PROMAC Newsletter

Dear readers,

I am pleased to introduce the last newsletter of the PROMAC project. During the last few months, several notable actions were conducted to complete our project.

In March 2018, the PROMAC partners were visiting the Energy Center of the Netherlands, the Amsterdam Science Park and Wageningen University to investigate further the biorefineries processes of macroalgae.

Ten peer-reviewed papers have already been published and are available on our research gate PROMAC page. Most of the deliverables in the project have been completed. However, the collection of the biomass and the extraction of the products to be tested in animal trials took longer time to achieve and were in less quantity than expected. These challenges delayed the attempts needed to assess the health and environmental impacts of these new seaweed products. The project was therefore granted a one-year extension to deliver all the results within the assigned budget.

Finally, the results of the project and its spin-offs (ISBIT, Makroterm projects) will be presented at the PROMAC-conference in Ålesund from 8th to 9th of November 2018.

More information about the project and scientific publications can be found on our webpage, **www.promac.no.**

Céline Rebours

PROMAC

Palmaria Palmata Stimulated Immune Responses and Diminished Gut Microbial Diversity in Zebrafish

Zebrafish (*Danio rerio*, Figure 1A) is an attractive animal model for the investigation of immune and other health-related functions in monogastric animals, including fish as well as humans. The tools available for the study of the biology of this model are numerous, allowing thorough investigations of basic mechanisms.

Thus, a feeding trial was conducted with zebrafish to evaluate effects on the immune apparatus of two seaweeds: S*accharina latissima* and *Palmaria palmata*. A total of five diets were evaluated, one control diet without seaweed, two diets containing *S. latissima* products, i.e. whole product and a protein concentrate, and similar for the *P. palmata*. All the seaweed products had an inclusion level in the diet of 15%. The feeding experiment lasted for 8 weeks and samples were taken for evaluation of immune and stress-related markers, histomorphology and gut microbiota profiles.

The results of this study indicated that a 15% inclusion of the seaweeds products evaluated did not affect growth performance and survival. All sampled fish showed a normal (healthy) intestinal morphology. On the other hand, a decrease in diversity of the gut microbiota, i.e. in number of species, was observed in the fish fed the concentrate *P. palmata* diet (Figure 1B). Moreover, whole *P. palmata* products caused modulations of immune- and stress-related gene expression in the intestine (Figure 1C,D). These results suggest that bioactive compounds in *P. palmate* trigger expression of genes involved in the immune apparatus as well as in stress responses.

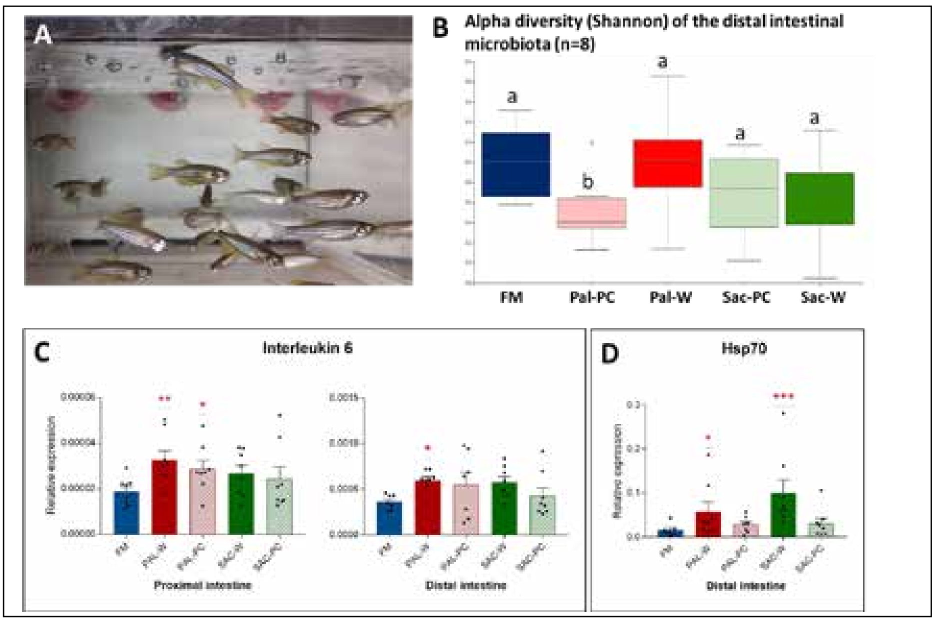

Figure 1

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Forskningsrådet

Two additional zebrafish experiments, one to be completed before the end of the year 2018, the other starting early 2019, are follow-ups with the aim to further characterize the observations made so far.

