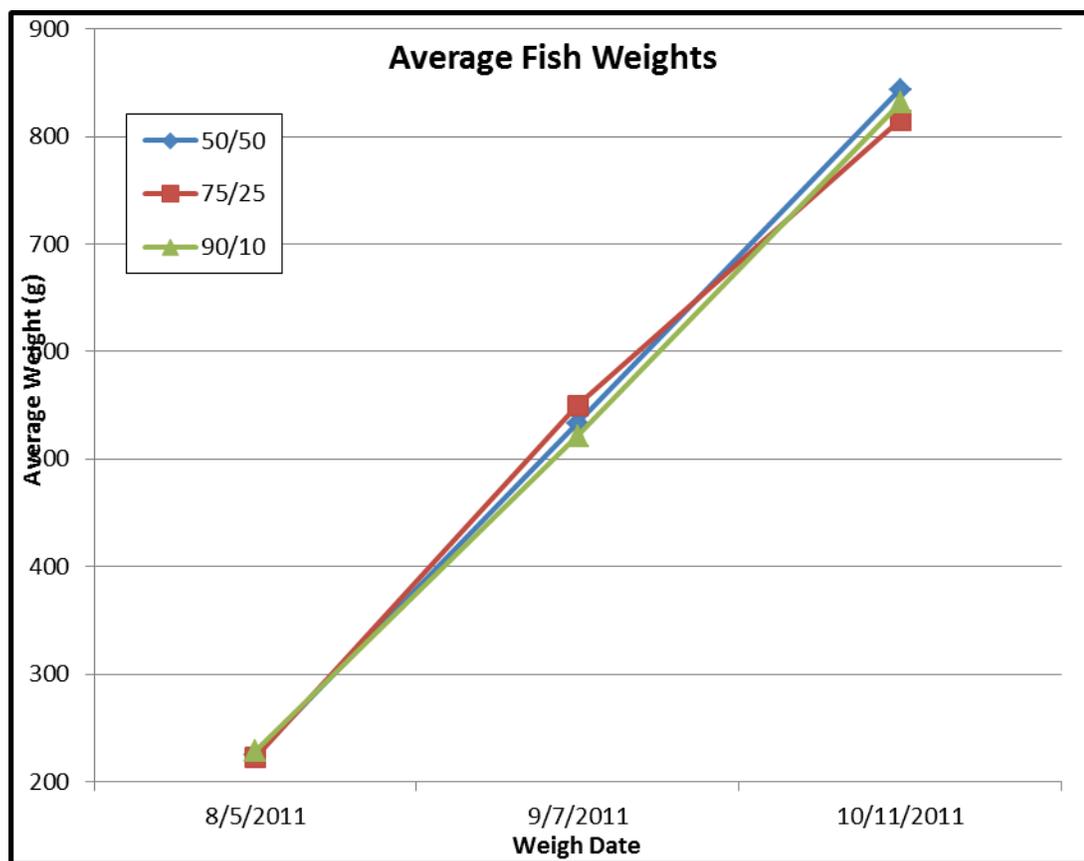


Figure 7: Growth chart showing average individual fish weights by diet, for all 3 weighing events.

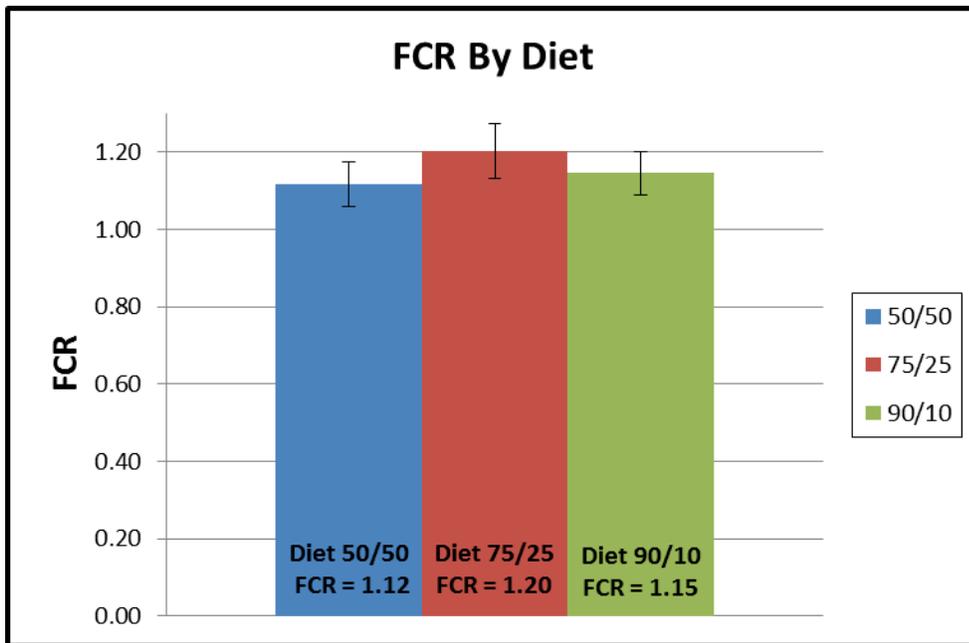


Figure 8: Mean FCR by diet, including error bars. Numerical values are shown on each column.

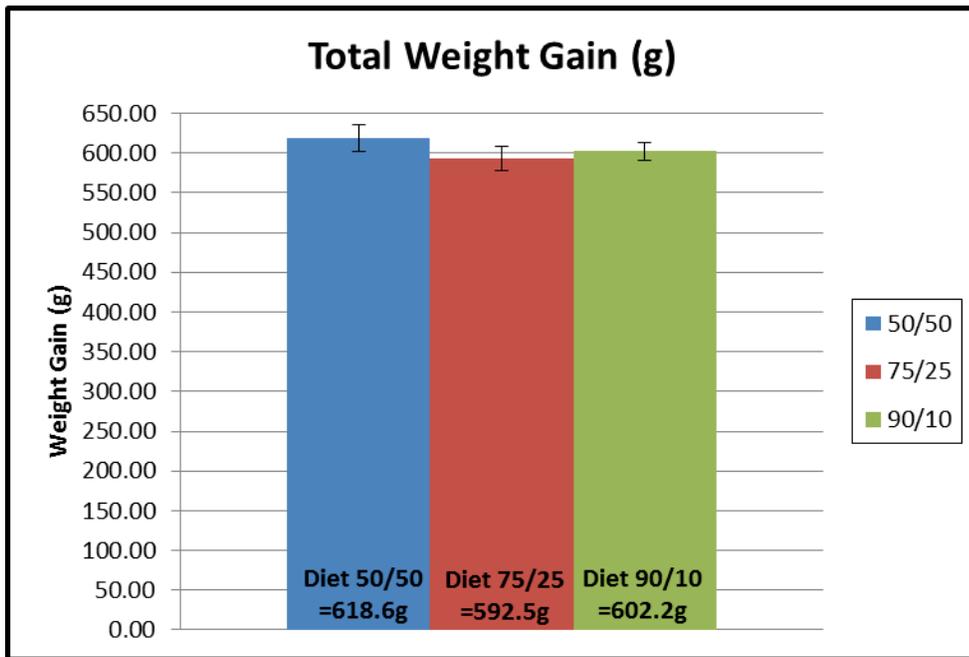


Figure 9: Final mean total weight gain by diet, including error bars. Numerical values for diets are shown on each column.