Visit to the laboratory of Professor Douglas Tocher (Institute of Aquaculture, University of Stirling, Scotland)

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2012/750

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NON-TECHNICAL SUMMARY

PROJECT NO: 2012/750: Visit to the laboratory of Professor Douglas Tocher (Institute of Aquaculture, University of Stirling, Scotland) to undertake collaborative research into the lipid and polyunsaturated fatty acid (PUFA) metabolism of Southern Bluefin Tuna

PRINCIPAL INVESTIGATOR: Andrew Scholefield

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(PROJECT) OBJECTIVES OF RESEARCH TRAVEL GRANT/ INDUSTRY BURSARY

1. Investigate the incorporation of various PUFA into cellular lipids in the SBT cell line. In particular, to test the incorporation α-linolenic acid (ALA) and linoleic acid (LNA), the predominant PUFA in vegetable oils (the main oils replacing fish oil), and eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), the predominant PUFA in fish oils.
2. Investigate the *de novo* synthesis of EPA and DHA from ALA in the SBT cell line. Fish species differ in their capacity for *de novo* synthesis of EPA/DHA.
3. Investigate the mitochondrial and peroxisomal β-oxidation of ALA, LNA, EPA and DHA by the SBT cell line.

NON TECHNICAL SUMMARY:

(PROJECT) OUTPUTS DEVELOPED AS RESULT OF TRAVEL GRANT/ INDUSTRY BURSARY:

A paper documenting the research conducted at the University of Stirling is currently being written (please see attached). It is expected to be submitted by December 2013.

ABOUT THE PROJECT/ACTIVITY

BACKGROUND AND NEED

The purpose of this grant was to undertake collaborative research into the lipid and polyunsaturated fatty acid (PUFA) metabolism of Southern Bluefin Tuna (SBT). This research was conducted using an SBT cell line that was recently produced. We took an *in vitro* approach because it is currently impossible to undertake statistically robust feeding trials with either hatchery-reared or wild-caught SBT. This is due to the limited number of the former and the strict quota limiting the capture of the latter. Our experiments using the *in vitro* approach were designed to provide data to inform the design of future feeding trials with live SBT. This project followed an international trend towards increasing fish oil replacement in feeds for farmed fish to improve the sustainability of the target industry. Very little is known about dietary fish oil replacement in Bluefin tuna species so the purpose of this research was to obtain data which may be used to predict the impacts of fish oil replacement in future manufactured feeds for farmed SBT. Since it was logistically difficult and expensive to obtain such data from traditional feeding trials with SBT, our approach using the SBT cell lines offered a more rapid and cost-effective alternative.

RESULTS

Desaturation and elongation of [1-14C] fatty acid substrates

The cells fed [1-14C] 18:3 n-3 converted approximately 13% of this substrate to desaturation/elongation products (Fig. 2A). The majority of this conversion was elongation to 20:3 n-3, but there was some desaturation to 18:4 n-3, and approximately 1% was both desaturated and elongated to yield 20:4 n-3 (Fig. 2A). Similarly, the cells supplemented with [1-14C] 18:2 n-6 showed evidence of both Δ6 desaturation and elovl5 activity but the amount of substrate converted was significantly lower (5.6% conversion; $P < 0.001$). Approximately 2.6% of the radioactivity recovered was from the Δ6 desaturation product, 18:3 n-6 and 3.0% was from the elongation product, 20:2 n-6 (Fig. 2B). Interestingly, the cells converted significantly more 20:5 n-3 than either of the C18 substrates with nearly 17% conversion (Fig. 2C, $P \leq 0.011$), all of which was seen as the elongation product 22:5 n-3.

Oxidation of [1-14C] fatty acid substrates

The oxidation of the [1-14C] fatty acid substrates is shown in Fig. 3. The lowest level of oxidation was seen for the saturated fatty acid 18:0. The 18:1 n-9 and 18:2 n-6 fatty acids were slightly higher, and the n-3 fatty acids showed the highest levels of oxidation. Interestingly, the highest level of cellular oxidation products and most

evidence of complete oxidation was seen in the cells fed 20:5 n-3 which was significantly higher than all other substrates ($p < 0.01$), with the exception of 18:3 n-3 ($p = 0.071$).

Incorporation of [1-^{14}C] fatty acid substrates into cellular lipids

Most treatments displayed a similar incorporation of [1-^{14}C] fatty acid substrates into the different polar lipid classes. For example, Phosphatidylcholine (PC) represented the greatest proportion of radioactivity recovered in every treatment (Fig. 4). This was presumably due to the abundance of PC in normal cells. Before publication, a mass profile of polar lipids from untreated cells will be created and the relative abundances of each polar lipid class will calculated by dividing the observed radioactivity in each lipid class by the amount of that class present in normal cells. This will correct for the 'mass effect' that causes a similar profile to be seen for all the fatty acid supplements.

