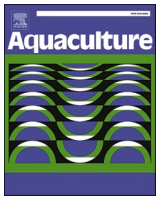
Dietary replacement of fishmeal with marine proteins recovered from shrimp and herring process waters promising in Atlantic salmon

Aquaculture 574 (2023) 739735

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture



Dietary replacement of fishmeal with marine proteins recovered from shrimp and herring process waters promising in Atlantic salmon aquaculture

Ida Hedén [a,*], Bita Forghani Targhi [b], Gunvor Baardsen [c], Bjørge Westereng [d], Tore Svendsen [e], Elisabeth Jönsson [a], Linda Hasselberg Frank [a], Ingrid Undeland [b], Kristina Sundell [a], Henrik Sundh [a]

[a] Department of Biological and Environmental Sciences, SWEMARC, University of Gothenburg, Box 463, 405 30 Gothenburg, Sweden
[b] Department of Biology and Biological Engineering–Food and Nutrition Science, Chalmers University of Technology, SE 412 96 Gothenburg, Sweden
[c] Skretting ARC, P.O. Box 48, 4001 Stavanger, Norway
[d] Faculty of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, 1433, Aas, Norway
[e] Vandrensning.com, Kildemarksvej 3, 4200 Slagelse, Denmark

ARTICLE INFO

Keywords:
Aquaculture
Alternative feed
Side stream
Circular economy

ABSTRACT

There is a general agreement that fish meal (FM) and fish oil (FO) are valuable resources for aquafeed, but that the production cannot keep the same pace as the current growth of the aquaculture industry. Therefore, there is a need to find alternative sources for lipids and protein. This study examines the possibility of using proteins recovered from seafood industry side stream waters as a complement to FM in feed for Atlantic salmon. To recover the proteins, herring and shrimp process side streams waters were flocculated then treated with dissolved air flotation (DAF), which is considered a gentle technology. Shrimp steam water was used to obtain shrimp protein (SP) and herring salt brine to obtain herring protein (HP). The recovered semi-solid protein fraction was spray dried and formulated into experimental diets at inclusion levels of 1.9–10% (dw/dw). Two feeding trials were conducted to investigate; 1) the properties of different flocculants to recover SP from shrimp steam water, alginate (Alg; 1.9% inclusion), carrageenan (Carr; 2.0% inclusion) and a synthetic flocculant from Kemira (Kem; 3.8% inclusion). 2) total or partial replacement of FM with SP-Alg (10% and no FM) and HP-Alg (3% and 8% FM). For both feeding trials each diet was provided to triplicate tanks ($n = 31$ and 30/tank) of Atlantic salmon with a start weight of 193 g (duration 10 weeks) and 304 g (duration 7 weeks) respectively.

All fish showed similar feed intake, feed conversion ratio (on tank basis) and weight gain. The inclusion of SP or HP as total or partial replacement of FM did neither influence adiposity of the fish, as measured by condition factor, nor heposomatic index (HSI). No negative effect of the alternative protein could be found through histological examination of the intestine. In feeding trial two, diets did not affect the adaptive immune indicators CD8α and MHC II. The SP-Alg diet did not affect intestinal barrier and transporting functions, assessed using Ussing-chamber technology. However, HP-Alg affected the trans-epithelial resistance, which indicate that the intestinal barrier function could be affected by low inclusions. We conclude that from a biological perspective, SP recovered from shrimp steaming waters using Alg and DAF technology represents a new marine biomass with potential as a replacement for FM in Atlantic salmon feed. To diversify the possibility of using flocculants to retrieve proteins we also suggest further investigation of the potential to use Carr in larger inclusions.

1. Introduction

The production of Atlantic salmon has increased with 140% between 2000 and 2017 and is expected to increase to a yearly production of 3.300 t by 2025 (FAO, 2022; Tacon, 2020). The current use of fish meal (FM) from wild fisheries in fish feed has its limitations because of stagnated catches, and unpredictable availability due to natural events, and increasing feed production, not only for aquaculture (FAO, 2022).

* Corresponding author.
E-mail address: ida.heden@bioenv.gu.se (I. Hedén).

https://doi.org/10.1016/j.aquaculture.2023.739735
Received 3 March 2023; Received in revised form 19 May 2023; Accepted 28 May 2023
Available online 30 May 2023

The inclusion of FM in feed for Atlantic salmon has been significantly reduced the past decades, and mainly replaced by products from terrestrial plants and today only approximately 25% of the ingredients come from marine origin (Aas et al., 2019; FAO, 2022; Ytrestøyl et al., 2015). However, inclusion of plant-derived ingredients in Atlantic salmon diets can have negative effects on growth performance, feed utilization and fish health due to imbalanced nutrient composition and anti-nutritional factors (Gatlin et al., 2007; Turchini et al., 2009; Krogdahl et al., 2010). Replacing FM with land-based alternatives also raise concerns because of conflicts with other human interests such as direct food production for human consumption and that they depend on land- and water supplies (FAO, 2022; Froehlich et al., 2018; Pahlow et al., 2015; Tacon et al., 2022). As the aquaculture industry continues to expand, the current replacement does not cover the predicted future need for sustainable protein sources in feed production (FAO, 2022; Gatlin et al., 2007; Hardy, 2010; Sprague et al., 2016; Stone et al., 2011). Therefore, there is a demand for alternative protein sources with adequate nutritional profile and palatability.

One marine resource that is currently being underutilized is process water emerging from the seafood processing industry. Water is used during handling, transport, processing (e.g. boiling, peeling) and storage (e.g. brining) (Chowdhury et al., 2010). The volume of process water consumed in shrimp and herring industries is estimated to be ~50 m^3/ton peeled shrimp and ~ 7 m^3/ton final marinated herring product, respectively (Forghani et al., 2020a). Upon use, these waters get enriched in proteins (≤90 kg/m3 water) and fatty acids (≤20 kg/m3) (Amado et al., 2016; Forghani et al., 2020a; Jarrault et al., 2017; Rosa and Nunes, 2004), but with common practices, these nutrients are lost from the food chain. Normally, seafood process waters are treated with synthetic hazardous coagulant-flocculants or synthetic flocculants to remove organic matter. This routine makes the recovered biomass unsuitable for food or feed purposes (Chowdhury et al., 2010; Fahim et al., 2000). However, replacing these flocculants with natural flocculants such as carrageenan or alginate can turn this currently wasted resource into a valuable food/feed raw material at the same time as the environmental impact of the water discharge is reduce.

The fish intestine is one of the first organ to be exposed to new ingredients in feed and its compositions may affect the intestinal health, including barrier and transporting functions (Knudsen et al., 2008; Krogdahl et al., 2010). In fish, the intestine is a multifunctional organ, being responsible for nutrient uptake and osmoregulation at the same time as it acts as a barrier for harmful luminal content such as antigens and pathogens (Jutfelt, 2011; Sundell and Sundh, 2012; Taylor and Grosell, 2006; Whittamore, 2012). The barrier function is mediated by different layers. 1) The extrinsic mucus barrier consisting of secreted mucins alongside antimicrobial and antiviral substances. 2) The intrinsic physical barrier with the intestinal epithelial cells connected at the apical membrane through tight junctions (Günzel and Yu, 2013). Antigens and pathogens that manage to breach the extrinsic and intrinsic barrier will be exposed to the third part of the barrier, the gut-associated lymphoid tissue (GALT) (Rombout et al., 2014). A well-established example of when dietary content has negative effect on the intestinal health in Atlantic salmon is the case of extracted soybean meal (SBM). SBM leads to an inflammatory response that results in reduced mucosal fold height, loss of absorptive vacuolization and widening of the central stroma (Krogdahl et al., 2010). This is accompanied by an increased epithelial permeability (Knudsen et al., 2008), and upregulated intestinal immune response (Bakke-McKellep et al., 2007a; Lilleeng et al., 2009; Marjara et al., 2012; Romarheim et al., 2013).

Maximal conversion of the feed into somatic growth is a prerequisite for any novel protein source to ensure economic and biological feed efficiency (Tacon et al., 2022). The somatic growth in fish is regulated by the growth hormone (GH)/insulin-like growth factor-1 (IGF—I) system (Norbeck et al., 2007; Reindl and Sheridan, 2012). IGF-I is a peptide hormone that belongs to the family of polypeptide growth factors related to proinsulin and mediates growth effects of growth hormone (GH) (Duan, 1997; Maures et al., 2002). Increased IGF-I plasma concentrations correlate with increased growth performance and has therefore been suggested to be used as an growth indicator (Duan, 1997; Duan et al., 1995; Dyer et al., 2004; McCormick et al., 1992; Pérez-Sánchez et al., 1992).

The aim of this study was to examine if proteins recovered from shrimp and herring process waters using natural flocculants are suitable to use in feed for Atlantic salmon. The objectives were to 1) compare the effect on fish growth and welfare after feed inclusion of proteins recovered using three flocculants; alginate (Alg), carrageenan (Carr) and Kemira (Kem) from shrimp process water and 2) select the most promising flocculant and evaluate complete or partial replacement of fish meal by inclusion of proteins derived from shrimp and herring process waters.

2. Material and method

2.1. Ingredient processing

Shrimp and herring protein biomass were produced at two separate occasions and used in two different feeding trials. The protein was recovered according to the principal described by Forghani et al. (2020a). In short, proteins of the process water were aggregated using flocculants. The aggregates were then recovered through dissolved air flotation (DAF) and spray dried (Anhydro Lab s3 dryer) resulting in protein-rich powder. For inclusion in feeding trial I, shrimp protein (SP) was recovered from shrimp steaming water in pilot scale using three different flocculants: two natural flocculants Alg, Carr and a synthetic flocculant from Kem (C-592). In feeding trial II, Alg was chosen as main flocculant because it was considered a natural, food grade flocculant and had beneficial properties for retrieval of proteins, and that it had no negative effects on fish growth. Further, even though Carr performed equally well in terms of protein retrieval and fish growth it was excluded since it was not approved within the European Union (EU 2017/1145) as a technological additive at the time of the trials. For the second feeding trial herring protein (HP) was also recovered from herring salt brine using Alg and the previously mentioned method. Both SP-Alg and HP-Alg produced were frozen after their recovery. In the spray drying, ~400 L of frozen flocculated biomass recovered from ~8000 L shrimp processing water and 170 L frozen flocculated biomass recovered from ~2500 L herring salt brine was thawed, homogenized with a pilot scale blender, and dried on a Gea FSDTM-6.3 Fluidized Spray Dryer, with 180 °C inlet temperature, 70 °C outlet temperature and 50 °C fluid bed temperature. The drying yielded 19.5 and 3.7 kg dried material of SP-Alg and HP-Alg concentrate respectively, containing 55.9–62.1 and 48.1% protein (dry weight (dw) basis), respectively (Table 1). The proximate composition of the protein powders produced for feeding trial I and II is summarised in Table 1 and the amino acid profile is presented in Table 2.

