Total replacement of fish meal with black soldier fly (Hermetia illucens) larvae meal does not compromise the gut health of Atlantic salmon (Salmo salar)

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Total replacement of fish meal with black soldier fly (*Hermetia illucens*) larvae meal does not compromise the gut health of Atlantic salmon (*Salmo salar*)

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ABSTRACT

Limited availability of sustainable feed ingredients is a significant concern in salmon aquaculture. Insects may become an important, sustainable resource for expanding the raw material repertoire. Herein, we present data from a 16-week seawater feeding trial with Atlantic salmon (initial body weight, 1.4 kg) fed either a reference diet with a combination of fish meal, soy protein concentrate, pea protein concentrate, corn gluten and wheat gluten as protein source, or a test diet wherein all the fish meal and most of the pea protein concentrate were replaced by black soldier fly larvae meal. The gut health of fish was evaluated using endpoints including organ and tissue indices, histopathology variables and gene expression indicative of lipid metabolism, immune responses, barrier functions and detoxification/stress responses. A higher relative weight of distal intestine was found in fish fed the insect meal diet. Steatosis of enterocytes was observed in the proximal and mid intestine in both diet groups, albeit, less severe in the proximal intestine of fish fed the insect meal diet. Inflammatory morphological changes, similar to those induced in the distal intestine by standard soybean meal, were present in all the examined intestinal segments, with a higher degree of submucosa cellularity in the proximal intestine of insect meal diet fed fish, the only notable diet effect. Few differentially expressed genes were identified in the proximal or distal intestine. In summary, total replacement of fish meal with black soldier fly larvae meal did not compromise the gut health of Atlantic salmon.

1. Introduction

Marine ingredients in the Norwegian salmon diet have gradually been replaced by plant sources, decreasing from ~90% in 1990 to ~25% in 2016. Among the plant-based protein sources, soy protein concentrate accounted for 19.2% of the total diet ingredients followed by wheat gluten (9.0%), corn gluten (3.4%), horse beans (2.0%), pea protein concentrate (1.4%), faba beans (1.3%), sunflower meal (1.2%) and other marginally used plant proteins (2.7%) (Aas et al., 2018). While the future availability of plant proteins is guaranteed in the short-term (Shepherd et al., 2017), there is a need for new nutrient sources in Norwegian salmon aquaculture as the production volume is expected to grow. Moreover, as the world population is projected to reach 9.8 billion in 2050 (UN, 2017), global food production must maximize the nutritional output for human consumption and minimize the input of resources, with the lowest possible impact on the environment (Ytrestøyl et al., 2015). Hence, the salmon feed producers need to reduce their dependency on terrestrial plant products that may be used directly for human consumption, and seek new, sustainable feed ingredients for the future salmon aquaculture.

Insects possess an outstanding capacity to upgrade low-quality organic material, require minimal water and cultivable land, and emit little greenhouse gases (van Huis, 2013). At present, exploiting insects as feed ingredients is not in direct competition with food production. Black soldier fly (BSF; *Hermetia illucens*) is being produced at industrial scale in Europe due to its exceptionally good nutritional value and suitability for massive production. On a dry matter basis, BSF larvae contain about 42% protein and 35% lipid (Newton et al., 1977). In terms of protein quality, BSF larvae contains a favorable essential amino acid profile closer to fishmeal than that of soybean meal (Barroso

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et al., 2014). Moreover, the fat level and fatty acid profile are diet-dependent, allowing for control using different feed substrates (St-Hilaire et al., 2007a, 2007b). The potential of BSF larvae as an alternative feed ingredient for fish has been evaluated in several omnivorous and carnivorous species including Atlantic salmon (Belghit et al., 2018; Bondari and Sheppard, 1987; Borgogno et al., 2017; Devic et al., 2017; Hu et al., 2017; Kroeckel et al., 2012; Li et al., 2016; Li et al., 2017; Lock et al., 2016; Magalhaes et al., 2017; Renna et al., 2017; Sealey et al., 2011; St-Hilaire et al., 2007a, 2007b). The optimal substitution level of fishmeal in the diet by BSF larvae meal varies considerably in different studies ranging from 25% to 100%, possibly due to differences in the larvae meal quality, fish species and diet formulation. While the nutritional value of BSF larvae meal has been extensively studied, its impact on fish health, the gut health in particular, has not been investigated.

