



# 13F516 - The anti-inflammatory and microbial modulating effects of marine derived laminarin and omega-3 fatty acids on inflammatory bowel disease in an experimental porcine model.

## Final Report

## **SUMMARY**

The objective of this project was to determine if natural bioactives with anti-inflammatory activity could alleviate the recurrence of inflammatory conditions in humans, such as Inflammatory Bowel Disease. The bioactives tested were laminarin, chitosan and omega-3 fish oil. All three bioactives are well characterised in the literature as having anti-inflammatory activity and can be sustainably harvested from nature. The pig was the experimental model. The bioactives were given either directly to the pig as a model for the adult human or to the dam during the suckling period as a model for the child. IBD was induced in the pigs by orally administering Sodium Dextran Sulphate once daily for 4 days. Faecal scores and health checks were recorded twice daily. Serological markers of epithelial permeability, colonic histopathology and immune cell infiltrate profiles were assessed. Treatments that provided a significant improvement in severity of colitis were selected for detailed transcriptome analysis and microbiome profiling. Of the three bioactives that were initially compared in the pig, laminarin showed the greatest potential as it was associated with lower pathology scores and expression of gene pathways associated with Tissue Repair and Organismal Injury. To lengthen the time period of supplementation and to supplement the animals while the gastrointestinal tract was developing/maturing, Laminarin was supplemented to suckling sows. Piglets were challenged with DSS after 5 weeks of supplementation. A similar effect was observed with a proportion of animals displaying evidence of reduced pathology and tissue repair. Unfortunately, due to the fact that only a proportion of the animals responded to laminarin and that their response was not deemed a sufficient reduction in inflammation and tissue damage, it was concluded that these three bioactives that have anti-inflammatory effects in gastrointestinal tracts with mild inflammation, would not provide sufficient protection for acute cases of Irritable Bowel Syndrome.

## **KEYWORDS**

Inflammation; Omega-3; Laminarin.

## **ACRONYM**

Marine-IBD

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## **PUBLICATION DATE**

October 2022.

## Section 1 - Research Approach & Results

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### Start Date

01 September 2014

### End Date

30 April 2017

### Research Programme

Food Institutional Research Measure

### TRL Scale

TRL 5: Technology validated in relevant environment

### NRPE Priority area Food

for Health

### Total DAFM Award

€493,064.00

### Total Project Expenditure

€412,310.53

### Rationale for undertaking the Research

Internationally, the incidence of IBD is increasing in both adults and children, and a recent report shows that is unfortunately occurring in the Irish population at a worryingly accelerating rate. The National Children's Research Centre runs a prospective study on paediatric IBD (DOCHAS) which has identified a threefold increase in incidence since 2001. A key challenge in IBD is to resolve the exaggerated inflammatory response provoked through dysregulated interactions of the host immune system with enteric microorganisms. Inflammatory models of IBD provide a useful approach with which to test possible avenues towards addressing these problems. Previously, we demonstrated a capacity for seaweed derived polysaccharides, chitosan and Omega-3 fish oil to influence the gut-associated immune status. These effects include improvements in maternally derived immunoglobulins, reductions in maternal and progeny faecal coliforms, and improvements in appetite and bodyweight gain of offspring following weaning. Furthermore, our research group has shown in a porcine model that direct consumption of a brown algae extract broadly down-regulates the colonic gene expression of the interleukin-17 cytokines. The use of omega-3 as an anti-inflammatory biomolecule in IBD has been tested in murine models and has shown promising effects in alleviating the pathology associated with colitis, including modifications to adaptive immune responses in colonic mucosal surfaces. Hence the overall objective of this project was to determine if these molecules, could alleviate the symptoms of experimentally induced IBD in the pig model.

### Methodology

The pig is considered a suitable model for studies on IBD due to the anatomical and physiological similarities with humans. The use of dextran sulphate (DS) to induce colitis results in an inflammatory response in the colon which bears many of the hallmarks the histopathology associated with IBD. Hence, an experimental porcine model of IBD was used, whereby IBD was induced with an oral gavage of dextran sulphate (DS) once daily for four days. The bioactives were given either directly to the pig as a model for the adult human, or to the dam during the suckling period as a model for the human child. Experimental procedures were approved under University College Dublin