A: Zebrafish was used as model organism to study effects on the immune apparatus of the seaweeds *Saccharina latissima* and *Palmaria palmata*.

B: Microbiota diversity was decreased in fish fed the diet with concentrated *P. palmata*.

C and D: Transcript levels of inflammatory and stress-related genes, such as interleukin 6 and heat shock protein 70 (Hsp70), were significantly higher in fish fed diets with whole *P. palmata* than those given fish meal-based diets. FM: diet based on fish meal; PAL-W: diet with whole *P. palmata*; PAL-PC: diet with protein concentrate of *P. palmata*; SAC-W: diet with whole *S. latissima*; SAC-PC; diet with protein concentrate of *S. latissima*. Significant differences are indicated by *: p<0.05; **: p<0.01; ***: p<0.001.

By: Alexander Jaramillo Torres, Guro Løkka, Trond Kortner, Åshild Krogdahl.

The Nutritive Value of a Protein Enriched Fraction of the Seaweed *Saccharina Latissima* for Ruminants

An important objective of the PROMAC project is to evaluate the nutritive value of refined macroalgal products in ruminants. Of particular interest was to test protein-rich fractions as alternative to conventional protein feeds. Fractions of *Saccharina latissima*, *Alaria esculenta*, and *Palmaria palmata* were first evaluated *in vitro* with rumen fluid in a previous experiment in PROMAC. Based on the results of this *in vitro* screening, a protein enriched fraction of *S. latissima* was selected to be tested in a feed digestibility trial in sheep. The product was produced by SINTEF in WP3 of the PROMAC project. The sheep were fed a basal ration with hay, and was in addition supplemented with either the *S. latissima* product, commercially available *Porphyra* spp. (a red macroalgae) or soybean meal. A control with only hay was also included. The protein diets were planned to be similar in protein level.

Our results show that the protein digestibility of a diet containing *S. latissima* was lower than that of *Porphyra spp*. and soybean meal. That means relatively more of the ingested protein was excreted in the faeces on the diet with *S. latissima* than on the other protein enriched diets. We observed that the concentration of volatile fatty acids and ammonia in the rumen was lower in the sheep that were fed *S. latissima* than on the other diets. This indicates that the *S. latissima* product had low degradability in the rumen. The protein that is not degraded in the rumen may be digested and absorbed in the intestine, which is regarded as beneficial. However, the increased loss of protein in faeces with *S. latissima* indicates that the intestinal digestion was poor. Analysis of amino acid composition in blood plasma, showed similar values for all diets, and suggests similar quality of the protein absorbed from the different diets and that most of it were of microbial origin.

It is important to note that the *S. latissima* used in this trial was rinsed during processing to remove salt. This process potentially also removed other water-soluble compounds, such as free amino acids. If so, the fraction of protein resistant to rumen degradation and intestinal absorption may have increased. We recommend that further studies explore the extent to which processing of macroalgae affects its nutritive properties and rumen degradability, in addition to those measuring the intestinal absorption of these macroalgae species.

By: Şeyda Özkan Gülzari, Vibeke Lind, Inga Marie Aasen and Håvard Steinshamn.

Establishing a Large-scale Seaweed Industry in Norway: Strategic, Environmental and Economic Considerations

Research is being poured into developing both potential products and the processes required to convert seaweed, or macroalgae, into products. So far, the results are products for high-end markets, such as restaurants, but in very modest volumes. To understand what it will take to create a large-scale seaweed industry, research is conducted into the strategic-, environmental- and economic realities such an industry would have to deal with as suppliers of fish-feed. The idea is that unless the industry has something to offer that competing products do not, in terms of cost, performance or environmental- and economic footprint, the seaweed industry will be delegated to small volumes for special needs and high value products. These topics are discussed below.