The present study was part of a larger investigation consisting of a freshwater and seawater feeding trial that aimed to reveal the nutritional value and possible health effects for Atlantic salmon, of a protein-rich insect meal (IM) produced from BSF larvae. In the 8-week freshwater trial, pre-smolt salmon were fed either a reference diet or a test diet wherein 85% of the dietary protein was supplied by BSF larvae meal. The gut health of fish was evaluated using endpoints including organ and tissue indices, histopathology variables and gene expressions (Li et al., 2019). Results from the freshwater trial showed no indications that dietary inclusion of insect meal may affect the gut health of Atlantic salmon negatively. The insect meal diet seemed to reduce excessive lipid deposition in the pyloric caeca enterocytes and stimulate xenobiotic metabolism (Li et al., 2019). The present study focuses on the gut health in the seawater-phase salmon fed BSF larvae meal for 16 weeks. Post-smolt Atlantic salmon was fed either a reference diet with a combination of fish meal, soy protein concentrate, pea protein concentrate, corn gluten and wheat gluten as protein sources, or a test diet wherein all the fish meal and most of pea protein concentrate were replaced by BSF larvae meal. The gut health of seawater-phase salmon fed a commercially-relevant reference diet and an insect meal test diet was evaluated using the same endpoints measured in the freshwater trial (Li et al., 2019).

2. Materials and methods

2.1. Diets and fish husbandry

A feeding trial with seawater-phase Atlantic salmon (initial body weight 1.40 kg, S.D. = 0.043 kg) was conducted at the Gildeskål Research Station (GIFAS), Nordland, Norway, in accordance with laws regulating the experimentation with live animals in Norway. Fish were fed either a commercially-relevant reference diet (REF) with a combination of fish meal, soy protein concentrate, pea protein concentrate, corn gluten and wheat gluten as protein source, or an insect meal diet (IM) wherein all the fish meal and most of the pea protein concentrate were replaced by BSF larvae meal (Table 1). The insect meal was produced from BSF larvae by Protix Biosystems BV (Dongen, The Netherlands). The larvae were grown on media partially containing seaweed (*Ascophyllum nodosum*) mixed with organic plant-derived waste (60:40). At the end of an eight-day growth period, the larvae were mechanically separated from the feeding media, washed and partially defatted before being dried and ground to produce the insect meal. Each diet was randomly allocated to triplicate net pens (5 × 5 × 5 m; 125 m^3) each containing 90 fish. Fish were fed by hand until apparent satiation twice daily (or once due to the light conditions). The feeding trial lasted for 16 weeks. Within this period, the salmon reached a mean weight of 3.7 kg that is suitable for sensory testing. Further details on the insect meal, diet composition and fish husbandry were reported elsewhere (Belghit et al., 2019).

Table 1
Formulation and proximate composition of the experimental diets (previously published in Belghit et al., 2019).

	REF	IM
Ingredients (% wet-weight)		
Fishmeal LT94	10	0.0
Black soldier fly larva meal[a]	0.0	14.75
Soy protein concentrate	25	25
Corn gluten meal	7.5	7.5
Wheat gluten meal	3.35	6.88
Pea protein concentrate 55	8.8	2.84
Fish oil	10.18	14.76
Rapeseed oil	20.95	14.73
Binder	12.32	11.24
Additives[b]	1.89	2.29
Yttrium	1.0	1.0
Chemical composition (wet-weight basis)		
Dry matter (%)	93	95
Crude Protein (%)	38	39
Crude Lipid (%)	29	29
Ash (%)	4.6	4.5
Carbohydrates (%)	11.6	11.4
Gross energy (MJ/kg)	24.6	25.0
TBARS (nmol/g)	3.0	4.9

REF, reference diet; IM, insect meal diet; TBARS, Thiobarbituric acid reactive substances.

[a] Partially defatted. Produced by the Protix Biosystems BV (Dongen, The Netherlands).

[b] Supplemented to meet the nutrient requirements of salmon, mostly consist of vitamin/mineral mix, amino acids (methionine and lysine) and phosphorus.

2.2. Sample collection

At the termination of the feeding trial, fish were randomly taken from the net pens, anesthetized with tricaine methanesulfonate (MS222®; Argent Chemical Laboratories, Redmond, WA, USA) and euthanized by a sharp blow to the head. Body weight was registered for all the fish sampled. From 6 fish per net pen, the whole digestive tract was dissected, cleaned free of attached adipose tissue and opened longitudinally. Only fish with chyme present along the whole intestine were sampled to ensure exposure to the diets until the point of sampling. The chyme was gently removed using a spatula. The emptied intestine was divided into proximal (PI), mid (MI) and distal (DI) segments and weighed, respectively. The gut tissue was rinsed in phosphate buffered saline three times to remove traces of remaining chyme and cut into pieces for RNA extraction and histological evaluation. The gut tissue for RNA extraction was preserved in RNAlater solution at room temperature for < 12 h, incubated at 4 °C for 48 h and stored at −20 °C after arrival at the lab, whereas the gut tissue for the latter purpose was fixed in 4% phosphate-buffered formaldehyde solution for 24 h and transferred to 70% ethanol for storage at room temperature.