Animal Research Ethics Committee (AREC-14-14-O'Doherty) and were conducted in accordance with Irish legislation (SI no. 534/2012) and the EU directive 2010/63/EU for animal experimentation. Health and faecal scores were observed twice daily. At 7 days post-DS administration, pigs were euthanized with sodium pentobarbitone. Serum was collected for xylose and lactulose assessment by spectroscopy and cytokines by ELISA. Macroscopic assessment of the distal gastrointestinal tract was carried out and assigned a pathology score. The colon was emptied of digesta, weighed and tissue from the second loop of the proximal colon was fixed for microscopic histopathology. For downstream transcriptomic analysis, tissues were excised from the proximal colon, and stored in RNAlater™, prior to RNA sequencing/Nanotechnology. To facilitate gut microbiota profiling, digesta samples taken adjacent to the colonic lumen were snap frozen at -80°C for subsequent microbial DNA extraction by analysing the V3 region of the 16SrRNA. Measures of immune cell infiltrate, colonocyte damage, lesions, odema, goblet cells, eosinophils, epithelial cells and macrophages were determined by analysing 15 sections of tissue per animals, sampled 100µm apart. Data was analysed with SAS. Regression and correlation analyses were performed between the different variables.

## **Project Results**

There were significant correlations observed between faecal scores, histopathology and transcriptome analysis in all experimental groups such that animals with higher faecal scores (more diarrhoea) had greater evidence of colonic ulceration and significant up-regulation of networks of genes involved in inflammation and immune cell function. The dextran sulphate treatment consistently resulted in a range of symptoms reflective of IBD. These animals had higher faecal scores which was reflective of more diarrhoea in this group. There was increased gut permeability and mucosal damage as evidenced by increased immune cell infiltrate, colonocyte damage, lesions, bleeding, odema, increased goblet cells and eosinophils and decreased epithelial cells in the histology sections. There was a significant increase in the expression of genes involved in the pathways of cell-to-cell signalling, Immune cell trafficking and inflammation with significant changes to components of the NFK-beta, Toll like receptor, JAK STAT, TNF and T-Cell receptor signalling pathways.

When the bioactives were administered directly to the pig, they only had very slight effects on the DSS challenge and these effects were variable between the bioactives. Gut permeability and plasma cytokines were similar between all of the groups. Similar patterns of gross and histological damage were observed. However, a number of the animals that had been supplemented with laminarin had evidence of tissue repair, with an increase in the number of genes assigned to the pathways of Tissue Repair and Organismal Injury. While this was not statistically evident in the histology sections, none of the animals in the laminarin supplemented group had the higher scores in pathology (4-5). Hence, while the response was variable, it was concluded that laminarin was the most promising bioactive, but that it was possible that the supplementation period was too short and that it would be beneficial to give the supplement while the gastrointestinal tract was developing.

Hence, laminarin was subsequently administered to suckling sows and DSS was administered after 5 weeks of laminarin supplementation. This time period is a time of great elasticity of cellular development in the gastrointestinal tract. While there was no statistical difference in faecal score following the DSS challenge, there was again evidence of tissue repair in the histopathological analysis which was highly correlated with the expression levels of the two tissue repair genes highlighted in the top five differentially expressed genes (FGF2 and EGF). This was supported with the network 'tissue repair' highlighted in the top five networks in the network analysis.

While the two experiments cannot be statistically compared, it seems that maternal supplementation with laminarin prior to DSS challenge enhance the potential of the gut to undergo rapid tissue repair in comparison to piglets that got the supplementation after the DSS challenge. Unfortunately, this effect was only observed in a proportion of the animals, suggesting that even in a relatively homogenous population of commercial pigs, there is considerable genetic influence on the potential of the bioactives to work in this challenging inflammatory model.

## Section 2 - Research Outputs

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### Summary of Project Findings

This data is of interest to nutritionists and health practitioners involved with gut health. We have developed a model of IBD that could be used by other interested parties who believe that they may have a therapeutic intervention. There is no doubt that the bioactives used in this project (Laminarin, chitosan and omega-3 fish oil) have anti-inflammatory potential. However, the complex etiology of Irritable Bowel Syndrome underlies the observation that only a proportion of individuals respond to the dietary supplementation. It is difficult to predict which individuals that any specific treatment will benefit and thereby how it could be incorporated into a treatment regimen.

While Omega-3 fish oils have been widely promoted for their anti-inflammatory potential for IBD, our data suggests that it is no better than chitosan and probably inferior to Laminarin.

With regard to the use of bioactives to alleviate milder inflammatory conditions, the results suggest that a longer supplementation time period is beneficial. This suggests that a natural bioactive would be something that would need to be taken daily, even when the individual would be in remission.

