Small grant award SG416: Molecular analysis of Vitamin D utilisation in triploid Atlantic salmon.

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Background and Rationale

The sterile physiological state of triploid Atlantic salmon mitigates the risk of reproductively competent escapees, whilst lack of energy diverted towards maturation harbours the potential for improved growth (Piferrer et al. 2009). Faster growth and shorted cycle time improves seawater farming efficacy and overall sustainability of the industry. Yet, despite the potential benefits to the Scottish aquaculture sector triploid usage has been hindered by a historically high prevalence of deformities. In comparison to diploids, triploid salmon have a greater dietary mineral requirement (Fjelldal et al. 2016; Taylor et al. 2015). In particular, phosphorus (P) is of considerable importance to bone homeostasis and increased dietary levels are necessary to reduce the prevalence of deformities (Fjelldal et al. 2016). Phosphorus is a costly feed additive and has considerable environmental implications in freshwater (i.e. eutrophication) and is a rate limiting factor for production consents and biomass capacity. To this end, as part of the SALMOTRIP+ project, our research has begun to focus on the use of micronutrients to improve dietary P assimilation and reduce bone malformations. Vitamin D is an essential micronutrient that mediates dietary P and calcium (Ca) absorption in the intestine via Vitamin D receptor (VDR) and Retinoid X receptor (RXR). In addition VDR directly regulates the transcriptome via VDR response elements in the promoters of specific genes, including genes involved in bone mineralisation and homeostasis. As a result Vitamin D is likely to have a profound impact both directly and indirectly on bone health.

In a recently completed trial the effects of increased levels of dietary vitamin D (D3) on growth and prevalence of skeletal malformations was investigated over a 12 week period. Triploid

Atlantic salmon were fed 6 different dietary levels of Vitamin D3 in a classic dose-response regression design. Results showed an improvement in whole body mineral retention, a reduced prevalence and severity of vertebral malformations (Fig. 1), an increase in fillet D3 retention, and a linear increase in growth rate when fed diets with incremental vitamin D inclusion. These significant improvements are hypothesised to be as a consequence of Vitamin D3's role in facilitating the uptake of P and Ca in the intestine and direct actions on bone homeostasis, as well as the actions of vitamin D on myogenasis. Before developing this research further and direct implementation in commercial diets it is essential to understand the fundamental molecular mechanisms underpinning vitamin D metabolism.

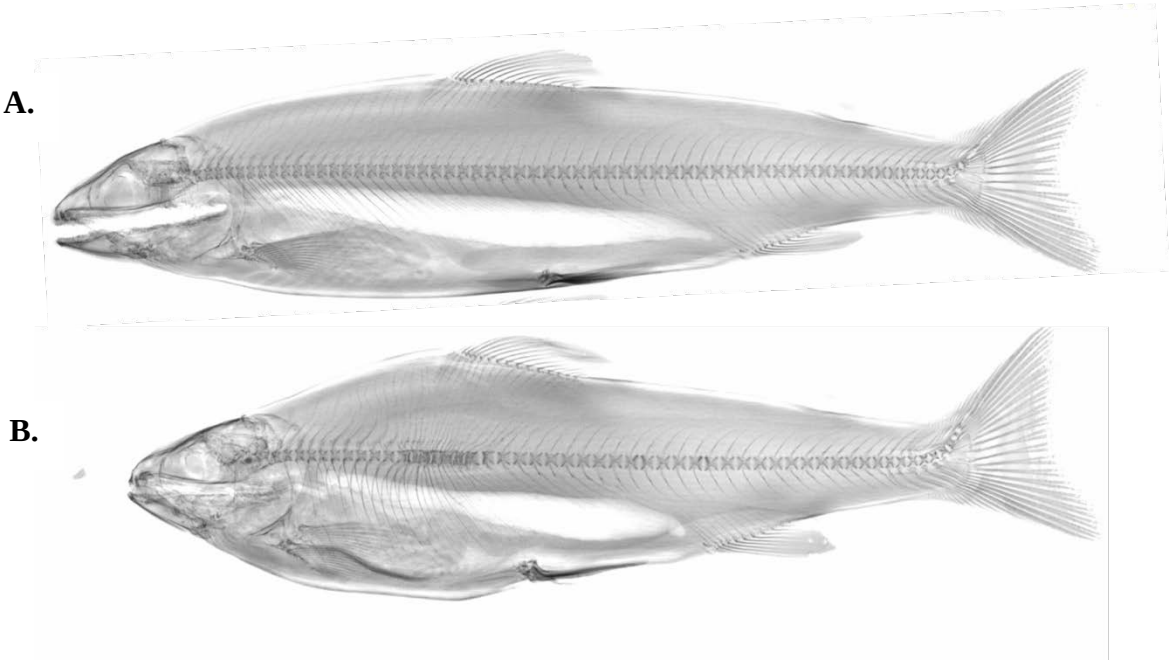

Figure 1. X-ray image of Atlantic salmon smolts from this study: A, an individual fed a diet with high vitamin D content and with no visible skeletal malformations and B, an individual fed a low vitamin D diet displaying severe skeletal malformations in region 2 of the vertebrae.

Objectives:

The primary objective of this study, supported by MASTS funding, is to utilise gene expression in order characterise the molecular mechanisms underpinning vitamin D mediated regulation of bone health and growth. This includes RNA to cDNA processing from tissue samples (378)

previously collected and qPCR for genes in the pathways described above. In particular this study investigates how increased dietary vitamin D3 impacts gene expression in the following pathways in a variety of tissue types:

- Molecular pathways of Vitamin D absorption and hydroxylation
- Define changes in P and Ca handling and subsequent mineral composition
- Characterise expression of genes involved in bone homeostasis
- Examine impact on genes involved in myogenesis

Relevance to Masts:

A key priority for MASTS is the continued growth and improve sustainability of the Scottish aquaculture industry. Use of triploid Atlantic salmon is a fundamental component of this as their sterile status has substantial benefits with regard to increased environmental and economic sustainability. However, to date the optimisation of triploids diets has relied heavily on the addition of dietary P. This study investigates how the inclusion of Vitamin D can optimise absorption of essential minerals such as P and results will provide a comprehensive understanding of the molecular mechanism underpinning such processes. An enhanced understanding of this will have a direct impact in the production of triploid specific diets leading to reduced P excretion into the environment and improving health and welfare during production. Collectively this data generation will form essential components in improving the sustainability within the industry.

Impacts and Outputs:

In conjunction with phenotypic and metabolic data already generated, gene pathway characterisation from this study will provide a fundamental insight into the effects Vitamin D at a molecular level leading to:

- **Comprehensive understanding of the mechanisms underpinning growth and bone homeostasis.**

- **Production of a high impact scientific publication and dissemination at scientific meetings**
- **As part of the SALMOTRIP+ project will contribute to development of a Triploid Farming Handbook Code of Best Practice**

In terms of wider industrial applications the inclusion of vitamin D in Atlantic salmon feed has a wide variety of potential benefits including:

- **Implementation of vitamin D supplementation in triploid salmon feeds at a commercial scale.**
- **Enhanced revenue and welfare for fish farmers through improved growth and reduced deformity.**
- **Increased environmental sustainability and reduced costs associated with lower reliance on phosphorus.**
- **Vitamin D inclusion in salmon feed may have a positive impact on human health as fillet yield increased with dietary inclusion**
- **Further research will forge further collaboration between academia and industry**

Collectively, this will improve customer perception of triploid production and the aquaculture industry and contribute towards greater economic and environmental sustainability within the aquaculture sector.