2.3. Organosomatic indices

Organosomatic indices (OSI) of the PI, MI and DI were calculated as percentages of the weight of intestinal segments relative to the fish body weight; OSI = 100 * TW/BW, where TW is the tissue weight and BW is the fish body weight.

2.4. Histology

After fixation, PI, MI, and DI samples were processed according to standard histological techniques to produce sections of 3 μm thickness from each intestinal segment and stained with hematoxylin and eosin. The sections were then examined blindly with a light microscope paying attention to typical inflammatory morphological changes commonly observed in salmonid intestine: that is, shortening and fusion of

mucosal folds, cellular infiltration within the lamina propria and submucosa, reduced enterocyte vacuolization and nucleus position disparity (Baeverfjord and Krogdahl, 1996). Normally, little to no vacuolization is present in the enterocytes of the PI and MI whereas enterocytes of the DI show varying degrees of supranuclear vacuolization that diminishes or disappears during inflammation. Shortening and fusion of mucosal folds are usually absent when signs of inflammation, such as immune cell infiltration within the lamina propria and submucosa, are observed in the PI and MI. Therefore, mucosal fold morphology (height and fusion) was only evaluated for the severity of inflammation in the DI. For each histological characteristic evaluated, a value of normal, mild, moderate, marked or severe was assigned.

2.5. Quantitative real-time PCR

Real-time qPCR assays were performed following the MIQE guidelines (Bustin et al., 2009) as described in the freshwater feeding trial (Li et al., 2019). In brief, total RNA was extracted from PI and DI samples with a mean A_{260}/A_{280} ratio of 2.2 (S.D. = 0.01). The RNA integrity was evaluated by agarose gel electrophoresis using the NorthernMax®-Gly sample loading dye (catalog no., AM8551; Ambion, Austin, TX, USA). Based on the gel electrophoresis results, the RNA integrity of 24 representative samples was further confirmed by the 2100 Bioanalyzer using the 6000 Nano LabChip kit (Agilent Technologies, Palo Alto, CA, USA). The average RIN (RNA integrity number) value for the selected samples was 9.5 (S.D. = 0.38). The cDNA synthesis was performed using 1.0 μg total RNA from all samples using a Superscript™ IV VILO™ cDNA synthesis kit (Invitrogen, Carlsbad, CA, USA). A negative control was set up by omitting RNA and the obtained cDNA was diluted 1:10 before use. The qPCR assays were performed using the LightCycler 96 (Roche Applied Science, Basel, Switzerland) and a 10-μL reaction volume was used, which contained 2 μL of PCR-grade water, 2 μL diluted cDNA template, 5 μL LightCycler 480 SYBR Green I Master (Roche Applied Science) and 0.5 μL (10 μM) of each forward and reverse primer. Samples were run in duplicates in addition to a no-reverse-transcription control and a no-template control for each gene. A three-step qPCR programme was applied incorporating an enzyme activation step at 95 °C (5 min) and 45 cycles of 95 °C (10 s), 55–63 °C (10 s) and 72 °C (15 s). Quantification cycle (Cq) values were determined using the second derivative method. Beta-actin (*actb*), glyceraldehyde-3-phosphate dehydrogenase (*gapdh*), RNA polymerase 2 (*rnapo2*) and hypoxanthine phosphoribosyltransferase 1 (*hprt1*) were evaluated for use as reference genes according to their stability across and within the treatments as described by (Kortner et al., 2011). The expression of target genes in the PI and DI were normalized to the geometric mean of the 4 reference genes evaluated. The mean normalized expression of the target genes was calculated from raw Cq values (Muller et al., 2002). The genes profiled and the primers used for the qPCR assays are given in Table S1.