### Summary of Staff Outputs

Research Output	Male	Female	Total Number
Post Doctorates	4	1	5
Research Technicians/ Assistants	1	0	1

### Summary of Academic Outputs

Outputs	Total Number	Details
Publications in Peer Reviewed Scientific Journals	3	<ol style="list-style-type: none"><li>1. Rattigan R, O'Doherty JV, Vigors S, Ryan MT, Sebastiano RS, Callanan JJ, Thornton K, Rajauria G, Margassery LM, Dobson ADW, O'Leary ND, Sweeney T. The Effects of the Marine-Derived Polysaccharides Laminarin and Chitosan on Aspects of Colonic Health in Pigs Challenged with Dextran Sodium Sulphate. <i>Mar Drugs</i>. 2020 May 16;18(5):262. doi: 10.3390/md18050262. PMID: 32429425.</li><li>2. Vigors S, O'Doherty JV, Rattigan R, McDonnell MJ, Rajauria G, Sweeney T. Effect of a Laminarin Rich Macroalgal Extract on the Caecal and Colonic Microbiota in the Post-Weaned Pig. <i>Mar Drugs</i>. 2020 Mar 11;18(3):157. doi: 10.3390/md18030157. PMID: 32168972</li><li>3. O'Shea CJ, O'Doherty JV, Callanan JJ, Doyle D, Thornton K, Sweeney T. The effect of algal polysaccharides laminarin and fucoidan on colonic pathology, cytokine gene expression and Enterobacteriaceae in a dextran sodium sulfate-challenged porcine model. <i>J Nutr Sci</i>. 2016 Mar 28;5: e15. doi: 10.1017/jns.2016.4. eCollection 2016. PMID:27110358</li></ol>

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1. Rattigan R, O'Doherty J.V., and Sweeney T. (2018). Comparing the effects of supplementation with marine derived polysaccharides laminarin and chitosan on the growth performance and faecal consistency of pigs post-weaning. BSAS, Dublin 2018.
2. M. McDonnell, J.V. O'Doherty, and T. Sweeney (2018) Effects of seaweed extract laminarin supplementation on weaned piglet hindgut microbiota. Microbiology Society Conference, Dublin 2018.
3. Margassery, L.M., Jackson, S. Sweeney, T., Dobson, A.D.W., O' Leary, N. Microbiome profiling of porcine susceptibility to experimental IBD among offspring of sows receiving seaweed extracts; evaluation of a maternal mode of dietary intervention. Microbiology Society Annual Conference Edinburgh 2017.
4. Margassery, L.M. , Jackson, S.A., Sweeney, T., Dobson, A.D.W., O' Leary, N. Microbiome profiling of porcine susceptibility to experimental IBD among offspring of sows receiving seaweed extracts; evaluation of a maternal mode of dietary intervention. 11<sup>th</sup> International Marine Biotechnology conference 2016, Baltimore, Maryland 2016.
5. Sweeney T., McDonnell M. , Mukhopadhyaya A. ,Ryan M. , Thornton K. , Venardou B. , Vigors S. and J O'Doherty .V. (2018). Supporting the symbiotic relationship between mucosal morphology, immunity and the gut microbiome in the pig. BSAS, Dublin 2018.
6. Jackson, S. Approaches to exploit the uncultivable microbial majority in industrial and environmental processes. International Symposium on Functional Genomics and Systems Biology 2017.

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## Intellectual Property

We had lengthy discussions with NOVA UCD to ascertain if some of these supplements, in particular Laminarin, could be utilised in a health setting to support individuals prone to Irritable Bowel Syndrome. This project has identified that compounds that are considered to be anti-inflammatory in a healthy intact gut are of benefit to a proportion of individuals when the structure of the gut is damaged, but not all individuals. In addition, the population of pigs used in this study would be considerably more homogenous than the human population, both in terms of genetics and environmental variables including diet, and so the results would probably be even more variable in a human setting. In addition, the benefits are subtle and may not yield and identifiable benefits to an individual suffering a current bout of IBD. Unfortunately, it was decided not to proceed with protecting or exploiting the results.

## Summary of other Project Outputs

Project	Details	Total No.
New Industry Collaborations Developed	This project formed the basis of a project application to DAFM that included three seaweed companies that had never worked together before. These industry partners were Bioatlantis Ltd, Bantry Marine Research Station and This is Seaweed Ltd.	1

## Potential Impact related to Policy, Practice and Other Impacts

Impact	Details
Environmental Sustainability	This project highlighted the potential of natural sustainable bioactives harvested from the marine environment. All three bioactives were marine derived.

