Soy Aquaculture Alliance & United Soybean Board
From baitfish feeding to balanced nutrition: A leap forward in sustainable tuna farming

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Final Report - Technical

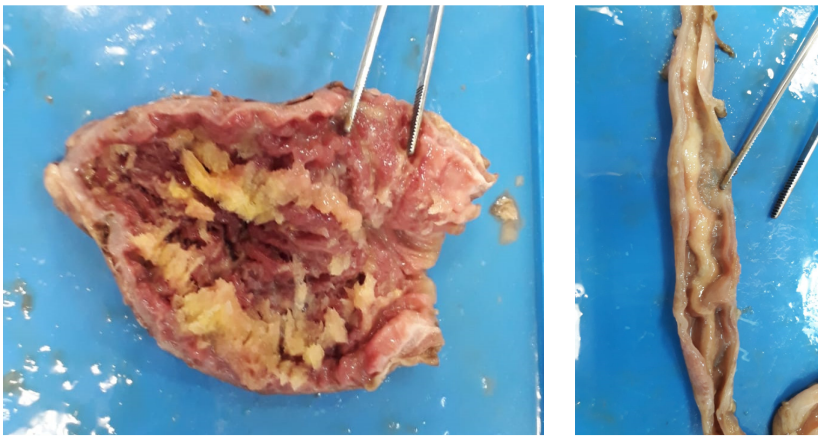

Figure 1. Yellowfin tuna stomach and proximal intestine of fish fed compound, soy-based diets. No signs of enteritis or inflammation were detected. Positive results of acid proteases in the stomach and alkaline proteases in pyloric caecum and intestine. Photos Manuel Yufera

1. Feeding trial – Long-term feeding (3 months) with compound diets composed of high levels of soy-based ingredients including protein concentrates and soy oil has been proven feasible with no loss of tuna performance or quality of the end-product. A feeding strategy which utilizes a diet with high level of soy oil during an initial extended fattening period, followed by a short finishing period with high levels of marine oils has been validated. This strategy not only results in higher consumption of soy oil while maintaining optimal growth but, it also has the potential to reduce feed cost by ~30% over a complete fattening cycle (8 months). After the finishing period, the nutritive value for the consumer of fish fed the compound diets was superior to that of fish fed baitfish based on overall DHA and EPA enrichment in formula-fed fish.

Composition	Diet 1	Diet 2	Diet 3	Bait
		%		
Dry matter	49.9	49.2	48.8	28.6
Protein	22.3	22.9	22.0	17.2
Lipid	17.7	16.7	17.8	10.8
Ash	3.3	3.8	3.7	4.4
Essential Amino Acids				
Arginine	1.2	1.4	1.3	-
Histidine	0.4	0.5	0.4	-
Isoleucine	0.7	0.8	0.8	-
Leucine	1.4	1.6	1.5	-
Lysine	1.3	1.4	1.3	-
Methionine	0.5	0.5	0.5	-
Phenylalanine	0.8	0.9	0.8	-
Threonine	0.8	0.8	0.8	-
Tryptophan	0.2	0.2	0.2	-
Valine	1.0	1.1	1.0	-
Long chain PUFAS				
Arachidonic	0.05	0.08	0.09	-
Eicosapentaenoic (EPA)	0.59	1.17	1.21	1.13
Docosahexaenoic (DHA)	0.39	0.79	0.75	0.63

Figure 2. Composition of experimental diets. 20 yellowfin tuna received diet 1 with high soy oil for 9 weeks. Then, fish were switched for 2 weeks to a ~100% marine oil diet (diet 2). Finally, fish were fed diet 3, which was identical to diet 2 except it had 0.01% yttrium oxide as inert marker, for one more week. A different group of 20 fish received a baitfish diet for the duration of the experiment (13 weeks). Tissue samples were secured at the beginning of the experiment and right after each dietary change.

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strategy not only results in higher consumption of soy oil while maintaining optimal growth but, it also has the potential to reduce feed cost by ~30% over a complete fattening cycle (8 months).