2.6. Statistics

Statistical analyses and creation of graphs were performed in R 3.5.2 (R Core Team, 2013). The *tidyverse* package (Wickham, 2017) was used to import, tidy, transform and visualize data. After exploratory analyses, continuous response variables were fitted by linear mixed effect model via the *lme4* package (Bates et al., 2015), treating diet as fixed effect and net pen as random effect. The model diagnostics were performed by plotting residuals against the fitted values and against each covariate in the model to assess homogeneity, by making a QQ-plot to check normality and by detecting influential observations using the *influence.ME* package (Nieuwenhuis et al., 2012). The *p* value of diet effect was obtained via parametric bootstrap comparisons using the *pbkrtest* package (Halekoh and Højsgaard, 2014). When the fitted models were singular, the Welch's *t*-test was run to compare group means and the normality assumption was visually checked via QQ-plots. For ordinal response variables, data were fitted by cumulative link mixed model via the *ordinal* package (Christensen, 2019), treating diet as fixed effect and net pen as random effect. The random effect was dropped when the full model produced singular fits or huge Hessian numbers, or when the random effect was not significant. The proportional odds assumption was checked by comparing models against ones that relax this assumption (i.e., allow nominal/scale effect) via likelihood-ratio tests. The random effect was visually inspected via conditional modes with 95% confidence intervals based on the conditional variance. The statistical model outputs were tidied using the *broom* package (Robinson and Hayes, 2019) when needed. Multiple comparisons were adjusted by the Holm-Bonferroni correction (controlling family-wise error rate) or Benjamini-Hochberg procedure (controlling false discovery rate) where applicable. Differences were regarded as significant when $p < .05$. Plots were generated using *ComplexHeatmap* (Gu et al., 2016), *ggplot2* of the *tidyverse* and extension packages of *ggplot2* including *cowplot* (Wilke, 2019), *ggpubr* (Kassambara, 2018) and *ggsignif* (Ahlmann-Eltze, 2019). Multiple figure panels were combined using the *cowplot* or *gridExtra* package (Auguie, 2017).

3. Results

To aid readers in interpreting data reported here, results on general fish performance and nutrients utilization, which have been published elsewhere (Belghit et al., 2019), are summarized below.

Both diets were readily accepted by the salmon throughout the whole feeding trial. No differences between the diet groups were recorded for feed intake, feed conversion ratio, body weight gain, protein productive value or whole-body proximate composition. Condition factor, hepatosomatic and viscerosomatic indices were not affected by dietary replacement of fish meal with IM. In line with absence of diet effect on the proteinase activity (trypsin and leucine aminopeptidase) and total bile salts level in the chyme, the apparent digestibility of crude protein, crude lipid, amino acids and fatty acids was not affected by dietary IM inclusion.

3.1. Somatic indices of intestinal sections

No significant diet effect was observed for PI-somatic index or MI-somatic index. However, DI-somatic index was significantly higher in fish fed the IM diet ($p < .05$) (Fig. 1).

3.2. Histological appearance

Enterocyte hypervacuolization, suggestive of excessive lipid accumulation (steatosis), was observed in the PI and MI in both diet groups (Fig. 2). It was, however, less severe in the PI of fish fed the IM diet ($p < .05$). Typical signs of enteritis commonly observed in salmonid intestine fed soybean meal diets, including shortening and fusion of mucosal folds (only evaluated for DI), cellular infiltration within submucosa and lamina propria and reduced enterocyte vacuolization (only applicable to DI), were observed in all the intestinal segments in both diet groups (Fig. 2). The only significant diet effect was a higher degree of submucosa cellularity in the PI of fish fed the IM diet ($p < .05$).

3.3. Gene expression

In total, we profiled 36 genes related to immune modulation, lipid metabolism, barrier function and xenobiotic metabolism in the intestine. The diet effect on the gene expression profile was quite minor in the PI and DI. In the PI, matrix metalloproteinase 13 (*mmp13*), a marker gene involved in tissue reorganization, was the only differential expressed gene which showed lower expression levels in fish fed the IM diet ($p < .05$) (Fig. 3). In the DI, choline kinase (*chk*), a marker gene involved in de novo synthesis of phosphatidylcholine, was the only differential expressed gene which showed lower expression levels in

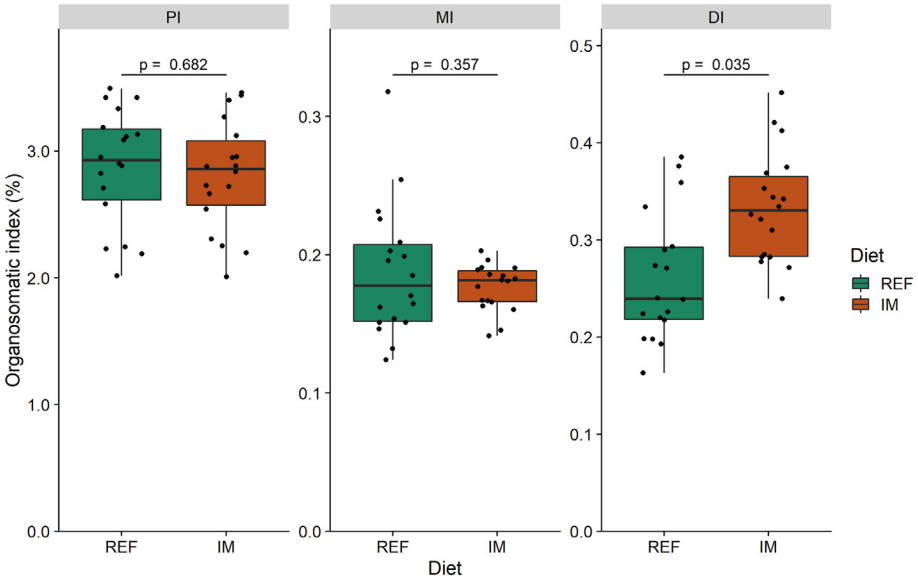

Fig. 1. Somatic indices of intestinal sections of Atlantic salmon fed the experimental diets. The boxplots are in the style of Tukey. PI, proximal intestine; MI, mid intestine; DI, distal intestine; REF, reference diet; IM, insect meal diet.