Table 1
Proximate composition of protein powders produced for **feeding trial I** and **II**, SP Alg = alginate, SP Carr = carrageenan, SP Kem = a synthetic flocculant from Kemira, recovered from shrimp process water, HP Alg = alginate recovered from herring salt brine, dw = dry weight.

		Protein % dw	Lipids % dw	Ash % dw	Moisture %
Feeding trial I	SP Alg	61.6	21.4	9.6	6.1
	SP Carr	66.2	14.0	8.2	8.1
	SP Kem	55.9	24.2	9.4	9.8
Feeding trial II	HP Alg	48.1	11.2	33.6	7.4
	SP Alg	62.1	26.7	2.7	7.0

Table 2
Amino acid profile of protein powders produced for **feeding trial I** and **II**, SP Alg = alginate, SP Carr = carrageenan, SP Kem = a syntehtic flocculant from Kemira, recovered from shrimp process water, HP Alg = alginate recovered from herring salt brine, dw = dry weight.

	Feeding trial I			Feeding trial II	
	SP Carr	SP Alg	SP Kem	HP Alg	SP Alg
Amino acid	Amount (mg/g dw)				
GLY	53.0	51.9	33.3	16.1	31.6
ALA	36.1	28.7	24.9	22.0	27.1
SER	17.1	12.9	11.9	14.1	21.3
PRO	17.8	17.7	14.5	12.8	19.5
VAL	28.3	23.4	19.5	17.7	14.7
THR	21.4	17.4	15.2	13.0	12.2
TAU	0.8	1.3	0.5	–	–
HYP	0.8	0.5	0.6	0.3	0.2
ILE	29.7	25.2	20.2	23.0	14.6
LEU	49.9	39.1	32.1	36.3	31.2
ASN	1.0	1.0	0.8	–	–
ASP	75.0	54.8	41.6	34.7	37.6
LYS	55.2	41.7	33.3	31.3	26.8
GLU	79.4	56.3	43.6	44.2	50.5
MET	15.5	12.4	10.5	11.6	11.4
HIS	14.1	10.4	7.8	13.4	7.8
PHE	30.2	24.2	19.3	15.5	17.8
ARG	49.6	37.0	27.1	52.2	33.5
TYR	3.2	2.3	1.9	15.1	16.6

2.2. Experimental animals and holding conditions

2.2.1. Feeding trial I

SalmoBreed Atlantic salmon, Erfjord stamfisk (Rogaland, Norway), was transferred to Lerang research station, Norway as eyed eggs 13 of January 2016 (fertilized 5 of November 15). The fish was deemed at the peak of smoltification and transferred to seawater on 3 October 2016. Atlantic salmon of both sexes were weighed and divided into four dietary treatment groups, 31 individuals ($n = 93$/diet) with a standardized initial average weight of 193 ± 1.7 g in triplicate tanks (circular, 450 L) (Table 3). The fish were kept in seawater flow through system with a temperature of 12 °C. The experiment was conducted over 72 days (January to March 2017). The feed was produced using extrusion technology as sinking pellets by Skretting ARC and had a formulated content of 43% protein, 28% fat and 8% moisture (Table 3). The control diet contained 10% FM and 30% soybean protein concentrate and the three experimental diets were composed by 10% FM, whereas the inclusion of soybean protein concentrate (SPC) and wheat gluten were reduced when SP was included (Carr;1.86%, Alg; 2.00% and Kem; 3.84%) with the aim of reaching the same protein replacement. The fish were fed in excess three times per day. Un-eaten pellets were collected after each feeding in a strainer and dried. Some fish sexually matured during the trial with an average gonadosomatic index of 6.5% at the end sampling, these fish were included in the analysis since they were equally spread across diets. No mortality was observed during the feeding trial.

2.2.2. Feeding trial II - total and partial replacement of FM

A new cohort of Atlantic salmon, SalmoBreed, Erfjord stamfisk (Rogaland, Norway), was transferred to Lerang research station as eyed eggs 5 of March 2016 (fertilized 4 of October 2016). Peak of smoltification reached and fish was transferred to SW on 6 September 2017. Atlantic salmon of mixed sexes was kept in the same conditions previously mentioned in section 2.2.1. The experiment was conducted over 52 days (48 days of feeding; January to February 2018). Fish were divided into three dietary groups kept in triplicate tanks, and each tank (circular, 450 L) contained 30 individuals ($n = 90$/diet) with an initial mean weight of 304 ± 3.4 g. Feed was produced as mentioned in the previous section. The feed was formulated to contain 44% protein and 27% fat (Table 4). The control diet contained 10% FM and ~ 28% soy protein concentrate (SPC) while in the experimental diets, the FM was partially or fully replaced by HP-Alg or SP-Alg. The experimental diets contained 3% HP-Alg and 8% FM, or 10% SP-Alg. The fish were feed three times per day. Un-eaten pellets were collected after each feeding and dried. There was no mortality during the feeding trial and no signs of maturity was observed.

2.3. Growth assessment

Measurement of length and weight were done at the start and end sampling. Feed intake was assessed continuously in both feeding trial I and II.

Growth rate, feed conversion ratio (FCR), and condition factor (K) were calculated on a tank basis, and hepatosomatic and gonadosomatic index (on fish that had sexually matured during the experiment) on individual basis, according to the following formulas:

$$\text{Specific growth rate (SGR, \%/d)} : \left[\left(\frac{Ln\ W_{Final}}{Ln\ W_{Initial}}\right)^{1/\Delta T} - 1\right] * 100.$$

Where W_{Final} was the final fish Ln of weight (g) per tank and $W_{Initial}$ was the initial fish Ln of weight (g) per tank, and T is the time in days.

$$\text{Relative growth rate (RGR)} : \frac{\left(\frac{W_{Final} - W_{Initial}}{W_{Initial}} * 100\right)}{T}$$

Where W_{Final} was the final fish weight (g) per tank, $W_{Initial}$ was the initial fish weight (g) per tank, and T is the time in days

Table 3
Feed formulation in **feeding trial I** with extraction of shrimp protein powder from shrimp processwater using three different flocculants SP Alg = alginate, SP Carr = carrageenan, SP Kem = a syntehtic flocculant from Kemira.

Ingredient (%)	Control	SP Carr	SP Alg	SP Kem
Shrimp powder with alginate	–	2.00	–	–
Shrimp powder with carrageenan	–	–	1.86	–
Shrimp powder with Kemira	–	–	–	3.84
Fish meal Nordic	10.00	10.00	10.00	10.00
Wheat	6.74	7.21	7.09	7.36
Corn gluten, CG*	4.89	4.71	4.72	4.57
Wheat gluten, WG*	14.31	13.77	13.79	13.37
Faba beans, dehulled*	3.30	3.17	3.18	3.08
Soya protein concentrate, SPC*	30.00	28.87	28.92	28.04
Rapeseed oil	13.82	13.82	13.82	13.82
Fish oil Nordic	10.62	10.26	10.42	9.81
Water/Moister change	1.62	1.48	1.53	1.40
Astaxanthin 10%	0.04	0.04	0.04	0.04
Premixes/micro ingredients	4.72	4.67	4.63	4.65

Table 4
Feed formulation of diets in **feeding trial II** with extraction of herring protein powder (HP Alg) from herring salt brine and shrimp protein powder (SP Alg) from process water using alginate.

Ingredient (%)	Control	HP Alg	SP Alg
Shrimp powder with alginate	–	–	10.00
Herring powder with alginate	–	3.00	–
Fish meal Nordic	10.00	8.00	–
Wheat	8.65	7.75	7.66
Corn gluten, CG	4.00	4.02	4.10
Wheat gluten, WG	18.04	18.05	18.41
Faba beans, dehulled	3.00	3.02	3.08
Soya protein concentrate, SPC	28.00	28.15	28.72
Rapeseed oil	11.70	11.75	10.99
Fish oil Nordic	11.89	11.94	11.17
Water/Moister change	0.96	1.02	1.42
Astaxanthin 10%	0.04	0.04	0.04
Premixes/micro ingredients	3.71	3.25	4.41

$$\text{Feed conversion ratio (FCR)} : FCR = \frac{C_T}{W_{Final} - W_{Initial}}$$

Where C_T is total feed consumption (kg), W_{Final} was the final fish weight (kg) per tank and $W_{Initial}$ was the initial fish weight (kg) per tank.

$$\text{Fulton}'\text{s condition factor (K)} : K = 100 \Big/ \left(\frac{W}{L^3}\right)$$

Where W is mass (g), and length is L (cm).

$$\text{Weight gain (\%)} : WG = \frac{(W_{Final} - W_{initial})}{W_{Initial}} * 100$$

Where W_{Final} is the final fish weight (g) per tank and $W_{Initial}$ is the initial fish weight (g) per tank.

$$\text{Heposomatic index (HSI)} : HSI = \frac{L_W}{B_W} * 100$$

Where L_W is the individual liver mass (g), and B_W is the individual body weight (g)

$$\text{Gonadosomatic index (GSI; \%)} : GSI = \frac{G_W}{B_W} * 100$$

Where G_W is the individual gonad mass (g), and B_W is the individual body weight (g)

2.4. Tissue sampling

2.4.1. Feeding trial I

On the sampling day, 12 Atlantic salmon from each diet, four individuals from each of the triplicate tank, were euthanized with MS-222 (200 mg l^{-1}) followed by a blow to the head. The fish was then opened laterally, and the intestine and mesenteric fat carefully removed. A 1 cm vertical section was sampled from the proximal (from pyloric caeca to distal intestine), and distal intestine (from the base of proximal intestine to anus) and fixated in 4% buffered paraformaldehyde (pH 7.2) for 24 h, dehydrated and embedded in paraffin wax. Length and weight were measured from all fish in each dietary treatment.

