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ABSTRACTS

GUT HEALTH AND MICROBIOTA IN POST-SMOLT ATLANTIC SALMON (*SALMO SALAR*) FED LARVAE MEAL FROM BLACK SOLDIER FLY (*Hermetia illucens*)

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Introduction

In salmon aquaculture, the limited availability of sustainable feed ingredients is a major obstacle. Insects, being part of the natural diet of salmonids, may become a sustainable resource and expand the raw material repertoire. In the “AquaFly” project, the potential of black soldier fly (*Hermetia illucens*) grown on low-quality organic matter as a source of sustainable feed ingredients for Atlantic salmon (*Salmo salar*) was assessed in one freshwater and one seawater feeding trial. Herein, we summarize data from the 16-week seawater feeding trial with salmon (initial body weight, 1.4kg) fed either a reference diet (REF) with a combination of fish meal, soy protein concentrate, pea protein concentrate, corn gluten and wheat gluten as protein source, or a test diet (IM) wherein all the fish meal and most of the pea protein concentrate were replaced by black soldier fly larvae meal

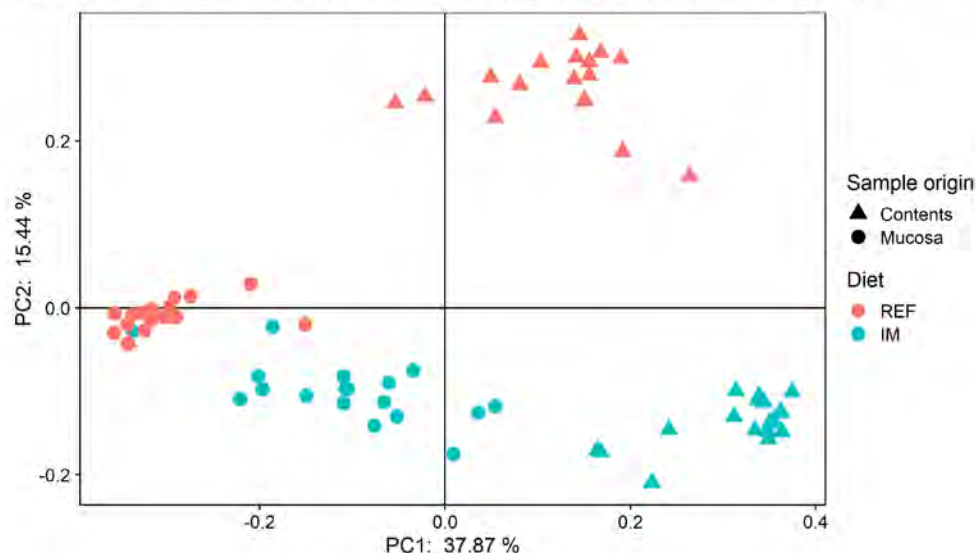
Materials and methods

The gut health of fish was evaluated using endpoints including organ and tissue indices, digestive enzyme activity, histopathological parameters and gene expression. In total, 36 genes under different functional categories were profiled via qPCR, including those indicative of lipid metabolism, immune responses, barrier functions and detoxification/stress responses. Microbiota in the distal intestine, both gut contents and mucosa associated, was analyzed via 16S rRNA gene sequencing.

Results

1. Histology. Hypervacuolization of enterocytes, suggestive of lipid accumulation, was observed in the proximal and mid intestine in both diet groups. It was, however, less severe in the proximal intestine of fish fed the insect meal diet ($p < 0.05$). Typical signs of enteritis commonly observed in salmonid intestine fed soybean meal diets were present in all the intestinal segments in both diet groups. A higher degree of submucosa cellularity was observed in the proximal intestine of fish fed the insect meal diet ($p < 0.05$).

Fig.1. Beta-diversity of distal intestine microbiota visualized by principle coordinate analysis (PCoA) of unweighted UniFrac distance. Each point represents one sample. The closer the



points, the more similar their microbial communities are.

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2. Gene expression. Of the 36 genes profiled in the proximal and distal intestine, a few genes showed differential expressions. The only genes showing differential expressions were *mmp13* (matrix metalloproteinase 13) and *chk* (choline kinase), whose expression level was significantly lower in the proximal and distal intestine, respectively, in fish fed the insect meal diet ($p < 0.05$).

3. Gut microbiota. Both diet and sample origin (gut contents vs mucosa) affected microbiota in the distal intestine. All the measured alpha-diversity indices, including observed OTUs, Shannon index, Pielou's evenness and Faith's phylogenetic distance, showed higher values in samples exposed to insect meal diet or originated from gut contents ($p < 0.05$). For the beta-diversity, samples formed 4 distinctive clusters in the PCoA plot separated by the diet and sample origin (Fig.1), confirmed by permutational multivariate analysis of variance (*PERMANOVA*, $p < 0.05$). The diet showed a more profound effect on the samples originated from gut contents than those from mucosa.

Discussion and conclusion

In the present study, total replacement of fish meal with black soldier fly larvae meal showed no appreciable negative effects on the gut health and function of Atlantic salmon. In line with what we reported in the freshwater trial (Li et al., 2019), insect meal inclusion was associated with a lower degree of enterocyte hypervacuolization in the proximal intestine, which is suggestive of lower lipid accumulation. In the context of frequent occurrence of steatosis found in the proximal intestine of farmed salmon in field surveys (Chikwati et al., 2018), the potential beneficial effect of insect meal inclusion on reducing lipid deposition in the gut deserves further attention. In contrast to the previous finding in the freshwater trial that insect meal diet increased the expression of *foxp3* (Li et al., 2019), a transcription factor for the differentiation of naïve CD4 T-cells into regulatory T cells, no such effect was observed in the seawater trial. On the other hand, the present study showed increased submucosa cellularity in the proximal intestine. The effect of insect meal inclusion on the gut immune response is also worth of attention in future studies.

Reference

- Chikwati, E.M., Midtlyng, P.J., Krogdahl, Å., 2018. Gut health monitoring during the seawater phase of farmed Atlantic salmon in different production regions of Norway – The Gutmatters Project, 8th International Symposium on Aquatic Animal Health, Charlottetown, Prince Edward Island, Canada, pp. 400.
- Li, Y., Kortner, T.M., Chikwati, E.M., Munang'andu, H.M., Lock, E.-J., Krogdahl, Å., 2019. Gut health and vaccination response in pre-smolt Atlantic salmon (*Salmo salar*) fed black soldier fly (*Hermetia illucens*) larvae meal. *Fish & Shellfish Immunology*, 86, 1106-1113.