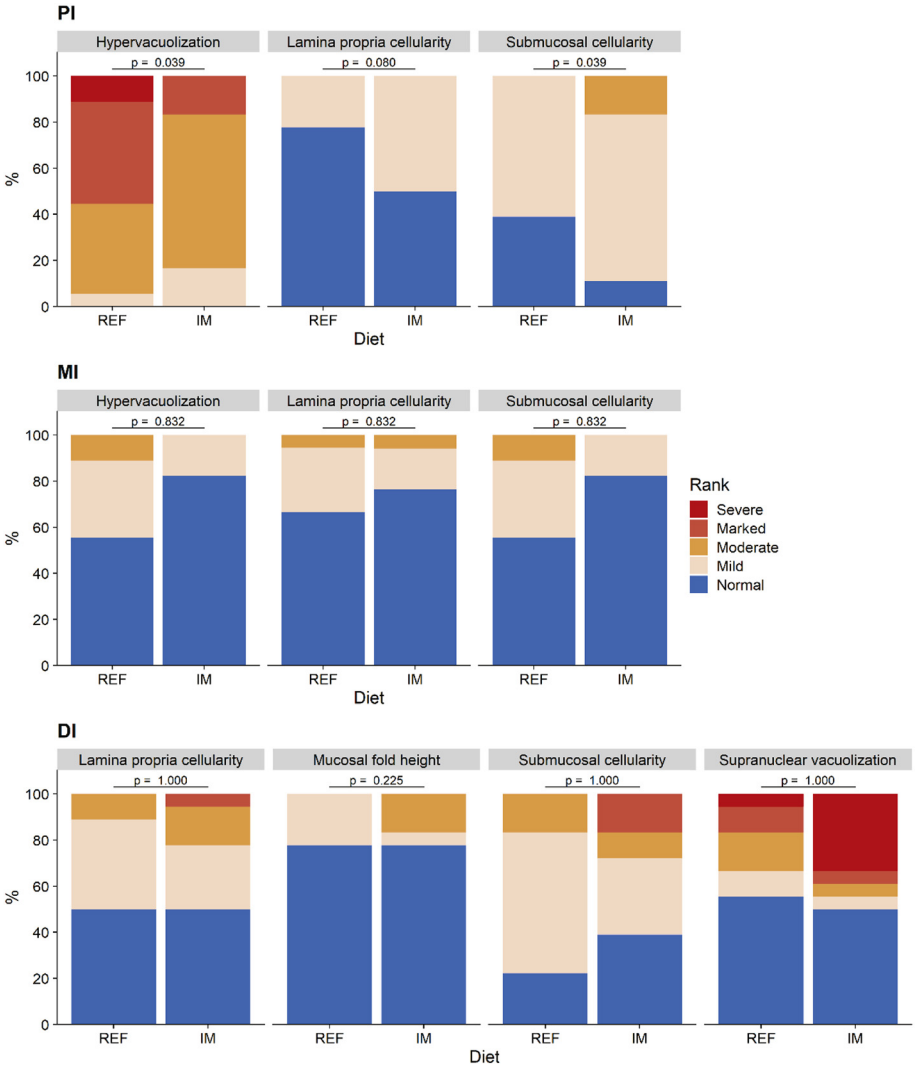

Fig. 2. Contingency chart showing percentages of sampled fish scored normal, mild, moderate, marked and severe regarding enterocyte hypervacuolization (steatosis) and inflammation in different gut segments. PI, proximal intestine; MI, mid intestine; DI, distal intestine; REF, reference diet; IM, insect meal diet.