INDUSTRY IMPACT

PROJECT OUTCOMES (THAT INITIATED CHANGE IN INDUSTRY)

The results generated through this project have not currently initiated a change in industry primarily due to the nature of the research. It is expected that the data generated regarding fatty acid metabolism in SBT will contribute to understanding of SBT nutrition but any impact will only be realised in the medium to long term. The high growth of cells fed EPA combined with high oxidation of EPA suggests that this fatty acid may be a growth enhancer. However, this would need to be validated *in vivo.*

SUMMARY OF CHANGE IN INDUSTRY

The nature of the results generated cause all impact to only be realised in the medium to long term.

WHAT FUTURE AND ONGOING CHANGES ARE EXPECTED?

Future changes in the industry could include modification of feeds to increase levels of EPA. Our data suggests that this modification may increase growth rates and n-3 LC-PUFA content of the fish flesh. However, it should be noted that no increases in flesh levels of DHA can be expected from the addition of EPA.

WHAT BARRIERS ARE THERE FOR CHANGES TO OCCUR?

The main barrier is the fact that this work was conducted *in vitro*. This is, of course, due to the high cost of feeding trials for SBT. However, if a higher level of growth may be expected in fish fed EPA enriched diets, then feeding trials may prove to be profitable in the long-term.

IF NOT ALREADY HAPPENING, WHEN WILL THE CHANGES OCCUR?

The changes to industry practice will depend on the willingness of industry participants to conduct feeding trials. However, if feeding trials are approved, and are successful, then modifications may be expected within 3-4 years.

WHAT IS THE LIKELIHOOD THAT THESE CHANGES WILL OCCUR?

At this stage, there is only a 10-15% chance that this work will result in a direct change to industry practice. However, since SBT are an extremely valuable species, even modest growth increases have the potential to increase profitability. Therefore with continuing research and communication of results to industry stakeholders, the likelihood that changes will occur may be much higher.

WHAT BARRIERS ARE THERE TO DOPTION OF THESE CHANGES AND WHAT ACTION COULD BE TAKEN TO OVERCOME THESE?

The continuation of my PhD research will focus on further elucidating the dynamics of fatty acid metabolism in SBT. Further results and discovery of additional factors that influence growth and fatty acid composition of SBT will provide more evidence and further motivation for industry to conduct feeding trials with SBT.

COMMUNICATION OF PROJECT/EXTENSION ACTIVITIES

WHAT IS THE OUTPUT THAT NEEDS TO BE COMMUNICATED?

SBT cells are heavily influenced by the fatty acids in their feeds. High levels of EPA can result in modest increases in growth of SBT cells in culture which may translate to increases in growth in farmed fish.

WHO IS/ARE THE TARGET AUDIENCE/S?

The target audiences are the industry stakeholders involved in SBT ranching and culture and other researchers investigating fatty acid and lipid metabolism in farmed fish.

WHAT ARE THE KEY MESSAGES?

- All fatty acids fed to SBT cells are incorporated into cellular lipids. Therefore the precise fatty acid composition of SBT feeds will be reflected in the composition of the flesh. This has influence on the texture, flavor and consistency of the flesh.
- Tuna cells show an increase in growth rate when fed EPA enriched diets. The oxidation of EPA in these cells is also significantly increased compared to any other diet. Therefore increasing EPA concentrations in feeds for farmed fish may result in increased growth rates.

WHAT IS THE CALL TO ACTION?

The data collected in this lab visit increased the current understanding of fatty acid uptake and metabolism in SBT. As future results come to light, this work may contribute to optimisation of fatty acid content for commercial SBT aquafeeds.

COMMUNICATION CHANNELS

Channel	*Who by*	*When*
Presentations to industry	Andrew Scholefield or Kathy Schuller	Depending on industry
Conference presentation	Kathy Schuller	International symposium on Fish Nutrition and Feeding, May 2014
Conference presentation	Andrew Scholefield	World Aquaculture Conference, June 2014
Conference presentation	Andrew Scholefield	International society for the study of fatty acids and lipids congress, July 2014

LESSONS LEARNED AND RECOMMENDED IMPROVEMENTS

WHAT IS YOUR FEEDBACK?

The biggest challenge in conducting this research was actually culturing the SBT cells themselves. The care required to grow them to a capacity sufficient for experiments was significant and often proved problematic. I sent some cells over before I arrived and nobody in the lab had any success keeping them alive for more

than 1 month. However, once I arrived, I was able to use my experience of working with this cell line to ensure survival and some advice from more experienced members of the lab in Scotland assisted in maintaining the health of the cells for the 5 months that I was there. This combined experience overcame the majority of the difficulties faced by working with this cell line. If further work was to be done in this area, a high level of competence in cell culture will be required along with additional information and experience of the SBT cell line specifically.

FURTHER ACTION REQUIRED IN REGARDS TO COMMERCIALISATION?
None at this stage.

ACKNOWLEDGEMENTS
Thank you to Prof. Douglas Tocher for hosting me and to Dr James Dick and Dr Matthew Sprague for their assistance with the laboratory work.

APPENDIX (IF APPLICABLE)
Please see attached for a draft of the manuscript that will be submitted as a result of this work.