2. Quality of resulting fillets (tuna steaks) – After the finishing period, the amino acid and fatty acid profile of the resulting tuna steaks had the same nutritive value for the human consumer as fish fed 100% marine oil. This value was superior to that of fish fed baitfish based on overall DHA and EPA enrichment in formula-fed fish.

Color: The Meat Science Laboratory of the Department of Animal Science at Texas A&M University conducted color assessment on fresh tuna steaks samples ~72 hours after harvest using a CR-400 Minolta Chroma meter. This instrument is specifically designed to evaluate the color of meat products with a high degree of accuracy and reliability by converting color spectra into a tridimensional scale and making the evaluation of color appearance an objective task. Results from hue analyses indicate that steak color from fish fed the compound feed placed consistently farther into the +a scale as well as into the dark L scale; whereas, those obtained from fish fed sardines were of a paler hue characterized by migration of the color spectrum to the L and +b axis. Therefore, steaks originating in fish fed the compound diets appear more agreeable to the sashimi/sushi market preference.

Residual mercury: The Trace Element Research Laboratory at Texas A&M University conducted total mercury quantification on tuna steaks after wet digestion in nitric acid via inductively coupled plasma mass spectroscopy (Perkin-Elmer-Elan 9000). Mercury is a heavy metal that reaches the atmosphere through emissions from coal-burning power plants. Bacterial conversion into methyl mercury is followed by bio-concentration through the food chain and accumulation in long-lived apex predators such as tuna. Methyl-mercury binds to tissue protein and becomes almost permanently attached to muscle tissue and other organs. However, this label can be diluted with the deposition of new, mercury-free tissue.

Mercury is a potent neurotoxin that poses substantial risks to developing fetuses and young children. Therefore, the World Health Organization (WHO) has defined maximum weekly intake levels for general population and pregnant woman at 3.3 and 1.6 µg/kg of body weight, respectively. Average total mercury in yellowfin tuna muscle (ppm = mg/kg) were 0.4 for baitfish-fed fish and only 0.2 for formula-fed fish. These results indicate that the mercury uptake risks may become smaller for humans consuming formula-fed fish versus sardine-fed fish.

Shelf-life: Eurofins Scientific (Des Moines, IA) conducted histamine evaluation of tuna steaks samples ~72 hours from harvest (t = 0) and seven days after (maintained under refrigeration 4.4 C = 40 F). Histamine fish poisoning is among the most common toxicities related to fish ingestion, constituting almost 40% of all seafood-related foodborne illnesses reported to the US Centers for Disease Control and Prevention where histamine fish poisoning results from the consumption of inadequately preserved and improperly refrigerated fish. Histamine is generated during decomposition of scombroid fish (tunas) by bacteria with high activities of histidine decarboxylase. The USDA – FDA considers that fish with 50 ppm histidine begin decomposition and 500 ppm of histamine is a hazardous level if consumed. Histamine levels were below detection upon arrival but climbed to 3.2 and 8.1 ppm in sardine and formula-fed tuna, respectively, at day 7. Although the 8.1 ppm level is still far below the 50 ppm that the USDA – FDA considers as the onset of decomposition, the formula-fed fish had a significantly lower histidine value which is indicative of an extended shelf-life.

Resistance to oxidation: Eurofins Scientific (Des Moines, IA) conducted antioxidant evaluations of tuna steaks. Fish were harvested in Panama on Dec 19, 2018 and placed in -80 C freezer before transport to the US in a cryo-shipper which maintains that temperature by pre-cooling the shipper with liquid nitrogen. Time 0 is when thawed samples were first analyzed (Dec 26). Peroxide values were measured again after three weeks had elapsed. After arrival at Eurofins samples were kept under refrigeration at 4.4 C.

Shelf life for harvested tuna is defined by the length of time that fish may be stored without becoming unfit for use. In the case of the sushi/sashimi grade – the highest quality fish a restaurant offers, and the one they feel confident can be eaten raw. This only lasts for about 3 days until it must be cooked before consumption. Extending the shelf life for even one or two days adds significant value to the product.