Life Cycle Assessment for Seaweed Farming and Processing

One task in the PROMAC-project is to find solutions to produce feed-protein based on cultivated macroalgae. While other work-packages are working on solutions and improvements on the way from producing seedlings to testing the protein rich feed-ingredient, the work-package 6 uses a Life Cycle Assessment (LCA) to describe the environmental impact of the entire production chain. In the LCA, the main energy and material inputs and environmental releases are included by taking account of the entire way from raw material extraction through materials processing. This approach allows not only to highlight where most material and energy is used and where high environmental loads are, but also to look for improvements and to compare the process with comparable products. In case of protein-rich feed ingredients, we compare seaweed and soy as sources to produce feed-protein from cradle to gate.

The material and substance flow analysis presented by Philis et al. (2018) for the energy consumption for producing feed-protein shows that most of the energy required is needed for drying the harvested seaweed. As a first step, the LCA uses a comparable approach where the energy required for the different processes is accounted for. The LCA includes, in addition, the amount of energy and environmental impact caused by providing this energy and all other products used. When 100 kWh of electricity from the Norwegian grid is used, 137 kWh have been necessary to produce this amount and the global warming potential equals 3 kg CO_2 (LCIA – CML 2015). These values are for the average Norwegian grid mix where over 96 % of electric energy is from hydropower and include energy use and environmental impact for the entire life cycle for materials in e.g. building and maintenance of dam, turbine and the grid as well as losses in the grid.

Focusing only on the protein in the two different protein products, the material and substance flow analysis used by Philis et al. (2018) shows that 24 MJ are needed to produce 1 kg of soy protein and 278 MJ per kg seaweed protein (12 times more energy for seaweed protein). Using a LCA-approach, the energy demand is higher, 160 MJ per kg of soy protein and 491 MJ per kg seaweed protein. The global warming potential (GWP), calculated on LCIA – CML 2015, results in 8 and 27 kg CO_2-equivalents respectively. An important reason for the minor differences between the two different products in the LCA is that the materials and energy used to produce seaweed concentrate have a lower environmental impact than those used to produce soy protein concentrate. As mentioned before, the main part of energy is needed to dry the harvested seaweed. When surplus energy is free available from incineration, the energy demand per kg of seaweed protein can be reduced to 201 MJ and the GWP to 11 kg CO_2-eq. However, it has to be taken into account that energy from incineration probably will not be totally free and thus a part of the CO_2 emission from the incineration has to be included into the calculations. The seaweed protein product has a protein content of about 31 % in dry matter. Further increase in protein content will also increase the energy demand as well as environmental costs.

In the analyses, only the production of protein was evaluated. If in addition to protein also high value components as lipids, fucoxanthin, fucoidan and laminaran would be extracted from seaweed, the produced value could be increased and thus the economic and environmental costs per unit protein product be reduced.

Life Cycle Costing for Seaweed Farming and Processing

As already mentioned, seaweed farming and processing is today largely handcraft for high-end usage such as pharmacy and restaurants. This approach will not work if we are to process the seaweed for Seaweed Protein Concentrate (SWPC) to be used in production of fish-feed. Our work shows the importance of scale of the biorefinery which is the single most important factor in this respect, and we are talking about thousands of tonnes per year for each biorefinery. For a facility with a 25,000 tonnes capacity, we can achieve a production cost of ca 0.4 EUR/kg.

The competing product – Soy Protein Concentrate (SPC) – contains 60 % protein and is traded at about 0.8 EUR/kg. This means that since SWPC contains only 10 % protein, it is still at a major cost-disadvantage as a source of protein only. However, since SWPC contains Laminaran and Mannitol at roughly 10 % each (there is large variations in the literature) the fish-feed producers may be interested in paying more for the 1 kg SWPC. Indeed, our analysis shows that there is approximately 30 % probability that SWPC will match or surpass SPC when these non-protein compounds are included. This is illustrated in Figure 1 below.