2.4.2. Feeding trial II

The sampling took place over three consecutive days. On each day, 8 Atlantic salmon were sampled from randomly chosen tanks at two time points. This procedure resulted in a total of $n = 12$ fish per diet. The fish was euthanized with MS-222 (200 mg l^{-1}). Length and mass were then recorded from each fish. Blood was sampled through caudal puncture with a heparinized syringe and transferred to a microtube that was kept on ice until it was centrifuged (5 min, 3000 *g* 4 °C). Plasma was transferred to a new microtube and stored in a − 80 °C freezer until analysis. The Atlantic salmon were then opened laterally, and the intestines were carefully dissected out and rinsed from mesenteric fat. The proximal (from pyloric caeca to distal intestine), and distal intestine (from the base of proximal intestine to anus) was sampled and opened longitudinally with a bull pointed scissor, and faeces was removed. The intestine was rinsed in ice cold ringer solution (SW; 150 mM NaCl; 2.5 mM KCl; 2.5 mM $CaCl_2$; 1 mM $MgCl_2$*$6H_2O$; 7 mM $NaHCO_3$; 0.7 mM NaH_2PO_4*$2H_2O$; 5 mM HEPES; 0,5 mM L-lysine HCl; 10 mM D-Glucose; 20 mM L-Glutamine; pH 7.8). The first 5 mm of each intestinal segment was sampled for histology and immunohistochemistry. These intestinal tissues were fixated in 4% PFA for 24 h in room temperature, and then transferred to 70% ETOH. Tissues were dehydrated through an alcohol gradient, Histolab-clear (Histolab Products AB, Gothenburg, Sweden), and embedded in paraffin wax using standard procedures. The rest of the proximal and distal intestine was kept in ice-cold ringer solution before being mounted in Ussing chambers. The liver was removed and weighed for calculation hepatosomatic index (HSI). Muscle was sampled using the standardized method, the so called "Norwegian quality cut" (NQC) (Johnsen et al., 2011), where a 2 cm trans-sectional cut of the whole fish was made from the same region, from the caudal to the dorsal fin, on all fish and stored in a − 80 °C freezer until further analysis.

2.5. Plasma IGF-I levels

IGF-I plasma concentrations in feeding trial II were determined using a homologous salmonid radioimmunoassay (RIA) on acid/ethanol-extracted plasma (Shimizu et al., 2000), following the general protocol of Moriyama et al., 1994.

2.6. Intestinal transport and barrier functions in feeding trial II

Intestinal transport and barrier function were assessed using the Ussing chambers methodology, previously described by Sundell et al., 2003 applying modifications reported by Sundell and Sundh, 2012. At each sampling, proximal and distal intestines from eight fish were mounted in Ussing chambers and each half-chamber filled with 4 ml Ringer's solution. Transepithelial resistance (TER), transepithelial potential (TEP), and short circuit current (SCC) were measured every 5 min. The intestines were allowed to acclimate in the chamber in fresh Ringer's solution for 60 min to reach steady state of the electrical parameters. At $T = 60$ the Ringer was exchanged with new fresh plain Ringer's on the serosal side. In the mucosal chamber, the fresh Ringer's contained ^{3}H-L-lysine in presence of 0.5 mM cold L-lysine (specific activity 3.1 × 10^3 MBq mol^{-1}) and the paracellular marker molecule ^{14}C-mannitol (specific activity 0.036 MBq mL^{-1}) (PerkinElmer, Waltham, MA, USA) for assessment of tissue integrity. Assessment of accumulated radioactivity over 60 min was initiated by withdrawing 100 μL from both chambers at $T = 0$. At $T = 20$, 25, 30, 60, 80, 85 and 90 min 100 μL was withdrawn from the serosal chamber only in exchange for new Ringer without ^{3}H-Lysine. The withdrawn volume of the serosal side was replaced by plain Ringer to sustain chamber volume during the experiment. Five mL scintillation fluid (Ultima Gold, PerkinElmer, Waltham, MA, USA) was added and radioactivity was determined using a β-counter (Wallac 1409 Liquid Scintillation Counter, Turku, Finland).

The mucosal to serosal L-lysine transport across the epithelium was calculated according to equation:

$$l - lysine\ transport = \frac{dQ/dt}{A}$$

Where dQ/dT is the accumulation of L-lysine (mol) over time (min) divided intestinal tissue area (A = 0.75 cm^2).

The apparent permeability coefficient (P_{app}) for mannitol was calculated using the equation:

$$P_{app} = \frac{dQ}{dt} * \frac{1}{AC_0}$$

Where dQ/dT is the accumulation of ^{14}C-mannitol (mol) on the serosal side over time (s) of, and C_0 is the initial concentration of ^{14}C-mannitol on the mucosal side (mol/ml) and exposed tissue area (A = 0.75 cm^2).

2.7. Histology and immunohistochemistry

Intestinal morphology was evaluated in feeding trial I and II. Sections (6 μm) of the proximal and distal intestine were produced with a Shandon finesse microtome (Thermo Fisher Scientific, Waltham, MA, USA), and mounted on APES (3-Aminoprepyl triethoxysilane, Sigma-Aldrich, Stockholm, Sweden) treated microscope slides, dried at 37 °C for 24 h. Slides were stained with a combination of haematoxylin-eosin and alcian blue 8 GX, pH 2.5. Photographs were taken at 10× magnification (manual WSI scanning software, Microvisioneer, Esslingen am Neckar, Germany) connected to a Nikon eclipse E1000 microscope. Signs of inflammation were graded according to the method described by (Baeverfjord and Krogdahl, 1996; Krogdahl et al., 2003). In short, the morphological traits were visually evaluated were 1. the length and

width of the intestinal folds, 2. loss of enterocytes and supranuclear vacuolization, 3. increased amount of connective tissue, and increased width of the central lamina propria in the intestinal folds, 4 increased presence of leucocytes in the lamina propria and submucosa. The assessed traits were graded with 1. for normal structure, with no signs of inflammations and 2. for moderate changes and 3. for severe signs of inflammation, the data is presented as average score ± SEM.

Species-specific monoclonal mouse antibodies directed towards Atlantic salmon cluster of differentiation 8 ($CD8\alpha^+$) and major histocompatibility complex II ($MHC\ II^+$), previously validated by Hetland et al. (2010), were used for assessment of diet effects on the mucosal immune system. Microscope slides with intestinal tissue were produced as described in the previous section. The tissue sections were placed in a 60 °C owen for 30 min, then rehydrated by removing the wax with Histoclear (Histolab Products AB, Gothenburg, Sweden), and transferred through a EtOH-gradient (100–70%). After rehydration, the tissue was subjected to heat-induced epitope retrieval in 10 mM Tris-Base, 1 mM EDTA, 0,05% Tween, pH 9.0 at boiling temperature for 30 min. Endogenous peroxidase activity was quenched for 30 min in 3% H_2O_2, 30% methanol in tris-buffer saline (TBS; 5% tween; pH 7.6). Un-specific protein interaction was blocked by incubation in blocking solution (TBST, 3% bovine serum albumin, 5% normal goat serum, 5% normal donkey serum: AH diagnostics, Sweden), for 1 h at room temperature. The blocking solution was removed, and the sections were incubated with primary antibodies CD8α + (1:200), and $MHC\ II^+$ (1:200) diluted in protein blocking solution in a humid chamber overnight at 4 °C. The tissues were rinsed in TBS. Secondary antibody, donkey anti-mouse (GE Healthcare, Buckinghamshire, UK) at a concentration of 1:1000 was applied and incubated for 1 h in room temperature. After incubation, the samples were rinsed in phosphate-buffer saline (PBS; 5% tween; pH 7.2) followed by incubation with Avidin-Biotin Complex according to protocol (Vector laboratories, Burlingame, USA). Sections were rinsed in PBS and stained in Vector NovaRed (Vector laboratories, Burlingame, USA) for 10 min according to the manufacturer's protocol. Photographs were taken as described in previous section. The pictures were taken with 10 x magnification and the length and number of cells was counted in approximately 10–15 mucosal folds in the proximal and distal intestine from each fish. Data is presented as cells / mm of epithelia ± SEM. Analyses of epithelial length and number of immune cells were performed using ImageJ (Rueden et al., 2017).

2.8. Colorimetric assessment

Pigment dispersal in the right and left NQC-fillets from Atlantic salmon in feeding trial II ($n = 12$) was determined using Konica Minolta Chroma Meter CR-200 (Osaka, Japan), each fillet was measured 6 times in different location to retrieve an average value per fillet. The equipment is computerized to measure L*values (brightness), a*values (green-red), and b*values (yellow-blue). The data was the handled with SpectraMagic NX software.

2.9. Compositional analyses of SP and HP

Crude protein of the dried SP/HP powders was analysed by a commercial lab (Eurofins Sweden) using the Kjeldahl method and a conversion factor of 6.25. Dry matter and ash contents were measured gravimetrically after drying the samples at 105 and 550 °C, for 24 and 3 h, respectively. Total lipids were analysed gravimetrically after extraction with chloroform and methanol according to the method described by Forghani et al. (2020a). Amino acids for study 1 were analysed by a commercial lab (Eurofins) using the method of Fontaine et al. (2000). Amino acids for study 2 were analysed in house with liquid chromatography-mass spectrometry (LC-MS) after acid digestion as described by Forghani et al. (2020b). Results were reported as mg amino acid/g dried powder. It should be stressed that levels of methionine and tryptophan could be underestimated due to susceptibility to degradation during the acid hydrolysis treatment.

2.10. Statistics

Growth data (SGR and FCR),HSI, IGF-I concentration, intestinal function, CD8 and MCH II cell count data as well as colorimetric data were assessed with One-way ANOVA followed by multiple comparison test (Student-Newman-Keuls test, *P*-value lower than 0.05, considered significant). Normal distribution and homogenous variances were assessed using visual histogram and QQ plots and Shapiro-Wilks test while Levene's test was used for assessment of homogeneity of variances. If the data failed to meet the criteria for parametric test, it was transformed in order of LOG and SQRT before further analysis. Non-parametric data such as histological score in feeding trial I and II was analysed using Kruskal-Wallis test. Statistical analysis was performed using GraphPad prism (version 9.3.1) or SPSS statistical software from (Version 24, IBM Corp, Armonk, NY, USA).

3. Results

3.1. Feeding trial I

None of the three different flocculants Carr, Alg and Kem used to recover SP for use in feed to Atlantic salmon in feeding trial I did affect the growth, FCR or condition factor (K) of the fish in comparison to the regular FM + SPC diet ($P > 0.05$) (Table 5). There were only minor changes in the mucosal morphological traits measured and those were spread across diets. There were no differences among groups in mucosal fold width or hight (Control ($n = 3$), 1.00 ± 0; Alg (n = 3), 1.00 ± 0; Carr (n = 3), 1.00 ± 0; Kem (n = 3), 1.00 ± 0), and no effect of diet on absorptive tissue (Control (n = 3), 1.00 ± 0; Alg (n = 3), 1.00 ± 0; Carr (n = 3) 1.00 ± 0; Kem (n = 3), 1.33 ± 0.16). Some fish in the control and Alg diet had moderate changes with swollen lamina propria and increased amount of connective tissue (Control (n = 3), 1.33 ± 0.14; Alg (n = 3), 1.33 ± 0.14; Carr (n = 3) 1.00 ± 0; Kem (n = 3); 1.00 ± 0), further, there was moderate non-significant infiltration of mixed leukocyte population in the lamina propria and submucosa (Control (n = 3), 1.33 ± 0.14; Alg (n = 3), 1.33 ± 0.14; Carr (n = 3) 1.00 ± 0; Kem (n = 3), 1.00 ± 0).

