

Project Title: Testing replacement of fishmeal and fish oil in *Seriola rivoliana* (Kona Kampachi™) diets with soy-based protein and oil

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Objective 1: Evaluation of inclusion rates of algal pastes feasibility of utilizing an algal-based feedstock for taurine production

Previously we have shown that in absence of taurine displacing fishmeal with soybean protein concentrate (SPC) greater than 10% leads to poor growth in *S. rivoliana*. The goal of this component of the program is to ascertain if an algal-paste inclusion, in which the algal cells can simultaneously supply both taurine requirement and aid in further displacement of fish oil in the diet of this high end fish. The current soybean-based diet formulation includes 40% SPC, and 50% high omega-3 fatty acid soybean oil. This translates to roughly 11% fishmeal remaining in the diet, and 50% fish oil. Hence, the question being addressed is, can an algal paste with omega-3 fatty acids and taurine, be incorporated to completely displace the remaining fish components of the feed. Thereby creating a feed with 100% terrestrial-based ingredients, with the majority of the protein and oil soybean based.

Our previous report communicated the ingredients in the respective formulated diets. Pellets from both formulations were analyzed for amino acid profile, total oil/protein and peroxide value. The algal paste is dried *Nannochloropsis* sp. It is noteworthy that the algal paste formulation displaced 100% of the fish-based protein. While the amino acid profiles, and total protein and crude fat levels were approximately equivalent with one of the commercial diets analyzed, taurine level in the commercial formulation was 1.1%, as compared to our test formulation of 5% and the peroxide value was significantly higher in our test formulation, 2.5 meq/kg and 25 meq/kg for the commercial (EWOS) and test formulations, respectively.

The feeding trial was initiated on June 6. Each treatment, 40% SPC, 40% SPC plus alga and two commercial diets, is set up in triplicate, with 45 fish per tank. Fish were 71 days old at the time of feeding, with an individual average weight of 29.8 ± 0.8 grams. Following 28 days of growth average fish weights were 121.5 g for our SPC formulation and 93, and 145.2 g for commercial diets, Expalsa and EOWS, respectively. Mean fish weight fed the SPC formulation with *Nannochloropsis* inclusion was 105.6 gr. However, in the fish fed the SPC plus algal inclusion eye disorders were observed, and this

treatment was subsequently aborted. Given the analyses of the feed indicated substantial equivalence among the test feeds and the commercial EWOS, with exceptions being taurine level and oxidative nature of the lipid, we hypothesize the underlying problem being digestibility of the *Nannochloropsis* paste inclusion. Moreover, our analysis of the flesh lipids indicated carotenoid supplementation in the EWOS feed treatment. Indeed direct analysis of the EWOS feed confirmed the carotenoid supplementation.

Fatty acid profile of the flesh was monitored in the fish fed the respective diets via GC following 28 days and 78 days of feeding (Note: only EWOS and SPC diet without algal paste inclusion at 78 day time point). The data is summarized in Tables 1 & 2 below. In addition proximate analysis was conducted on a subset of the fish following 78 days of feeding, and the data is summarized in Table 3.

We currently are formulating SPC diets with altering levels of taurine from 2.5% up to 7.5% to allow us to gain insight if the 5% taurine supplementation currently used is in excess, or if higher incorporation rates may translate to growth rate enhancement. This feeding trial will be initiated within the next two weeks and we will communicate our findings in the next reporting period.

Table 1: Fatty acid profile of flesh following 28 days of feeding

Diet	18:1	18:2	GLA	ALA	STA	EPA	DHA
SPC	16.6±1.5	9.1±0.2	4.6±0.1	7.8±0.2	6.5±0.3	7.0±0.4	7.2±1.4
SPC+Nano	14.2±1.7	9.2±0.3	3.7±0.3	7.0±0.5	5.1±0.4	8.2±0.3	6.5±0.9
EWOS	25.4±0.2	10.4±0.2	0.3±0.0	2.6±0.0	1.0±0.0	7.9±0.2	7.2±0.3

Numbers within each column refer to mean % of the respective fatty acid ± standard deviation about the mean. Note: no variation was observed in the monitored saturated fatty acids 14:0, 16:0 or 18:0.

Table 2: Fatty acid profile of flesh following 78 days of feeding

Diet	18:1	18:2	GLA	ALA	STA	EPA	DHA
SPC	18.9±0.4	9.7±0.4	4.8±0.1	8.7±0.3	6.5±0.3	6.3±0.4	6.6±0.2
EWOS	24.1±0.7	8.7±0.4	0.3±0.0	2.2±0.1	1.3±0.1	8.9±0.3	8.1±0.5

Numbers within each column refer to mean % of the respective fatty acid ± standard deviation about the mean. Note: no variation was observed in the monitored saturated fatty acids 14:0, 16:0 or 18:0.

Table 3: Proximate analysis of flesh

Diet	Protein	Fat	Ash
SPC	18.9±0.3	11.9±1.4	2.4±0.3
EWOS	18.2±0.5	9.1±0.4	2.6±0.6

Numbers within each column are mean %± standard deviation about the mean