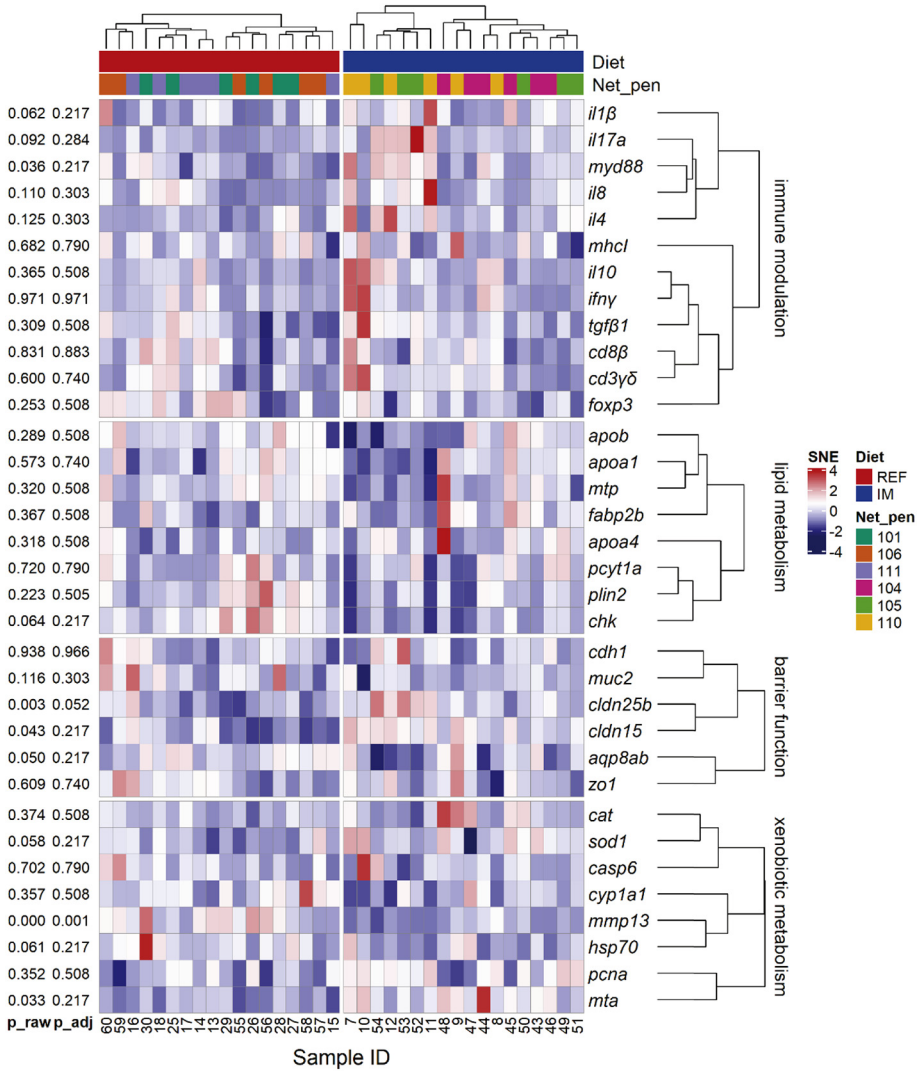

Fig. 3. Gene expression profile in the proximal intestine of Atlantic salmon fed the experimental diets. Data in the same row was scaled (each data point was subtracted by the row mean and divided by the standard deviation). Samples (columns) were clustered within each diet based on the Euclidean distance and genes (rows) were clustered within each functional category based on the Spearman's rank-order correlation. The Ward's minimum variance method was used for the linkage of clusters. For cells in the same row, the deeper the red color, the higher is the gene expression in the respective sample; similarly, the deeper the blue color, the lower is the gene expression in the respective sample. The raw (p_raw) and FDR-adjusted (p_adj) *p* value of diet effect for each gene are shown on the left side of the heatmap. The annotations for the samples (diet and net pen) are given on the top of the heatmap. A supplementary figure showing the normalized expression data before scaling is available as Fig. S1 which displays the data as boxplots overlaid by individual data point. Abbreviations: SNE, scaled normalized expression; REF, reference diet; IM, insect meal diet; see Table S1 for explanations of gene abbreviations. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

fish fed the IM diet as well ($p < .05$) (Fig. 4).

4. Discussion

In the present study, total replacement of fish meal with BSF larvae meal was associated with a lower degree of steatosis in the proximal intestine and a higher relative weight of distal intestine. Furthermore, replacing fish meal with insect meal in the diet of salmon was associated with increased submucosa cellularity in the proximal intestine.

In our previous experiment, pre-smolt Atlantic salmon were fed a test diet for 8 weeks wherein BSF larvae meal accounted for 60% of the total diet ingredients, replacing most of the fish meal and soy protein concentrate in the reference diet (Li et al., 2019). Gene expression analysis showed increased amount of transcripts indicative of uptake of fatty acids (*cd36*, *fabp2*) and cholesterol (*npc1l1*), immune tolerance (*foxp3*), stress response (*hsp70*) and detoxification activity (*cyp1a1*, *mta*, *sod* and *cat*) in the intestine of fish fed the insect meal diet (Li et al., 2019). Given the much lower inclusion level of insect meal in the present study (15% in the diet), it is not surprising that few genes showed differential expressions. Despite the substantial difference on inclusion level of insect meal between the previous study (Li et al., 2019) and the current trial, both studies showed that insect meal diet was associated with lower enterocyte steatosis in the proximal intestine and increased the relative weight of distal intestine.