Together with histamine levels, the degree of oxidation determines the possible length of shelf life in tuna slices. Peroxide value is used to determine the rancidity of a sample containing fat or oil subject to oxidation. Fresh oils have a peroxide value of <10 mEq/Kg while peroxide values in the 30-40 mEq/Kg range are associated with a rancid taste. In all cases tuna steaks could be classified as fresh according with the <10 mEq/Kg threshold. However, streaks from formula-fed fish always had smaller peroxide values and deteriorated little between time 0 and 7. In contrast, steaks from sardine-fed fish were close to the 10 mEq limit by day 7.

Blind sensory evaluation: The Food Science Department at the University of Arkansas in Fayetteville (Fayetteville, AK) conducted the blind sensory evaluation. Analyses were performed by a panel of ten spectrum method trained panelists, with randomized sample presentation and sashimi slices (raw, never frozen) at time 0 (Dec 26, 2018) and seven days after under refrigeration (Jan 2, ~4.4 C).

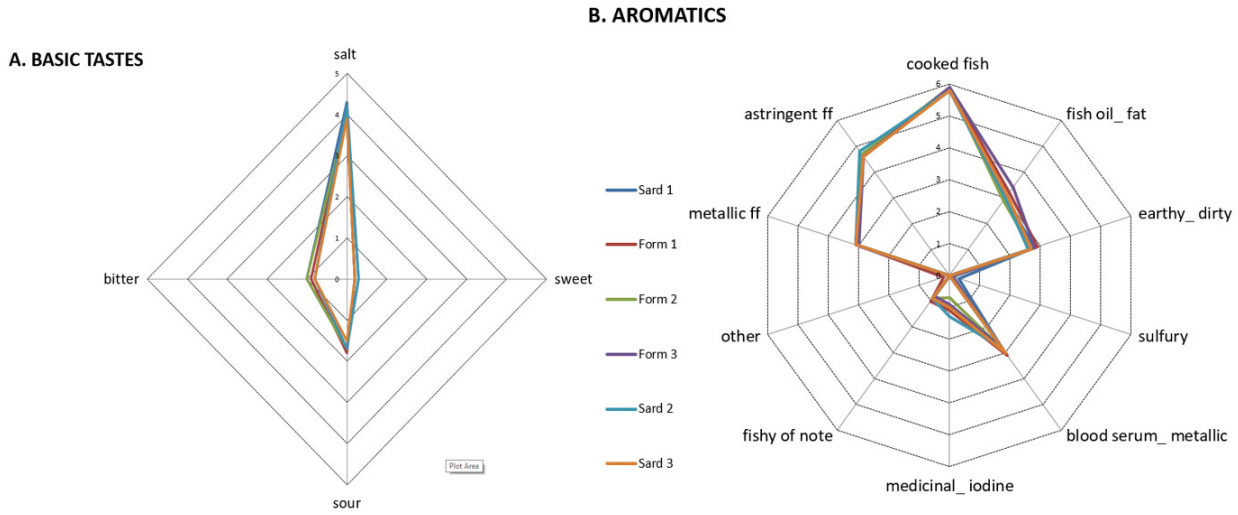

Figure 3. Blind sensory evaluation of stakes from tuna fed compound feed (Form) or sardines at T = 0

Results of the organoleptic analysis can be seen in Figure 3, above. Panel A presents the basic taste parameters reported by the testers with panel B showing the aromatics. No significant differences were detected between any of the seven diets regarding basic tastes or aromatics ($P < 0.05$). These tests were repeated on Jan 2, 2018 with very similar results. On the last day, an electronic nose (e-nose) was used to identify specific odor components and to analyze its chemical makeup (identify it). The trained panelists also performed an aroma test to corroborate results of the e-nose (Figure 4 below).