## Dissemination Activities

Activity	Details
Workshops at which results were presented	Dobson, A.D.W. Marine Biodiscovery: Deep Waters, Exciting Prospects, Marine Institute, Rinville, Oranmore, Galway. Screening, Culturing and Metagenomics. June 30th, 2016.

## Knowledge Transfer Activities

<b>Identify knowledge outputs generated during this project.</b>	<ol style="list-style-type: none"><li>1. This data is of interest to nutritionists and health practitioners involved with gut health and in particular IBD. We have developed a model of IBD in Ireland that could be used by other interested parties who believe that they may have a therapeutic intervention worth testing.</li><li>2. This project has identified that IBD has a complex etiology. We induced IBD in all of the animals using a similar protocol, however only a proportion of the animals responded to the natural anti-inflammatories. This shows that there is a genetic component to the ability of therapeutics to resolve the inflammation associated with IBD.</li><li>3. This project also identified that three compounds derived from very different marine sources have anti-inflammatory activity.</li></ol>
<b>Identify any knowledge transfer activities executed within the project.</b>	All of the members of the research team were actively involved in knowledge transfer, particularly by presenting talks and posters at conferences and workshops as well as peer-reviewed publications, as outlined in the dissemination activities.

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**List any impacts resulting from the knowledge transferred during the project.**

Probably the greatest impact of this study was the fact that three marine companies that had never collaborated before, agreed to come together to work collaboratively on the bioactivity of seaweeds. These industry partners were Bioatlantis Ltd, Bantry Marine Research Station and This is Seaweed Ltd. This was a very significant development and if the project had been funded, the collaborative venture would have been a significant role model for how Irish marine based companies could operate collectively for the benefit of all. This is a model that works very well for the dairy and meat industries in Ireland and would be extremely beneficial for the marine based companies that comparatively are small and have a less well developed research infrastructure.

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## Section 3 - Leveraging, Future Strategies & Reference

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### Leveraging Metrics

Type of Funding Resource	Funding €	Summary
Exchequer National Funding	€17,000,000.00	SFI 16/RC/3889: Biorbic Bioeconomy Research Centre. Two of the DAFM grant holders (T. Sweeney and JV O'Doherty) were two of the seven principal Investigators in this project.
	€1,296,000.00	SFI 14/1A/2548: The Macroalgal Fibre Initiative: 'natural molecules naturally'. The "Macroalgal Fibre Initiative: 'natural molecules naturally'". Two of the DAFM grant holders (T. Sweeney and JV O'Doherty) were the two principal Investigators in this project.

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### Future Strategies

#### DAFM:

We submitted an application to DAFM entitled: Novel 'climate friendly' seaweed bioactives to reduce ruminant methane emissions. It was a collaboration with three industry partners Bioatlantis Ltd, Bantry Marine Research Station and This is Seaweed Ltd. Unfortunately the application was not successful.

#### Disruptive Technology innovative Fund:

We submitted a Disruptive Technology innovative grant led by Bantry Marine Research Station in 2018 , which was not funded.

We submitted Disruptive Technology innovative grant led by Bioatlantis in 2020. It was titled ""Zero Inputs of Non-Compliant Compounds"- 2022 (Zinc-2022). The company plans to resubmit that project.

### Project Publications

1. Rattigan R, O'Doherty JV, Vigors S, Ryan MT, Sebastiano RS, Callanan JJ, Thornton K, Rajauria G, Margassery LM, Dobson ADW, O'Leary ND, Sweeney T. The Effects of the Marine-Derived Polysaccharides Laminarin and Chitosan on Aspects of Colonic Health in Pigs Challenged with Dextran Sodium Sulphate. *Mar Drugs*. 2020 May 16;18(5):262. doi: 10.3390/md18050262. PMID: 32429425.
2. Vigors S, O'Doherty JV, Rattigan R, McDonnell MJ, Rajauria G, Sweeney T. Effect of a Laminarin Rich Macroalgal Extract on the Caecal and Colonic Microbiota in the Post-Weaned Pig. *Mar Drugs*. 2020 Mar 11;18(3):157. doi: 10.3390/md18030157. PMID: 32168972.
3. O'Shea CJ, O'Doherty JV, Callanan JJ, Doyle D, Thornton K, Sweeney T. The effect of algal polysaccharides laminarin and fucoidan on colonic pathology, cytokine gene expression and Enterobacteriaceae in a dextran sodium sulfate-challenged porcine model. *J Nutr Sci*. 2016 Mar 28;5:e15. doi: 10.1017/jns.2016.4. eCollection 2016. PMID: 27110358