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| Organization: | Ichthus Unlimited, LLC |
| Project Title: | From baitfish feeding to balanced nutrition: A leap forward in sustainable tuna farming |
| Project Number: | 1830-352-0501-K |

Project Status - Completed

Key Activities

- 1. Feeding trial with juvenile yellowfin tuna (YFT, *Thunnus albacares*)
- 2. NMR spectroscopy and metabolomics
- 3. Preliminary economic evaluation
- 4. Sensory evaluation of resulting products

Key accomplishments

- 1. Long-term feeding 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
- 2. 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)
- 3. After the finishing period, the amino acid and fatty acid profile of the resulting tuna steaks had the same nutritive value for the 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
- 4. The sensory/organoleptic properties were not different in formula-fed fish in contrast to the baitfish-fed fish. Texture on the counter was slightly superior as well as color and resistance to oxidation. Feeding compound feeds to tuna also resulted in a decreased concentration of peroxides and histamines in tuna steaks. The combined reduction of these molecules in muscle tissue increases the shelf life of the end-product. Mercury analysis indicated that animals fed the compound feed had reduced levels of methyl-mercury in their tissues
- 5. NMR-based metabolomics shows a high similarity in metabolic profiles between tuna fed baitfish and those fed the compound diets. 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
- 6. Preliminary economics:
 - a. Polls conducted on recurring customers of sushi/sashimi restaurants indicate that they considered these products as nutritious (protein-rich), tasty and healthy (n-3 fatty acid-rich). Clients were concerned, however, with mercury levels in tuna products and try to follow World Health Organization recommendations to limit the consumption of tuna to one ration per week. The demonstrated reduction in mercury for the formula-fed fish makes it possible to increase this recommended intake to 2 or 3 rations per week, effectively multiplying the sushi/sashimi market
 - b. The value chain of sushi/sashimi restaurants operates under a strict cold-chain (never-frozen) and a limited number of days during which these products can be consumed raw before they need to be cooked for health reasons. A major component of the decision to cook raw fish is based on the concentration of histamines (scombrotoxins) which are indicative of product deterioration. Tissues from formula-fed fish have reduced levels of histamines increasing the

elapsed time during which a sushi/sashimi restaurant can safely serve these products in a raw form

Project progress: the project has been completed

Quantifiable results

Did this project meet the intended Key Performance Indicators (KPIs)?

Key performance indicators:

USB focus addressed: a) increase of soybean sales by creating new demand for soy protein and b) increase of soy oil sales by creating new demand.

The development and commercialization of compound tuna diets is an altogether new market that has the potential to absorb over 2.4 million bushels of soy per year. Results from the present project demonstrate that high-value soy protein isolates and concentrates can be used long-term in tuna diets providing not only an excellent amino acid profile but feed functionality as well. In addition, the use of soy oil in these diets at relatively high levels increases the use of US soy over other sources in aqua feed diets.

Results will be incorporated in a pilot production for a feeding demonstration to be conducted in Europe in 2020 in collaboration with tuna farmers of the Mediterranean Sea. Support from SAA for this trial is kindly requested.

Expected Outputs/Deliverables

- Increases in US soybean sales and soybean oil can be expected as early as Q1 2021 for the European market
- A tailored tuna aquaculture session was organized at the World Aquaculture Society Meeting in New Orleans, LA (Mar 2019) and included several presentations on tuna nutrition. Results from the trials in Panama were presented at this meeting
- Results are also contributing to one Ph.D. dissertation at Texas A&M University (Vladimir Muñoz). This document and associated information will be transformed into scientific manuscripts for publication in peer-reviewed journals

Outputs/Deliverables

• Similar presentations are planned for the Aquaculture Europe 2019, Berlin, Germany (Oct 2019)

Describe any unforeseen events or circumstances that may have affected project timeline, costs, or deliverables (if applicable.)

Unforeseen event affecting projects: Operating in Panama was costly and logistically challenging. Ichthus Unlimited is starting to build a tuna hatchery facility in San Diego, CA which will greatly reduce cost and international export issues. This facility should be fully operational by the end on 2019.

Despite difficulties with international freight, weather events affecting power lines, riots blocking the only highway between Panama City and the Achotines Laboratory, and other challenges, the team was able to complete the experimental components, secure samples and ship them back to the US in a timely manner. The US government shutdown at the beginning of the year delayed some of the results also as the NMR machine (for metabolomic research) is shared with the federal entity NIST and sample processing is still backlogged.

What, if any, follow-up steps are required to capture benefits for all US soybean farmers?

Follow up steps to capture benefits to US soybean farmers

The demonstration trial in Europe described above is an excellent way to capitalize on the results for the following reasons: the Mediterranean basin is home to over 20 major tuna farms all avid for a compound feed. The Mediterranean Sea, in contrast to the sardine-rich California waters is a very oligotrophic (low primary productivity) environment with very scarce fisheries. In Ensenada, Mexico, farmers have several options of fresh baitfish they can use to feed their tuna. Only about 15% of the baitfish is imported from international sources. In Europe, over 95% of the baitfish used in tuna ranching is imported and used frozen. Thus, the high cost and shortage of options makes the compound tuna feed a more urgent need for Mediterranean farmers.

Presentation of the results of this research in European venues is essential to disseminate results and generate interest on compound tuna feeds, and US soybeans and oil.

List any relevant performance metrics not captured in KPI's.

Several press releases on tuna aquaculture, including this research, have inundated the specialized media (undercurrents, aquafeeds, hatchery magazine) and more interviews are being requested. In these, our acknowledgment and gratitude for the SAA-USB support has been and will continue to be very evident.

For the first time, the characterization of the digestive physiology of juvenile yellowfin tuna was made possible through the collaboration of IU with scientists from the Spanish National Research Council - Institute of Marine Sciences of Andalusia