3.2. Feeding trial II

3.2.1. Growth

There was no significant effect of diet on growth or on HSI ($P > 0.05$; Table 6). IGF-I plasma concentrations were significantly different among dietary groups ($P < 0.05$). Post hoc test revealed that IGF-I levels were higher in the Atlantic salmon fed with SP-Alg in comparison to the HP-Alg diet ($P < 0.05$; Fig. 1).

Table 5
Growth performance and feed intake of Atlantic salmon fed with experimental diets in **feeding trial I**: Control (10% fish meal) (n = 3), and SP Alg (2.0% alginate; n = 3), SP Carr (1.86% carrageenan; n = 3), and SP Kem (3.84% kemira; n = 3) in replacement for soy bean concentrate.

Diet	SGR %/d Mean ± SD	RGR %/d Mean ± SD	FCR - Mean ± SD	Final Weight g Mean ± SD	Weight gain g Mean ± SD	K g/cm^3 Mean ± SD
Control	1.37 ± 0.02	2.38 ± 0.05	0.73 ± 0.01	519 ± 1.3	327 ± 7.4	1.34 ± 0.02
SP Alg	1.34 ± 0.03	2.29 ± 0.08	0.75 ± 0.00	507 ± 1.3	314 ± 14.5	1.33 ± 0.01
SP Carr	1.33 ± 0.04	2.27 ± 0.10	0.73 ± 0.01	504 ± 1.4	311 ± 17.9	1.30 ± 0.01
SP Kem	1.33 ± 0.03	2.26 ± 0.06	0.74 ± 0.01	505 ± 1.4	311 ± 10.1	1.32 ± 0.03

Table 6
Growth performance and feed intake of Atlantic salmon fed with experimental diets in **feeding trial II**: herring protein powder – HP Alg (3%) and shrimp protein powder – SP Alg (10%) as partial or total replacement of fish meal. Data shown as mean ± SD, $n = 3$.

	SGR %/d Mean ± SD	RGR %/d Mean ± SD	FCR ratio, g/g Mean ± SD	Final weight g Mean ± SD	Weight gain g Mean ± SD	K g/cm^3 Mean ± SD	HSI - Mean ± SD
Control	1.39 ± 0.03	1.98 ± 0.06	0.79 ± 0.00	594 ± 65	289 ± 8.5	140 ± 0.01	1.11 ± 0.05
HP Alg	1.33 ± 0.01	1.87 ± 0.02	0.79 ± 0.01	581 ± 67	275 ± 3.5	1.38 ± 0.00	1.13 ± 0.04
SP Alg	1.41 ± 0.03	2.02 ± 0.06	0.78 ± 0.01	591 ± 77	291 ± 10.8	1.40 ± 0.01	1.15 ± 0.04

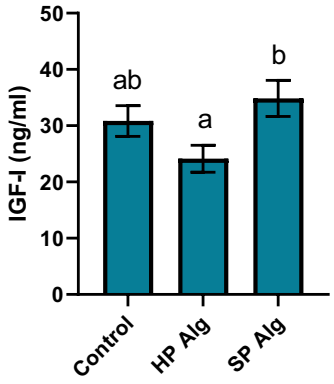


Fig. 1. IGF-I plasma concentration if fish in **feeding trial II** with herring protein powder – HP Alg (3%) and shrimp protein powder – SP Alg (10%) as partial or total replacement of fish meal. Data presented as mean ± SEM, $n = 12$. Different letters illustrate significant difference.

3.2.2. *Intestinal functions*

The intestinal barrier function assessed as TER was significantly reduced in the distal intestine ($P < 0.02$) but not in the proximal intestine, in the dietary group exposed to partial replacement with HP-Alg compared with the FM-containing control diet. Whereas SP- Alg did not significantly affect TER in the proximal or distal intestine ($P > 0.05$; Fig. 2a). Mannitol diffusion rate (Papp) was not significantly affected by the diets in any of the intestinal segments ($P > 0.05$; Fig. 2b). The active transport of L-lysine or net ion-transport (TEP and SCC) across the proximal or distal intestine was not affected by the diet ($P > 0.05$ Table 7).

Table 7
Results from the ussing chamber methology from **feeding trial II** with herring protein powder – HP Alg (3%) and shrimp protein powder – SP Alg (10%) as partial or total replacement of fish meal. Data presented as mean ± SEM, n = 12.

Diet		TEP (mV)	SCC (μA)	L-Lysine transport (mol/min*cm^2)
Control	Proximal intestine	2.6 ± 0.01	26.6 ± 0.3	1.6E-09 ± 2.3E-10
	Distal intestine	0.7 ± 0.01	2.2 ± 0.05	6.1E-11 ± 1.7E-10
HP Alg	Proximal intestine	2.9 ± 0.01	26.0 ± 0.2	1.3E-09 ± 1.2E-10
	Distal intestine	0.8 ± 0.01	3.0 ± 0.06	1.1E-10 ± 1.4E-11
SP Alg	Proximal intestine	2.4 ± 0.02	21.6 ± 0.2	1.2E-09 ± 3.6E-11
	Distal intestine	0.6 ± 0.01	2.0 ± 0.07	1.2E-10 ± 3.1E-11

3.2.3. *Histology and immunohistochemistry*

Histological scoring of the distal intestine did not reveal any significant signs of inflammation in relation to the different diets. Some individuals had moderate changes with increased width and shorter mucosal folds (Control ($n = 11$), 1.0 ± 0; HP-Alg ($n = 10$), 1.0 ± 0; SP-Alg (n = 11), 1.33 ± 0.16) and reduced supranuclear vacuolization in the enterocytes (Control (n = 11), 1.41 ± 0.14; HP-Alg (n = 10), 1.36 ± 0.14; SP-Alg (n = 11), 1.56 ± 0.17), and to some extent widening of lamina propria in the intestinal folds (Control (n = 11), 1.17 ± 0.11; HP-Alg (n = 10), 1.18 ± 0.12; SP-Alg (n = 11), 1.56 ± 0.23). There were also some individuals with infiltrated leukocyte in the lamina propria and submucosa, evenly spread among the dietary groups (Control (n = 11), 1.25 ± 0.13; HP-Alg (n = 10), 1.45 ± 0.16; SP-Alg (n = 11), 1.33 ± 0.16).

CD8α lymphocytes were found to be primarily intra-epithelial in the proximal and distal intestine of almost all experimental fish (Fig. 3a and c). In general, there were higher cell counts of CD8α in the proximal intestine than in the distal intestine, but there was no significant difference among dietary groups ($P > 0.05$) (Fig. 4a). MHC II staining resulted in intense staining of the brush boarder membrane and the apical region of the intestinal epithelium, and MHC II positive cells were mainly located in the lamina propria at the base of the mucosal folds while cells could occasionally be found in the secondary folds of the distal intestine (Fig. 3b and d). The morphology of the positive cells often showed distinct extensions or dendrites. The number of MHC II positive cells / mm epithelia was not significantly affected by diet ($P > 0.05$) (Fig. 4b).

3.2.4. *Fillet colouring*

There was no significant differentiation in colour dispersal in the NQC fillets in terms of L* spectrum, a* spectrum or b* spectrum among dietary groups (Table 8).

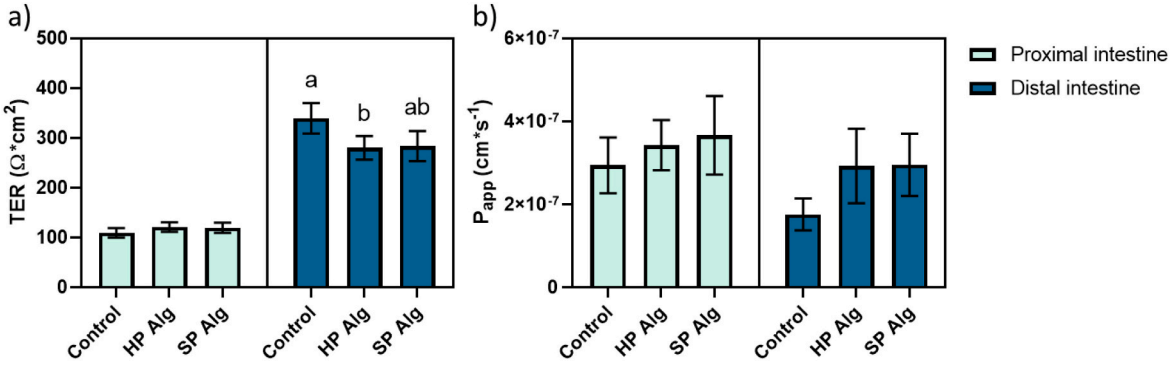


Fig. 2. a) Intestinal transepithelial resistance (TER), and b) mannitol permeability (Papp) across the intestinal tissue of Atlantic salmon from **feeding trial II** with herring protein powder – HP Alg (3%) and shrimp protein powder – SP Alg (10%) as partial or total replacement of fish meal. Data presented as mean ± SEM, n = 12. Different letters illustrate significant difference.