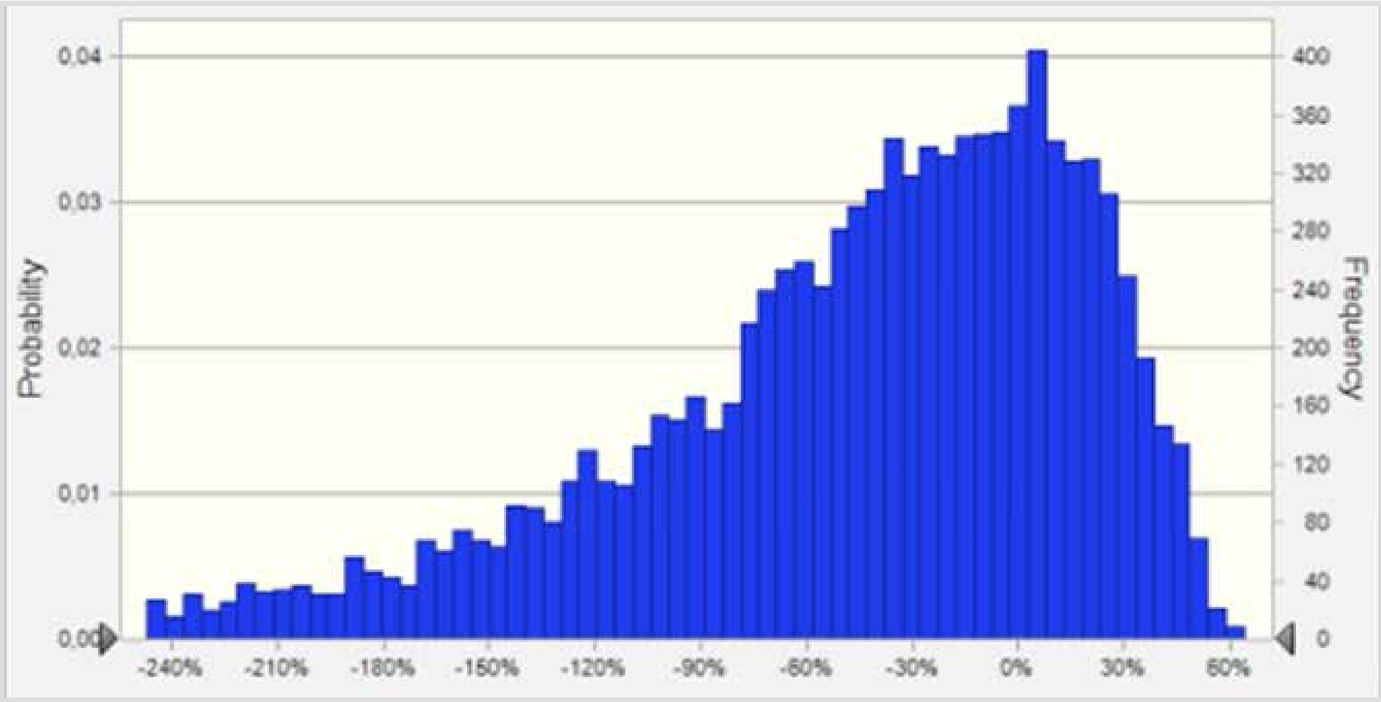

Figure 1: SWPC with Laminaran and Mannitol compounds.

Besides, if we test the model (deterministic) with an assumption that the scale of the biorefinery is larger than 65,000 tons per year, then the ROS > 0.9 %. With our modeling of having four such biorefineries in Norway, that means an industrial output in Norway of 260,000 tons. This therefore implies that the deterministic break-even point is about 65,000 tons per year for the biorefinery.

[1] Following the FAO standards for soy pertaining to protein concentrate, processed dried seaweed does not qualify to be called 'SWPC', see file:///M:/CXS_175e.pdf. Nevertheless, for consistency and simplicity sake in comparing protein content of soya to that of seaweed, the authors have used the term 'SWPC' in referring to the processed dried seaweed with protein content of about 10 %.

By: Jan Emblemsvåg, Nina Pereira Kvadsheim, Matthias Koesling, Jon Halfdanarson, Celine Rebours, Annik F. Magerholm.

Project facts

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- Duration: 2015 – 2018
- Project owner: Møreforsking
- Project Manager: Céline Rebours