Enterocyte steatosis is thought to represent a lipid transport or metabolism disorder in enterocytes which in severe cases may be accompanied by accumulations of lipidic materials inside the gut lumen and referred to as lipid malabsorption, eventually resulting in steatorrhea and the so-called “floating feces” on the surface of sea cages (Hanche-Olsen et al., 2013; Penn, 2011). In contrast to the freshwater trial where the enterocyte steatosis was confined to the proximal intestine (Li et al., 2019), it was observed in both proximal and mid intestine in the present seawater trial. Moreover, all the sampled fish showed varying degrees of steatosis in the proximal intestine enterocytes. The higher prevalence and severity of the enterocyte steatosis is possibly related to a higher feed intake of the seawater-phase salmon (Belghit et al., 2018; Belghit et al., 2019), which may exceed the capacity of enterocytes to transport the absorbed nutrients out of cytoplasm. Consistent with our previous finding in the freshwater trial (Li et al., 2019), fish fed the insect meal diet showed a lower degree of enterocyte steatosis in the proximal intestine, which is in line with a lower but insignificant expression level of *plin2*, a surface marker of lipid droplets (Heid et al., 1998). One should be reminded that there were no macroscopic appearances of lipid malabsorption in any fish at the time of sampling, and no apparent indications of reduced fish health as a result of the steatosis. Also, the analysis of total lipid content, lipid class and lipid droplet size and number in the liver showed no diet effect (Belghit et al., 2019).

Consistent with results from the freshwater trial (Li et al., 2019), increased relative weight of distal intestine was also observed in the

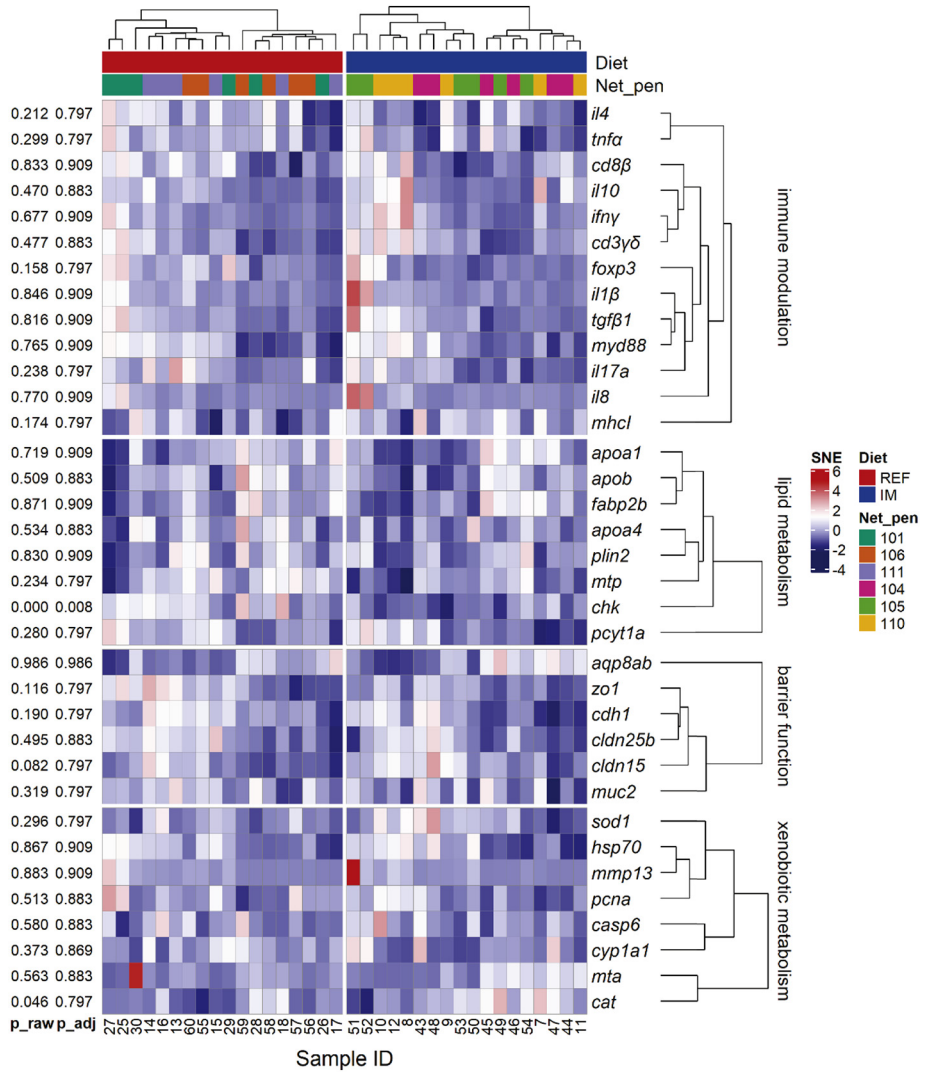

Fig. 4. Gene expression profile in the distal intestine of Atlantic salmon fed the experimental diets. See Fig. 3 for explanations of the graph and abbreviations. A supplementary figure showing the normalized expression data before scaling is available as Fig. S2 which displays the data as boxplots overlaid by individual data point.