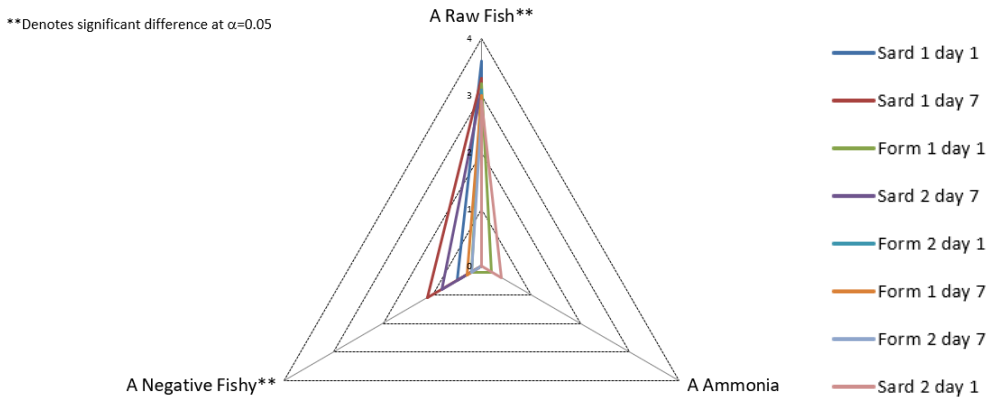

Figure 4. Electronic nose and aroma evaluation of steaks from tuna fed sausages (From) or sardines at T = 7

Both the e-nose and the trained panelists detected a significant departure in aroma from steaks (T = 0 vs. T = 7) collected from sardine-fed fish towards a negative fishy odor. These change in aroma was not apparent in steaks obtained from fish fed the formulated diets.

Taken together, these results indicate that stakes from fish fed the formulated diet had improved color and extended shelf life, as suggested by the various analyses conducted. However, the reader should not ascribe absolute certainty to these analyses because the sample size (n = 5) over a population of 439 fish is insufficient to make inferences about this population or to test the null hypothesis that the mean scores of sardine-fed tuna versus formula-fed fish on a specific test do not differ.

3. NMR-based metabolomics – Preliminary results (Figure 5, below) indicate a high similarity in metabolic profiles between tuna fed baitfish and those fed the compound diets.

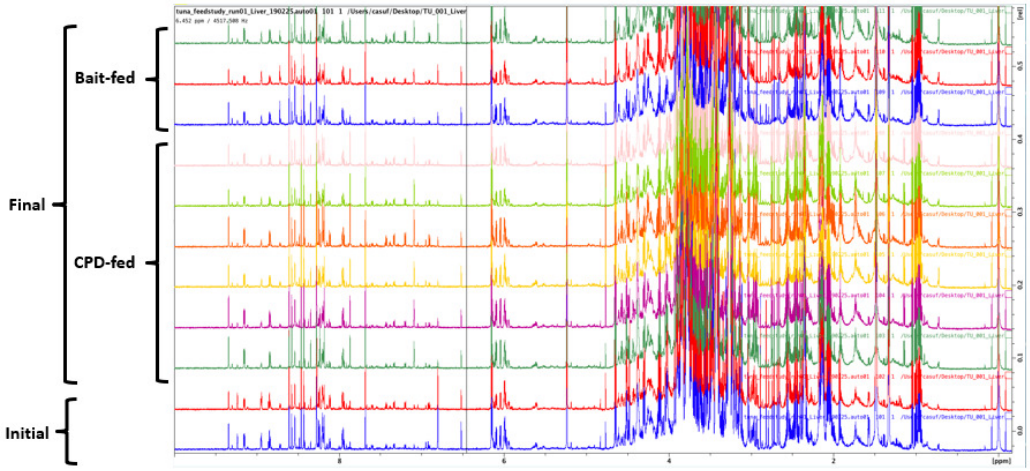

Figure 5. ^{1}H-NMR spectra of liver tissue extracts (polar extracts) for the different dietary treatments and sampling times appeared to be similar. Initial: n=2; Bait: n=3; CPD: n=6

The metabolic differences include the amino acids alanine, glycine, proline and threonine and metabolites such as glutathione, 2-aminobutyrate, methionine sulfoxide, lactate, taurine and succinate. A more comprehensive metabolic analysis will be included in the manuscript submitted to a peer-reviewed scientific journal in the coming months.