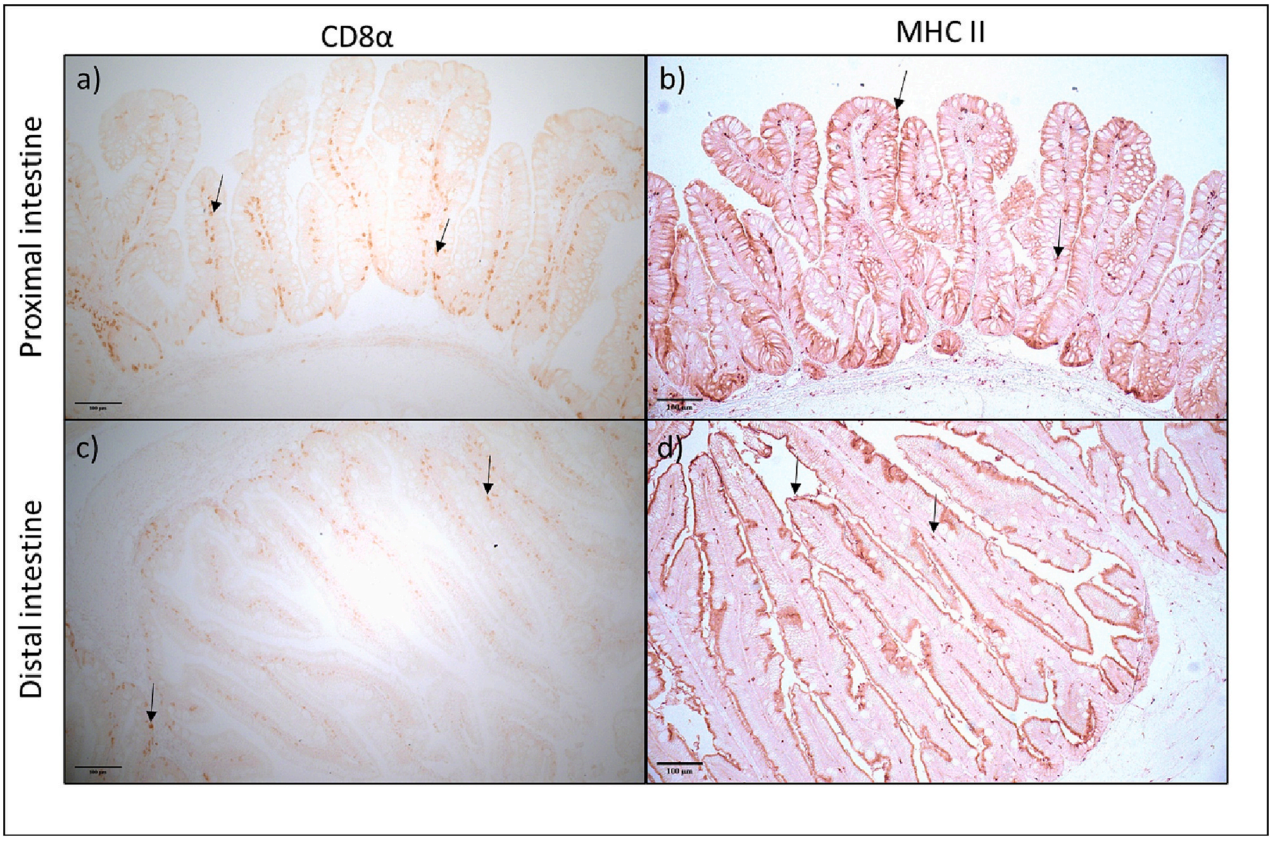


Fig. 3. Immunohistocemistry staining using CD8α (a and c) and MHC II (b and d) antibodies in the proximal and distal intestine of Atlantic salmon. Scale bar is representing 100 μm.

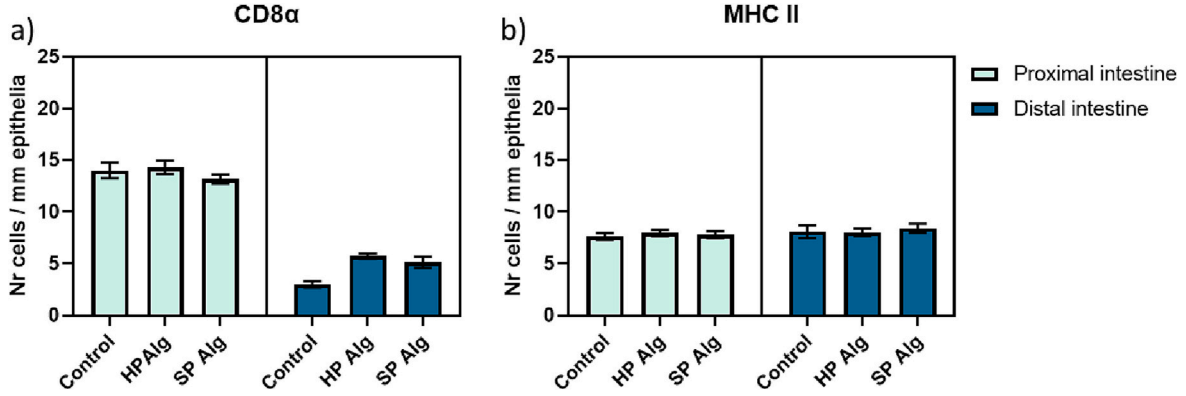


Fig. 4. Result from immunohistochemistry analysis of the number of T-cells (CD8) and major histocompatibility complex II (MHC II) in the proximal and distal intestine of Atlantic salmon from **feeding trial II** a) T-cells (CD8α) - Control (n = 12; 10) herring protein powder - HP Alg (3%) (*n* = 10; 8) and shrimp protein powder – SP Alg (10%) (n = 12; 8) as partial or total replacement of fish meal. b) MHCII - Control (*n* = 11; 9) herring protein powder – HP Alg (3%) (n = 10; 7) and shrimp protein powder – SP Alg (10%) (n = 11; 6) as partial or total replacement of fish meal. Data shown as mean ± SEM.

4. Discussion

Process water from the seafood industry is an abundant un-exploited resource of biomass, and recent studies show that protein enriched biomasses produced from these type of side-streams have potential as an alternative protein and fatty acid resource in fish feed (Amado et al., 2016; Forghani et al., 2020a; Jarrault et al., 2017). In the current study, all dietary groups of fish grew equally well during the experimental period. There was only one indication that inclusion of protein powders produced from flocculation, DAF-treatment and spray drying of herring and shrimp process waters, had any negative effect on fish health or welfare. Atlantic salmon fed HP-Alg diet had a reduced TER which indicate impaired intestinal barrier function. Taken together, our results show that most protein powders included in the two feeding trials performed well in terms of growth and physiological assessment. Hence, from a biological perspective all but HP-Alg water-derived protein powders evaluated herein may be recommended as alternative protein sources to replace FM or SPC partially or fully.

Alg was chosen as a flocculent to use in feeding trial II because of its efficiency properties during protein recovery from process waters and that it performed well in terms of growth and histological examination in feeding trial I (Forghani et al., 2020b). Further, at the time of this

Table 8
Results from the colour dispersal measuremnts in the right and left NQC fillets from **feeding trial II** with herring protein powder – HP Alg (3%) and shrimp protein powder – SP Alg (10%) as partial or total replacement of fish meal. L*values (brightness), a*values (green-red), and b*values (yellow-blue). Data presented as mean ± SEM, n = 12.

		L* Spectrum	a* Spectrum	b* Spectrum
Control	Right fillet	52.0 ± 0.69	9.5 ± 0.41	15.6 ± 0.55
	Left fillet	52.0 ± 0.55	10.5 ± 0.47	15.6 ± 0.62
HP Alg	Right fillet	52.4 ± 0.73	9.0 ± 0.43	15.4 ± 0.55
	Left fillet	52.4 ± 0.61	9.2 ± 0.35	15.2 ± 0.50
SP Alg	Right fillet	52.2 ± 0.66	9.0 ± 0.42	15.4 ± 0.62
	Left fillet	51.7 ± 0.57	10.4 ± 0.43	15.1 ± 0.54

feeding trial, carrageenan was not authorized for use as a technological additive in fish feed according to European regulation (EU) 2017/1145. It could however be interesting to use Carr as flocculent and include in feed in larger quantities since it also had efficient protein recovery and since it had not negative effects of fish growth in feeding trial I (Forghani et al., 2020b).

In the second feeding trial, fish fed the SP-Alg diet had 45% higher IGF-I concentrations in their plasma compared with fish fed the HP-Alg diet, suggesting that the SP-Alg diet have some beneficial properties and/or content in comparison to the HP-Alg diet that could result in higher long-term growth. IGF-I function in fish and its role as a growth promoter is well known (Beckman, 2011; Duan and Xu, 2005; Wood et al., 2005). Plasma IGF-I concentrations correlate positively with growth rates in Atlantic salmon (Breves et al., 2020; Dyer et al., 2004), and Coho salmon (Beckman et al., 2004), and rainbow trout (Izutsu et al., 2022). In addition, it has been reported that long-term food deprivation or intake of diets with imbalanced nutritional content reduce IGF-I (Karapanagiotidis et al., 2019; Singha et al., 2020; Triantaphyllopoulos et al., 2020). Our data on IGF-I levels in plasma support that SP-Alg meets the fish's nutritional requirement in terms of protein to sustain growth and growth endocrinology. HP-Alg on the other hand, seems to result in lower IGF-I concentrations and that may lead to lower growth performance in the long term. Even though not significant, there were tendencies for lower growth performance in fish eating the HP-Alg diet compared to the other dietary groups. This experiment had a relatively short duration, and a long-term experiment would be needed to clarify if consumption of a diet with HP-Alg inclusion may result in impaired growth.

The intestine is a multifunctional organ and maintained intestinal functions in response to different feed ingredients are a prerequisite for the health and welfare of farmed fish (Krogdahl et al., 2010; Sundh and Sundell, 2015; Taylor and Grosell, 2006). The inclusion of HP-Alg, despite being as low as 3%, in the second feeding trial resulted in decreased TER but this was not reflected in increased mannitol diffusion (Papp) in the distal intestine. Reduced TER (increased ion-permeability) and increased mannitol diffusion (increased permeability of un-charged molecules) have previously been seen in Atlantic salmon fed SBM and serve as indicators of "leaky gut" and inflammatory response (Knudsen et al., 2008). One cause for our result could be that herring brine has a high salt concentration (up to 10%, translating to 33.6% ash in the HP-Alg powder), resulting in higher ion-leakage across the intestine while the dietary content and passage of small-uncharged molecules remained unchanged. Similar, but non-significant trends, were seen in the fish that had been fed the diet where FM was totally replaced by SP-Alg. However, since no other indicator measured revealed any negative effect of SP-Alg these results clearly suggest that SP-Alg is a promising, sustainable, novel ingredient in salmon feed, while further studies are needed to clarify if HP-Alg safely can be included in higher levels.

