A toolset was developed to evaluate the effects of adding an organic acid salt to high plant-ingredient substituted diets of gilthead sea bream to alleviate some of the detrimental effects of replacing fish meal and fish oil. The toolset was tested and validated using sodium butyrate, one of the most promising feed additives to be used in aquaculture. The tools and intestinal health testing approach can be adapted for other species and feed additives in development.

**KNOWLEDGE NEED**

Plant-based fish food is seen as more sustainable than that containing fish meal and fish oil. While some farmed fish species can survive without any or with a very limited supply of marine feeding ingredients, they can suffer from detrimental effects such as decreased efficiency to digest food and increased susceptibility to diseases and stress. Feed additives that improve health and nutrition of farmed fish and lessen intestinal inflammation induced by plant-based ingredients are therefore needed.

**POTENTIAL IMPACT**

- Use of the developed toolset will help with the understanding and assessment of the benefits of a feed additive, such as sodium butyrate, to a plant-based diet. As a simple “treatment” it can act as a first line of defence against diseases, improving health and welfare of gilthead sea bream fed on high plant-ingredient substituted diets.
- Similar positive effects could be assessed by making use of the toolset for a variety of other aquaculture species fed on similar diets when supplementing with sodium butyrate.
- The toolset will allow a better understanding of overall fish intestinal health as a result of a modified diet by providing more insight into the involved processes and dynamics.

**EATiP - Strategic Research and Innovation Agenda (SRIA):** Thematic Area 4 – Sustainable Feed Production; Goal 3, Thematic Area 7 – Aquatic Animal Health and Welfare; Goal 4. To see the full list and descriptions of the thematic areas and goals, please visit: bit.ly/2xjx1AX
UNDERLYING SCIENCE
Feeding experiments were conducted, all using the same dose of sodium butyrate supplementation (i.e. 0.4% BP-70 @NOREL), applied to four different diets: a control diet containing 25% fish meal and three experimental plant-based diets containing only 5% fish meal and different levels of fish oil. The effects of these dietary changes were studied using the following toolset:

- Blood biochemical (Haemoglobin concentration)
- Histological
- Immunohistochemical
- Molecular (gene expression)
- Electrophysiological (trans-epithelial electrical resistance)

RESULTS
The toolset applied provided evidence that the plant-based diets had significant negative consequences on factors related to growth, immune-response, inflammation and defence against disease overall. In addition, the toolset also showed that with the addition of the sodium butyrate additive most of these modifications were returned to normal values. These results confirmed the potential of the combined toolset used in the experiment to verify that a given additive can improve or reverse the detrimental effects of fish diet formulations using a high plant replacement of fish meal and oil.

The tools and the intestinal health testing approach developed in this research and current studies can be used as a toolset for other species and for testing candidate feed additives or improving those already existing.

END- USERS & POTENTIAL APPLICATIONS

- **END-USER 1:** Aquaculture feed producers
  APPLICATION: The same histochemical, molecular, immunohistochemical, and electrophysiological toolset could be used to test the effect of plant-based diets and alternative feed additives.

- **END-USER 2:** Aquaculture fish farmers
  APPLICATION: The potential of an additive as a “treatment” can be tested in different aquaculture species or when disease risk in the production cycle is highest.

- **END-USER 3:** Fish physiologists and pathologists (scientific community)
  APPLICATION: The toolset will further investigations of the changes in intestinal health due to diet, infections and feed additives, to understand the fundamental processes across different species.

STATUS
Technology Readiness Level (TRL) 4 - the proof of concept has been successful on sea bream and butyrate under laboratory conditions.

Next steps include:

- Streamlining the toolset by optimisation e.g. inflammation assay, and reducing the test genes from 86 to the 20 most informative and reliable genes
- Investigating if the toolset can be extended to detect changes in the mucus protein and bacteria composition in a routine manner in the digestive system as a response to feed additives
- Understanding how these functional features are modified by genetic and epigenetic factors to establish a powerful model for the development of sustainable and healthy fish feeds
- Testing and validation of the toolset for different feed additives and species to confirm its universal applicability