2021 Progress report for the stakeholders

The reasons for the project. The global demand for fish as an essential component of a healthy and balanced diet continues to grow. In order to meet these demands, in times of plateauing wild fish catch, aquaculture has increased to bridge the supply gap until recently when it became the global main source of fish. However, the concerns related to its sustainability have increased in parallel with its growth.

The composition of fish feed represents a major aspect of such concerns and demands the search for new sustainable feed ingredients that do not deplete the planet resources, do not compete with human nutrition, meet the fish welfare and health requirements and remain economically viable.

Designing and conducting feeding trials to develop novel feed ingredients is challenging and often requires high numbers of experimental animals. In vitro tools that can realistically model the functions of the digestive tract would be extremely useful and have the potential to significantly reduce the numbers of animals used in feed trials.

The aim of the project. The FISH-AI project, funded by FET open H2020, is developing a 3D in vitro model of the complex digestive, immune and barrier functions of the fish intestine with an aim to predict the health and nutritional value of novel alternative fish feed ingredients. Furthermore, the model aims to and increase the knowledge of cellular and molecular mechanisms at play within the fish intestine.

A 360 degrees strategic approach. We work with the Rainbow trout (*Oncorhynchus mykiss*) because it is one of the most popular species in European aquaculture. At this stage the project is running along 3 parallel pathways that will converge in the assembly of a functional artificial intestine prototype.

1. Intestinal epithelial cell lines (isolating the building blocks)

We derived cell lines from the anterior and the posterior portion of the Rainbow trout intestine. We identified the genes expressed by the different cells that form the intestinal stem cell population whose role is to ensure the integrity of the intestinal wall. Although most genes were already known in Mammals, we found the role they play in trout to be substantially different. This discovery is important because it enables us to identify the stem cell population in vitro and monitor its differentiation towards an epithelium that more closely replicates the natural one. This is the first component of the Fish-AI platform.

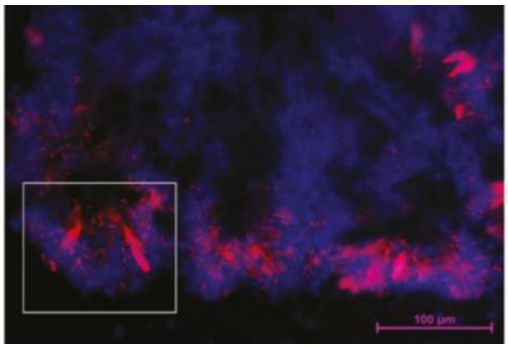

Figure 1. The pink cells are intestinal stem cells of Rainbow trout recognised for their intense expression of the sox9 gene (Verdile N et al. 2020. https://doi.org/10.3390/ijms21239192.)

This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 828835.

2. Scaffold development (creating the supporting frame)

The second component is a scaffold material that can mimic the properties of the intestinal wall layer, which separates stem cells and differentiated cells from the blood vessels. Hydrogels based on gelatine, which is a derivative of a major component of this layer in vivo, are promising candidates for use in the in vitro replica. Our goal is to find a gelatine which can offer the best combination of strength, permeability to nutrients, and interaction with the intestinal cell lines. In parallel, we aim to 3D print these hydrogels in a pillar-like structure, which is typical of the intestinal inner lining.

The last aspect, we have to address is to guarantee the natural flow of nutrients along the artificial intestinal lumen. In order to achieve this, we designed and printed a 3D cell culture chamber that allows the right positioning of the scaffold and the intestinal cell lines, enabling their exposure to an environment similar to the intestinal lumen.

3. In vitro digestion of complete fish feeds (testing the function)

Before arriving to the intestinal tract where the nutrients are absorbed, the pelleted feed that the fish eats, undergoes a process of digestion. This enables the nutrients to be separated and broken up into smaller fragments that cells can absorb. Therefore, our platform requires a parallel in vitro digestion procedure that produces the nutrients profile found in the intestine of fed rainbow trout. This is achieved by the production of rainbow trout digestive tract-based enzyme extracts and their use to in vitro digestion of fish feed, under different test conditions. By doing this, we will be able to compare the composition of this artificial digesta with the gut content, collected directly from the fish (Figure 2),

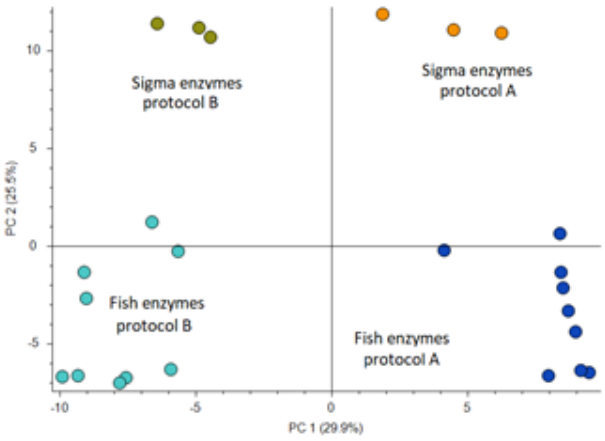

Figure 2. The patterns of amino acids, their metabolites and small peptides extracted from in vitro digested fish feed varies under different test conditions.

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