No signs of intestinal inflammation were observed during the histological examination. These findings were supported by the equal numbers of CD8α or MCH II positive cells across the dietary groups, suggesting that the proteins used in the feed in the current study do not impose an immune challenge in the intestinal tissue. It has previously been reported that there are higher numbers of CD8α positive cells (Romarheim et al., 2013), and increased mRNA expression of CD8β during SBM-induced inflammation in Atlantic salmon (Bakke-McKellep et al., 2007a; Marjara et al., 2012). Thus, the lack of increase in CD8α positive cells after intake of the experimental diets clearly suggest that no intestinal inflammation is induced. Further support is the observation of the higher number of CD8α cells found in the proximal compared to the distal intestine that is the opposite to what Niklasson et al. (2014) reported in Atlantic salmon exposed to IPVN, suggesting that an immune challenge would increase the amount of cells in the distal intestine. Subsequentially, the location of the CD8α and MHC II complexes, was confirmed according to previously reported data that the CD8α cells were intraepithelial, located on the basolateral side of the enterocytes in the Atlantic salmon intestine (Niklasson et al., 2014; Romarheim et al., 2013; Soleto et al., 2019). The location and appearance of the MHC II staining of positive cells showed distinct extensions or dendrites, corresponding to a reaction in dendritic cells or macrophages, found in the intestine of Atlantic salmon and is concurrent with the literature (Koppang et al., 2010, 2003; Løkka et al., 2014). Regarding MHC II positive cells during SBM-induced inflammation there are conflicting results in the literature. While an increase was reported by Romarheim et al. (2013), another study saw no differences in the number of MHC II cells (Bakke-McKellep et al., 2007b), where the latter was designed to test only SBM effects. Thus, the involvement of MHC II in intestinal inflammation of fish is not completely clear.

Shrimp process waters derived from steaming and or peeling processes are abundant resources with some limitations in that they contain relatively low concentrations of biomolecules and the composition can vary across the year (Amado et al., 2016; Forghani et al., 2020a; Jarrault et al., 2017). The upscale ability of the possible protein production from shrimp process water is connected to the profitability and cost for the companies. In many cases the water is already treated with chemical flocculants for removal of organic matter before discharge making it unsuitable for consumption (Chowdhury et al., 2010; Fahim et al., 2000). If these chemical flocculants would be replaced with alginate or carrageenan it could be beneficial for the company as an extra economic income of a side stream product. Nonetheless, the production cost of the protein is important, and it is hard to estimate the cost when done in pilot scale. Since the shrimp process waters is currently wasted the net cost for producing shrimp protein would come from labour and the use of flocculant with a price range between 10 and 100 Euro/kg (Forghani et al., 2020a). Further, the market value for the SP-Alg is hard to predict but in addition to yielding high quality protein and lipids, it also contains a considerable amount of astaxanthin 2.8–4.1 mg /L (Forghani et al., 2020a). Carotenoids and their importance as antioxidants in feed for salmon aquaculture is evident (Bjerkeng, 2000; Lara-flores, 2013). Also, it contributes to the reddish fillet colour of aquaculture salmon which is an important trait that can increase the attractiveness for the consumers (Alfnes et al., 2006). The lack of extra colour disposition after consuming the SP-Alg diet was perhaps not an unexpected result because of the low total contribution in the diet. Further, all the diets already had astaxanthin (40 mg/kg) as additives, which perhaps masked any possible extra colour dispersal coming from the SP-Alg. It has previously been found that carotenoids such as astaxanthin from various sources deposit in skin and fillet of Atlantic salmon (Buttle et al., 2001), rainbow trout (Gouveia et al., 1996; Jensen et al., 1998), and Nile tilapia (Valente et al., 2016), thus it would be interesting to further investigate if SP-Alg could be used as both an alternative protein and a natural colorant in feed.

The continued growth of the aquaculture industry and its dependency on the wild capture fisheries and other human food sources pose a great challenge for a sustainable development and expansion of the sector (Cashion et al., 2016; FAO, 2018; Jenkins et al., 2009). There is a general agreement that the FM and fish oil are valuable resources in

fish feed but that the production cannot keep the same pace as the increased demand (Gatlin et al., 2007; Glencross et al., 2007; Olsen and Hasan, 2012; Turchini et al., 2019). It has further been stated in EU that we need to change our perspective towards a circular economy where biomasses which are currently wasted become resources (EU, 2020; Gregson et al., 2015). Aligning to this strategy, research exploring sustainable, alternative feed ingredients is extensive. Focus is not that a single ingredient can replace all the FM and FO from wild caught fisheries but rather a combination of several sources that together reach the optimal nutritional composition (Glencross et al., 2007; Turchini et al., 2019). A next step in relation to the present study could be to evaluate combinations of protein enriched meals derived from different side streams.

5. Conclusion

Protein powders produced from seafood industry side stream waters, using natural flocculants, show high potential for inclusion in Atlantic salmon feed. There were a few indications of that fish eating the HP-Alg diet had both lower IGF-I concentration compared to the SP-Alg diet and a reduced intestinal barrier function, hence it needs further investigation if it is to be used as an alternative ingredient in fish feed. Nonetheless, the thorough examination of growth, endocrine growth regulation and intestinal health revealed the potential of SP-Alg as a replacement for FM and SPC. Increased IGF-I concentrations in fish provided with the SP-Alg diet compared to the HP-Alg diet suggest that the SP-Alg diet has beneficial properties equal to FM that could result in increased long-term growth rates. This concludes that SP-Alg clearly show potential as an alternative protein source in Atlantic salmon feed.

Ethical declaration

The experiments and analysis of the data was performed according the ARRIVE guidelines and according to the Norwegian, Forskrift om bruk av. dyr i forsøk - Lovdata.

Funding

The research leading to these results has received funding from Nordic Innovation (NoVAqua project, Mar-14322), University of Gothenburg, SWEMARC, and from the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning under grant agreement 223–2011-1073, Norwegian Research Council project 296083 and Helge Ax:son Johnsons Stiftelse F18–0359.

CRediT authorship contribution statement

Ida Hedén: Writing – original draft, Data curation, Investigation, Formal analysis, Visualization, Funding acquisition. **Bita Forghani Targhi:** Conceptualization, Methodology, Data curation, Resources. **Gunvor Baardsen:** Methodology, Data curation, Formal analysis, Resources. **Bjørge Westereng:** Methodology, Funding acquisition. **Tore Svendsen:** Methodology. **Elisabeth Jönsson:** Methodology, Data curation, Supervision, Writing – review & editing. **Linda Hasselberg Frank:** Data curation. **Ingrid Undeland:** Methodology, Conceptualization, Funding acquisition, Project administration. **Kristina Sundell:** Formal analysis, Conceptualization, Data curation, Funding acquisition, Project administration, Supervision, Writing – review & editing. **Henrik Sundh:** Supervision, Writing – original draft, Data curation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

We thank the all the financiers; Nordic Innovation (Mar-14322), University of Gothenburg, SWEMARC, and the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (223-2011-1073), and The Research Council of Norway (296083) and Helge Ax:son Johnsons Stiftelse (F18-0359) for their generous contribution to this study. We also thank Ola Tjåland at NMBU for his invaluable help for the conduction of drying shrimp and herring biomasses.

References

Aas, T.S., Ytrestøyl, T., Åsgård, T., 2019. Utilization of feed resources in the production of Atlantic salmon (*Salmo salar*) in Norway: an update for 2016. Aquac. Reports 15, 100216. https://doi.org/10.1016/j.aqrep.2019.100216.

Alfnes, F., Guttormsen, A.G., Steine, G., Kolstad, K., 2006. Consumers willingness to pay for the color of salmon: a choice experiment with real economic incentives. Am. J. Agr. Econ. 88 (4), 1050–1061.

Amado, I.R., González, M.P., Murado, M.A., Vázquez, J.A., 2016. Shrimp wastewater as a source of astaxanthin and bioactive peptides. J. Chem. Technol. Biotechnol. 91, 793–805. https://doi.org/10.1002/jctb.4647.

Baeverfjord, G., Krogdahl, Å., 1996. Development and regression of soybean meal induced enteritis in Atlantic salmon, *Salmo salar L.*, distal intestine: a comparison with the intestines of fasted fish. J. Fish Dis. 19, 375–387. https://doi.org/10.1111/j.1365-2761.1996.tb00376.x.

Bakke-McKellep, A.M., Frøystad, M.K., Lilleeng, E., Dapra, F., Refstie, S., Krogdahl, Å., Landsverk, T., 2007a. Response to soy: T-cell-like reactivity in the intestine of Atlantic salmon, *Salmo salar L.* J. Fish Dis. 30, 13–25. https://doi.org/10.1111/j.1365-2761.2007.00769.x.

Bakke-McKellep, A.M., Koppang, E.O., Gunnes, G., Sanden, M., Hemre, G.I., Landsverk, T., Krogdahl, Å., 2007b. Histological, digestive, metabolic, hormonal and some immune factor responses in Atlantic salmon, *Salmo salar L.*, fed genetically modified soybeans. J. Fish Dis. 30, 65–79. https://doi.org/10.1111/j.1365-2761.2007.00782.x.

Beckman, B.R., 2011. Perspectives on concordant and discordant relations between insulin-like growth factor 1 (IGF1) and growth in fishes. Gen. Comp. Endocrinol. 170, 233–252. https://doi.org/10.1016/j.ygcen.2010.08.009.

Beckman, B.R., Shimizu, M., Gadberry, B.A., Cooper, K.A., 2004. Response of the somatotropic axis of juvenile coho salmon to alterations in plane of nutrition with an analysis of the relationships among growth rate and circulating IGF-I and 41 kDa IGFBP. Gen. Comp. Endocrinol. 135, 334–344. https://doi.org/10.1016/j.ygcen.2003.10.013.

Bjerkeng, B., 2000. Carotenoid pigmentation of salmonid fishes - recent progress. Mem. V Simp. Int. Nutr. Acuícola 71–89.

Breves, J.P., Springer-Miller, R.H., Chenoweth, D.A., Paskavitz, A.L., Chang, A.Y.H., Regish, A.M., Einarsdottir, I.E., Björnsson, B.T., McCormick, S.D., 2020. Cortisol regulates insulin-like growth-factor binding protein (igfbp) gene expression in Atlantic salmon parr. Mol. Cell. Endocrinol. 518 https://doi.org/10.1016/j.mce.2020.110989.

Buttle, L.G., Crampton, V.O., Williams, P.D., 2001. The effect of feed pigment type on flesh pigment deposition and colour in farmed Atlantic salmon, *Salmo salar L.* Aquac. Res. 32, 103–111. https://doi.org/10.1046/j.1365-2109.2001.00536.x.

Cashion, T., Hornborg, S., Ziegler, F., Hognes, E.S., Tyedmers, P., 2016. Review and advancement of the marine biotic resource use metric in seafood LCAs: a case study of Norwegian salmon feed. Int. J. Life Cycle Assess. 21, 1106–1120. https://doi.org/10.1007/s11367-016-1092-y.