present seawater trial. Findings from experiments with chickens may be relevant in comparison. Dietary inclusion of BSF larvae meal (7.3% or 14.6%) was reported to increase the length of jejunum in laying hens (Bovera et al., 2018; Cutrignelli et al., 2018). In another study, laying hens fed a diet containing 17% BSF larvae meal for 21 weeks showed higher concentrations of short chain fatty acids (SCFAs) in the caecal content, including acetate, propionate, isobutyrate, butyrate, isovalerate and valerate. Notably, butyrate nearly tripled in its concentration increasing from 1.5 to 4.4 mmol/L (Borrelli et al., 2017). Butyrate is an important energy source for intestinal epithelial cells. It was estimated to provide 60–70% energy for colonic epithelium in human (Roediger, 1980) and is known to stimulate the proliferation of mucosal cells in colon (Kripke et al., 1989; Mortensen et al., 1999; Souleimani and Asselin, 1993; Whitehead et al., 1986). Whether dietary inclusion of BSF larvae meal may increase the production of SCFAs in the distal intestine of salmon and thus contribute to the increased organ weight remains further elucidation.

Opposed to the absence of gut inflammation in the freshwater trial (Li et al., 2019), signs of inflammation were observed in both diet groups in all the gut segments examined, which is a rare case. While gut inflammation has also been reported in farmed salmon fed commercial diets, it's usually only present in the distal intestine (Chikwati et al., 2018). The exception was when a parasitic infection occurs, such as tapeworm or nematode infection, causing inflammation throughout the whole intestine (Chikwati et al., 2018; Murphy et al., 2010). However, no parasitic infection was noted during the feeding trial or at the time of sampling. Given that the feeding trial was commenced with fish already at sizes averaging 1.4 kg, and no basal gut health assessment was conducted prior to start of the trial, it is hard to rule out historical exposure to inflammation-inducing diets and/or parasites. It is thus a good practice to conduct a basal gut health evaluation of experimental fish (> 100 g) before assignment to feed groups to minimize pre-existing gut health disorders that may diminish trial outcomes and goals.

Recent studies on the nutritional value of BSF larvae meal for rainbow trout (*Oncorhynchus mykiss*) (10.5%, 21%) (Cardinaletti et al., 2019), clownfish (*Amphiprion ocellaris*) (20%, 40%, 60%) (Vargas-Abúndez et al., 2019) and zebrafish (*Danio rerio*) (25%, 50%) (Zarantoniello et al., 2019) have not revealed signs of gut inflammation. Furthermore, its inclusion increased the expression of *foxp3*, a master transcription factor for the differentiation of naïve CD4 T cells into regulatory T cells, in the proximal and distal intestine of salmon in our freshwater trial (Li et al., 2019). In the present seawater trial, however, increased submucosa cellularity was found in the proximal intestine of salmon fed the insect meal diet. Possible explanations are: 1) Atlantic salmon prey on insects in the freshwater before they finish smoltification and migrate to the sea. Hence, the gut immune system of

salmon might have a higher tolerance of insect ingredients in the freshwater than in the seawater. 2) The increased submucosa cellularity was possibly, already present in the fish prior to start of the trial but the experimental diets improved the gut health in the proximal intestine to differing levels, with the reference diet performing better than the insect meal diet in reducing the severity of inflammatory changes. It should be noted that none of the proinflammatory marker genes profiled in the proximal intestine showed differential expressions. Neither did we observe comprised gut functions as a result of the increased submucosa cellularity.

In conclusion, total replacement of fish meal with black soldier fly larvae meal did not compromise the gut health of Atlantic salmon. Dietary insect meal inclusion seemed to reduce excessive lipid deposition within enterocytes (steatosis) in the proximal intestine. Possible interactions between insect meal inclusion and the development of gut inflammation in seawater-phase salmon is worth of attention in future studies.

Data and code availability

The data and code used for the statistical analyses and creation of figures are deposited at the GitHub repository (https://github.com/yanxianl/AquaFly-SeawaterGutHealth-Aquaculture-2019).

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Declaration of Competing Interest

The authors declare no competing financial interest.

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Appendix A. Supplementary data

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