Chowdhury, P., Viraraghavan, T., Srinivasan, A., 2010. Biological treatment processes for fish processing wastewater - a review. Bioresour. Technol. 101, 439–449. https://doi.org/10.1016/j.biortech.2009.08.065.

Duan, C., 1997. The insulin-like growth factor system and its biological actions in fish. Am. Zool. 37, 491–503. https://doi.org/10.1093/icb/37.6.491.

Duan, C., Xu, Q., 2005. Roles of insulin-like growth factor (IGF) binding proteins in regulating IGF actions. Gen. Comp. Endocrinol. 142, 44–52. https://doi.org/10.1016/j.ygcen.2004.12.022.

Duan, C., Plisetskaya, M.E., Dickhoff, W.W., 1995. Expression of insulin-like growth factor I in normally and abnormally developing Coho Salmon (*Oncorhynchus kisutch*). Endocrinology 136, 446–452.

Dyer, A.R., Barlow, C.G., Bransden, M.P., Carter, C.G., Glencross, B.D., Richardson, N., Thomas, P.M., Williams, K.C., Carragher, J.F., 2004. Correlation of plasma IGF-I concentrations and growth rate in aquacultured finfish: a tool for assessing the potential of new diets. Aquaculture 236, 583–592. https://doi.org/10.1016/j.aquaculture.2003.12.025.

EU, 2020. Circular Economy Action Plan. ENVE-VII-/006. Eur. Comm. 140, 1–12.

Fahim, F.A., Fleita, D.H., Ibrahim, A.M., 2000. Evaluation of some methods for fish canning wastewater treatment. Development of the food industry in Egypt is

essential in providing for the needs of its fast growing population (Ewiess and Mohammed, 1991; El-Gohary, 1993). The government's intere 205–226.
FAO, 2018. The State of World Fisheries and Aquaculture 2018 - Meeting the Sustainable Goals. FAO Fisheries and Aquaculture Department of the United Nations, Rome. https://doi.org/10.1016/j.fishres.2015.07.010.
FAO, 2022. The State of World Fisheries and Aquaculture 2022, In Brief to The State of World Fisheries and Aquaculture, 2022. https://doi.org/10.4060/cc0463en.
Fontaine, J., Eudaimon, M., Bargholz, J., Empie, M.W., Eriksson, S., Hoeg, T., Katayama, M., Kang, K.R., Lee, H., Legler, J., Llames, C.R., MacDonald, J.L., Pierre, F., Regnier, J.M., Schwadorf, K., Van Der Lee, R.A., Verbeek, K., 2000. Liquid chromatographic determination of lysine, methionine, and threonine in pure amino acids (feed grade) and premixes: collaborative study. J. AOAC Int. 83, 771–788. https://doi.org/10.1093/jaoac/83.4.771.
Forghani, B., Bordes, R., Ström, A., Undeland, I., 2020a. Recovery of a protein-rich biomass from shrimp (*Pandalus borealis*) boiling water: a colloidal study. Food Chem. 302, 125299 https://doi.org/10.1016/j.foodchem.2019.125299.
Forghani, B., Sørensen, A.D.M., Johannesson, J., Svendsen, T.C., Undeland, I., 2020b. Flocculation and flotation to recover protein-enriched biomasses from shrimp boiling and peeling process waters: a case study. ACS Sustain. Chem. Eng. 8, 9660–9668. https://doi.org/10.1021/acssuschemeng.0c01154.
Froehlich, H.E., Runge, C.A., Gentry, R.R., Gaines, S.D., Halpern, B.S., 2018. Comparative terrestrial feed and land use of an aquaculture-dominant world. Proc. Natl. Acad. Sci. U. S. A. 115, 5295–5300. https://doi.org/10.1073/pnas.1801692115.
Gatlin, D.M., Barrows, F.T., Brown, P., Dabrowski, K., Gaylord, T.G., Hardy, R.W., Herman, E., Hu, G., Krogdahl, Å., Nelson, R., Overturf, K., Rust, M., Sealey, W., Skonberg, D., Souza, E.J., Stone, D., Wilson, R., Wurtele, E., 2007. Expanding the utilization of sustainable plant products in aquafeeds: a review. Aquac. Res. 38, 551–579. https://doi.org/10.1111/j.1365-2109.2007.01704.x.
Glencross, B.D., Booth, M., Allan, G.L., 2007. A feed is only as good as its ingredients - a review of ingredient evaluation strategies for aquaculture feeds. Aquac. Nutr. 13, 17–34. https://doi.org/10.1111/j.1365-2095.2007.00450.x.
Gouveia, L., Gomes, E., Empis, J., 1996. Potential use of a microalga (*Chlorella vulgaris*) in the pigmentation of rainbow trout (*Oncorhynchus mykiss*) muscle. Eur. Food Res. Technol. 202, 75–79. https://doi.org/10.1007/BF01229690.
Gregson, N., Crang, M., Fuller, S., Holmes, H., 2015. Interrogating the circular economy: the moral economy of resource recovery in the EU. Econ. Soc. 44, 218–243. https://doi.org/10.1080/03085147.2015.1013353.
Günzel, D., Yu, A.S.L., 2013. Claudins and the modulation of tight junction permeability. Physiol. Rev. https://doi.org/10.1152/physrev.00019.2012.
Hardy, R.W., 2010. Utilization of plant proteins in fish diets: effects of global demand and supplies of fishmeal. Aquac. Res. 41, 770–776. https://doi.org/10.1111/j.1365-2109.2009.02349.x.
Hetland, D.L., Jørgensen, S.M., Skjødt, K., Dale, O.B., Falk, K., Xu, C., Mikalsen, A.B., Grimholt, U., Gjøen, T., Press, C.M.L., 2010. In situ localisation of major histocompatibility complex class I and class II and CD8 positive cells in infectious salmon anaemia virus (ISAV)-infected Atlantic salmon. Fish Shellfish Immunol. 28, 30–39. https://doi.org/10.1016/j.fsi.2009.09.011.
Izutsu, A., Tadokoro, D., Habara, S., Ugachi, Y., Shimizu, M., 2022. Evaluation of circulating insulin-like growth factor (IGF)-I and IGF-binding proteins as growth indices in rainbow trout (*Oncorhynchus mykiss*). Gen. Comp. Endocrinol. 320, 1–8. https://doi.org/10.1016/j.ygcen.2022.114008.
Jarrault, C., Dornier, M., Labatut, M.L., Giampaoli, P., Lameloise, M.L., 2017. Coupling nanofiltration and osmotic evaporation for the recovery of a natural flavouring concentrate from shrimp cooking juice. Innov. Food Sci. Emerg. Technol. 43, 182–190. https://doi.org/10.1016/j.ifset.2017.07.035.
Jenkins, D.J.A., Sievenpiper, J.L., Pauly, D., Sumaila, U.R., Kendall, C.W.C., Mowat, F. M., 2009. Are dietary recommendations for the use of fish oils sustainable? Cmaj 180, 633–637. https://doi.org/10.1503/cmaj.081274.
Jensen, C., Birk, E., Jokumsen, A., Skibsted, L.H., Bertelsen, G., 1998. Effect of dietary levels of fat, α-tocopherol and astaxanthin on colour and lipid oxidation during storage of frozen rainbow trout (*Oncorhynchus mykiss*) and during chill storage of smoked trout. Eur. Food Res. Technol. 207, 189–196. https://doi.org/10.1007/s002170050317.
Johnsen, C.A., Hagen, Ø., Adler, M., Jönsson, E., Kling, P., Bickerdike, R., Solberg, C., Björnsson, B.T., Bendiksen, E.Å., 2011. Effects of feed, feeding regime and growth rate on flesh quality, connective tissue and plasma hormones in farmed Atlantic salmon (*Salmo salar L.*). Aquaculture 318, 343–354. https://doi.org/10.1016/j.aquaculture.2011.05.040.
Jutfelt, F., 2011. Barrier function of the gut. Encycl. Fish Physiol. Genome Environ. 2, 1322–1331.
Karapanagiotidis, I.T., Psofakis, P., Mente, E., Malandrakis, E., Golomazou, E., 2019. Effect of fishmeal replacement by poultry by-product meal on growth performance, proximate composition, digestive enzyme activity, haematological parameters and gene expression of gilthead seabream (*Sparus aurata*). Aquac. Nutr. 25, 3–14. https://doi.org/10.1111/anu.12824.
Knudsen, D., Jutfelt, F., Sundh, H., Sundell, K., Koppe, W., Frøkiær, H., 2008. Dietary soya saponins increase gut permeability and play a key role in the onset of soyabean-induced enteritis in Atlantic salmon (*Salmo salar L.*). Br. J. Nutr. 100, 120–129. https://doi.org/10.1017/S0007114507886338.
Koppang, E.O., Hordvik, I., Bjerkås, I., Torvund, J., Aune, L., Thevarajan, J., Endresen, C., 2003. Production of rabbit antisera against recombinant MHC class II β chain and identification of immunoreactive cells in Atlantic salmon (*Salmo salar*). Fish Shellfish Immunol. 14, 115–132. https://doi.org/10.1006/fsim.2002.0424.
Koppang, E.O., Fischer, U., Moore, L., Tranulis, M.A., Dijkstra, J.M., Köllner, B., Aune, L., Jirillo, E., Hordvik, I., 2010. Salmonid T cells assemble in the thymus, spleen and in novel interbranchial lymphoid tissue. J. Anat. 217, 728–739. https://doi.org/10.1111/j.1469-7580.2010.01305.x.
Krogdahl, Å., Bakke-McKellep, A.M., Baeverfjord, G., 2003. Effects of graded levels of standard soybean meal on intestinal structure, mucosal enzyme activities, and pancreatic response in Atlantic salmon (*Salmo solar L.*). Aquac. Nutr. 9, 361–371. https://doi.org/10.1046/j.1365-2095.2003.00264.x.
Krogdahl, Å., Penn, M., Thorsen, J., Refstie, S., Bakke, A.M., 2010. Important antinutrients in plant feedstuffs for aquaculture: an update on recent findings regarding responses in salmonids. Aquac. Res. 41, 333–344. https://doi.org/10.1111/j.1365-2109.2009.02426.x.
Lara-flores, M., 2013. The use of carotenoid in aquaculture. Res. J. Fish. Hydrobiol. 8, 38–49.
Lilleeng, E., Penn, M.H., Haugland, Ø., Xu, C., Bakke, A.M., Krogdahl, Å., Landsverk, T., Frøystad-Saugen, M.K., 2009. Decreased expression of TGF-β, GILT and T-cell markers in the early stages of soybean enteropathy in Atlantic salmon (*Salmo salar L.*). Fish Shellfish Immunol. 27, 65–72. https://doi.org/10.1016/j.fsi.2009.04.007.
Løkka, G., Austbø, L., Falk, K., Bromage, E., Fjelldal, P.G., Hansen, T., Hordvik, I., Koppang, E.O., 2014. Immune parameters in the intestine of wild and reared unvaccinated and vaccinated Atlantic salmon (*Salmo salar L.*). Dev. Comp. Immunol. 47, 6–16. https://doi.org/10.1016/j.dci.2014.06.009.
Marjara, I.S., Chikwati, E.M., Valen, E.C., Krogdahl, Å., Bakke, A.M., 2012. Transcriptional regulation of IL-17A and other inflammatory markers during the development of soybean meal-induced enteropathy in the distal intestine of Atlantic salmon (*Salmo salar L.*). Cytokine 60, 186–196. https://doi.org/10.1016/j.cyto.2012.05.027.
Maures, T., Chan, S.J., Xu, B., Sun, H., Ding, J., Duan, C., 2002. Structural, biochemical, and expression analysis of two distinct insulin-like growth factor I receptors and their ligands in zebrafish. Endocrinology 143, 1858–1871. https://doi.org/10.1210/endo.143.5.8768.
McCormick, S.D., Kelley, K.M., Young, G., Nishioka, R.S., Bern, H.A., 1992. Stimulation of coho salmon growth by insulin-like growth factor I. Gen. Comp. Endocrinol. 86, 398–406. https://doi.org/10.1016/0016-6480(92)90064-Q.
Moriyama, S., Swanson, P., Nishii, M., Takahashi, A., Kawauchi, H., Dickhoff, W.W., Plisetskaya, M.E., 1994. Development of a homologous radioimmunoassay for Coho Salmon insulin-like growth factor-I. Gen. Comp. Endocrinol. 96, 149–161.
Niklasson, L., Sundh, H., Olsen, R.E., Jutfelt, F., Skjødt, K., Nilsen, T.O., Sundell, K.S., 2014. Effects of cortisol on the intestinal mucosal immune response during cohabitant challenge with IPNV in Atlantic salmon (*Salmo salar*). PLoS One 9, 20–22. https://doi.org/10.1371/journal.pone.0094288.
Norbeck, L.A., Kittilson, J.D., Sheridan, M.A., 2007. Resolving the growth-promoting and metabolic effects of growth hormone: differential regulation of GH-IGF-I system components. Gen. Comp. Endocrinol. 151, 332–341. https://doi.org/10.1016/j.ygcen.2007.01.039.
Olsen, R.L., Hasan, M.R., 2012. A limited supply of fishmeal: impact on future increases in global aquaculture production. Trends Food Sci. Technol. 27, 120–128. https://doi.org/10.1016/j.tifs.2012.06.003.
Pahlow, M., van Oel, P.R., Mekonnen, M.M., Hoekstra, A.Y., 2015. Increasing pressure on freshwater resources due to terrestrial feed ingredients for aquaculture production. Sci. Total Environ. 536, 847–857. https://doi.org/10.1016/j.scitotenv.2015.07.124.
Pérez-Sánchez, J., Weil, C., Le Bail, P.-Y., 1992. Effects of human insulin-like growth factor-I on release of growth hormone by rainbow trout (*Oncorhynchus mykiss*) pituitary cells. J. Exp. Zool. 262, 287–290. https://doi.org/10.1002/jez.1402620308.
Reindl, K.M., Sheridan, M.A., 2012. Peripheral regulation of the growth hormone-insulin-like growth factor system in fish and other vertebrates. Comp. Biochem. Physiol. - A Mol. Integr. Physiol. 163, 231–245. https://doi.org/10.1016/j.cbpa.2012.08.003.
Romarheim, O.H., Hetland, D.L., Skrede, A., Overland, M., Mydland, L.T., Landsverk, T., 2013. Prevention of soya-induced enteritis in Atlantic salmon (*Salmo salar*) by bacteria grown on natural gas is dose dependent and related to epithelial MHC II reactivity and CD8α+ intraepithelial lymphocytes. Br. J. Nutr. 109, 1062–1070. https://doi.org/10.1017/S0007114512002899.
Rombout, J.H.W.M., Yang, G., Kiron, V., 2014. Adaptive immune responses at mucosal surfaces of teleost fish. Fish Shellfish Immunol. 40, 634–643. https://doi.org/10.1016/j.fsi.2014.08.020.
Rosa, R., Nunes, M.L., 2004. Nutritional quality of red shrimp, *Aristeus antennatus* (Risso), pink shrimp, *Parapenaeus longirostris* (Lucas), and Norway lobster, *Nephrops norvegicus* (Linnaeus). J. Sci. Food Agric. 84, 89–94. https://doi.org/10.1002/jsfa.1619.
Rueden, C.T., Schindelin, J., Hiner, M.C., DeZonia, B.E., Walter, A.E., Arena, E.T., Eliceiri, K.W., 2017. ImageJ2: ImageJ for the next generation of scientific image data. BMC Bioinformatics 18, 1–26. https://doi.org/10.1186/s12859-017-1934-z.
Shimizu, M., Swanson, P., Fukada, H., Hara, A., Dickhoff, W.W., 2000. Comparison of extraction methods and assay validation for salmon insulin-like growth factor-I using commercially available components. Gen. Comp. Endocrinol. 119, 26–36. https://doi.org/10.1006/gcen.2000.7498.
Singha, K.P., Shamna, N., Sahu, N.P., Sardar, P., HariKrishna, V., Thirunavukkarasar, R., Kumar, M., Krishna, G., 2020. Feeding graded levels of protein to genetically improved farmed Tilapia (GIFT) juveniles reared in inland saline water: effects on growth and gene expression of IGF[sbnd]I, IGF-IR and IGF-BPI. Aquaculture 525, 735306. https://doi.org/10.1016/j.aquaculture.2020.735306.
Soleto, I., Granja, A.G., Simón, R., Morel, E., Díaz-Rosales, P., Tafalla, C., 2019. Identification of CD8α+ dendritic cells in rainbow trout (*Oncorhynchus mykiss*) intestine. Fish Shellfish Immunol. 89, 309–318. https://doi.org/10.1016/j.fsi.2019.04.001.

Sprague, M., Dick, J.R., Tocher, D.R., 2016. Impact of sustainable feeds on omega-3 long-chain fatty acid levels in farmed Atlantic salmon, 2006-2015. Sci. Rep. 6, 1–9. https://doi.org/10.1038/srep21892.

Stone, D.A.J., Oliveira, A.C.M., Ross, C.F., Plante, S., Smiley, S., Bechtel, P., Hardy, R.W., 2011. The effects of phase-feeding rainbow trout (*Oncorhynchus mykiss*) with canola oil and Alaskan Pollock fish oil on fillet fatty acid composition and sensory attributes. Aquac. Nutr. 17 https://doi.org/10.1111/j.1365-2095.2010.00792.x.

Sundell, K., Jutfelt, F., Ágústsson, T., Olsen, R.E., Sandblom, E., Hansen, T., Björnsson, B. T., 2003. Intestinal transport mechanisms and plasma cortisol levels during normal and out-of-season parr-smolt transformation of Atlantic salmon, *Salmo salar*. Aquaculture 222, 265–285. https://doi.org/10.1016/S0044-8486(03)00127-3.

Sundell, K.S., Sundh, H., 2012. Intestinal fluid absorption in anadromous salmonids: importance of tight junctions and aquaporins. Front. Physiol. 3 SEP, 1–13. https://doi.org/10.3389/fphys.2012.00388.

Sundh, H., Sundell, K.S., 2015. Mucosal Health in Aquaculture Environmental Impacts on Fish Mucosa, Mucosal Health in Aquaculture. Elsevier Inc. https://doi.org/10.1016/B978-0-12-417186-2.00007-8.

Tacon, A.G.J., 2020. Trends in global aquaculture and Aquafeed production: 2000–2017. Rev. Fish. Sci. Aquac. 28, 43–56. https://doi.org/10.1080/23308249.2019.1649634.

Tacon, A.G.J., Metian, M., McNevin, A.A., 2022. Future feeds: suggested guidelines for sustainable development. Rev. Fish. Sci. Aquac. 30, 135–142. https://doi.org/10.1080/23308249.2020.1860474.

Taylor, J.R., Grosell, M., 2006. Feeding and osmoregulation: dual function of the marine teleost intestine. J. Exp. Biol. 209, 2939–2951. https://doi.org/10.1242/jeb.02342.

Triantaphyllopoulos, K.A., Cartas, D., Miliou, H., 2020. Factors influencing GH and IGF-I gene expression on growth in teleost fish: how can aquaculture industry benefit? Rev. Aquac. 12, 1637–1662. https://doi.org/10.1111/raq.12402.

Turchini, G.M., Torstensen, B.E., Ng, W.K., 2009. Fish oil replacement in finfish nutrition. Rev. Aquac. 1, 10–57. https://doi.org/10.1111/j.1753-5131.2008.01001.x.

Turchini, G.M., Trushenski, J.T., Glencross, B.D., 2019. Thoughts for the future of aquaculture nutrition: realigning perspectives to reflect contemporary issues related to judicious use of marine resources in Aquafeeds. N. Am. J. Aquac. 81, 13–39. https://doi.org/10.1002/naaq.10067.

Valente, L.M.P., Araújo, M., Batista, S., Peixoto, M.J., Sousa-Pinto, I., Brotas, V., Cunha, L.M., Rema, P., 2016. Carotenoid deposition, flesh quality and immunological response of Nile tilapia fed increasing levels of IMTA-cultivated Ulva spp. J. Appl. Phycol. 28, 691–701. https://doi.org/10.1007/s10811-015-0590-9.

Whittamore, J.M., 2012. Osmoregulation and epithelial water transport: lessons from the intestine of marine teleost fish. J. Comp. Physiol. B Biochem. Syst. Environ. Physiol. 182, 1–39. https://doi.org/10.1007/s00360-011-0601-3.

Wood, A.W., Duan, C., Bern, H.A., 2005. Insulin-like growth factor signaling in fish. Int. Rev. Cytol. 243, 215–285. https://doi.org/10.1016/S0074-7696(05)43004-1.

Ytrestøyl, T., Aas, T.S., Åsgård, T., 2015. Utilisation of feed resources in production of Atlantic salmon (*Salmo salar*) in Norway. Aquaculture 448, 365–374. https://doi.org/10.1016/j.aquaculture.2015.06.023.