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Potential of insect-based diets for Atlantic salmon (*Salmo salar*)

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ABSTRACT

In the present study, we aimed to assess the effect of dietary insect meal (IM) and insect oil (IO) on growth performance, body composition and nutrient digestibility of freshwater reared Atlantic salmon. The IM and IO were produced from black soldier fly larvae (*Hermetia illucens*, L.; BSF) that had been grown on (1) media containing organic waste streams, or on (2) media partially containing seaweed (*Ascophyllum nodosum*). The feeding trial of the current study followed a factorial 2 × 3 way-ANOVA experimental design with six dietary groups of Atlantic salmon fed diets with insect-derived ingredients for 8 weeks. A typical industrial diet, with protein from fish meal and soy protein concentrate (SPC) (50:50) and lipids from fish oil and vegetable oil (33:66), was fed to a positive control group. Five experimental diets were formulated, where 85% of the dietary protein was replaced by IM and/or all the vegetable oil was replaced by IO (IM from insects grown on media 1, IO from insects grown on either media 1 (IO1) or media 2 (IO2)). Replacing the dietary fish meal and SPC with insect protein significantly reduced the apparent digestibility coefficients (ADC) of protein, lipid and all amino acids investigated, though remained highly digestible. There were, however, only small differences due to protein or lipid source in growth performance, and no effects of insect ingredients on feed intake or feed conversion ratio. Inclusion of IM-based diets significantly increased both hepatosomatic index and visceral somatic index of Atlantic salmon. Proteinase activity in the intestine was not affected by dietary inclusion of BSF larvae meal, while leucine aminopeptidase activity was lower in fish fed with insect ingredients than the control group. Whole-body protein, lipid, amino acids and minerals contents were not affected by protein or lipid source. In general, this study showed that protein meal and oil from BSF larvae hold a great potential as a source of nutrients for Atlantic salmon.

1. Introduction

The current global human consumption of fish is estimated to be 20 kg of fish annually per capita, of which approximately half is supplied by aquaculture (FAO, 2016). To meet the growing demand of fish and seafood for an increasing population, it is imperative to increase production efficiency of aquaculture while also respecting the environmental sustainability of the industry. The choice of ingredients and formulation of the fish diets can greatly influence the environmental impact of the aquaculture industry. Therefore, continuous improvement in this sector is crucial (Boyd and McNevin, 2015). Although insects, per today, are not produced in sufficient volumes to be used in commercial fish feed production, they show great promise as sustainable ingredients for future aquafeed production (Makkar et al., 2014; Tran et al., 2015).

In the last decade, there has been a growing interest in using insects as a protein source for animal feed production (FAO, 2013), largely due to the high quality and quantity of protein of many insect species (Sánchez-Muros et al., 2014). Insects used in commercial production have fast growth, reproduce easily, efficiently convert low-grade organic matter into high-value protein and fat and do not require cultivable land (Barroso et al., 2014; Henry et al., 2015; Sánchez-Muros et al., 2014). Insects are also part of the natural diets of many fish species (Tran et al., 2015; Van Huis, 2013). Insect larvae have successfully been used as a feed ingredient for a variety of fish species, e.g. European seabass (*Dicentrarchus labrax*) (Magalhães et al., 2017), blackspot seabream (*Pagellus bogaraveo*) (Iaconisi et al., 2017), Jian carp (*Cyprinus carpio* var. *Jian*) (Li et al., 2017), rainbow trout (*Oncorhynchus mykiss*) (Borgogno et al., 2017), juvenile turbot (*Psetta maxima*) (Kroeckel et al., 2012) and Atlantic salmon (*Salmo salar)* (Lock

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et al., 2016). In the western world, the three most promising insect species for feed purposes are the common housefly (*Musca domestica*), the yellow mealworm (*Tenebrio molitor*) and the black soldier fly (BSF) (*Hermetia illucens*) (Tran et al., 2015; Veldkamp et al., 2012). These species have received increasing attention in the last few years, due to their ability to grow well on organic waste, producing high-quality protein and fat (Čičková et al., 2015; Nguyen et al., 2015; Veldkamp et al., 2012).

The nutritional properties vary largely depending on the species and development stage of the insect (Alegbeleye et al., 2012; Barroso et al., 2014; Henry et al., 2015; Makkar et al., 2014; Sánchez-Muros et al., 2014; Tran et al., 2015; Van Huis, 2013). BSF larvae are rich in protein (≈40% of dry weight (DW)) and have a well-balanced essential amino acid profile, similar to the amino acids of fishmeal, and can thus provide high-value feedstuff (Barroso et al., 2014; Henry et al., 2015; Liland et al., 2017; Makkar et al., 2014). Moreover, BSF larvae are a good source of minerals such as iron, zinc, potassium, phosphorus, manganese and magnesium and contain a variety of vitamins (Henry et al., 2015; van Huis, 2013). BSF larvae also have a high fat content (≈30% of DW), which composition will vary according to the insect feeding medium (Sealey et al., 2011; St-Hilaire et al., 2007). For example, feeding BSF larvae a diet enriched with fish offal, rich in omega-3 long chain-polyunsaturated fatty acids (LC-PUFA), increased the concentration of these fatty acids in the larvae (Sealey et al., 2011; St-Hilaire et al., 2007). We recently demonstrated that feeding BSF larvae with media partially containing seaweed (the brown algae *Ascophyllum nodosum)*, enriched the larvae with marine nutrients, such as eicosapentaenoic acid (EPA) and iodine (Liland et al., 2017). The insect larvae can thus carry essential nutrients from sources which are not directly suitable for animal nutrition; such as seaweed, which is not ideally used in high concentrations in feed for carnivore fish species due to its high content of complex carbohydrates (De Jesus Raposo et al., 2015).

In the current study, we aimed to evaluate the effect of using BSF larvae grown on different media as feed ingredients for freshwater Atlantic salmon, focusing on growth performance, body composition, feed utilization and nutrient digestibility.

2. Materials and methods

2.1. Experimental diets and feeding trial

2.1.1. Diets

Insect Meal (IM) and Insect Oil (IO) used in this study were produced from BSF larvae by Protix Biosystems BV (Dongen, The Netherlands). The larvae were grown on (1) media containing organic waste streams, or on (2) media partially containing seaweed (ground seaweed (*Ascophyllum nodosum*) mixed with media 1 (50:50)). More details on the rearing and the chemical composition of the BSF larvae are presented in the supplementary tables (Tables S1-S2) and in Liland et al. (2017). At the end of an eight-day growth period, the larvae were mechanically separated from the feeding media, washed and processed immediately to separate IM and IO (Protix Biosystems BV). The experimental extruded diets (Table 1) were formulated and produced by Cargill (Dirdal, Norway), and supplemented with 2% yttrium oxide as an inert digestibility marker. Directly after production, the diets were shipped to the experimental facility and stored at −20 °C until they were fed to the fish. The control diet (IM-0/VO) represents a modern freshwater salmon diet, with protein from fish meal (FM) and soy protein concentrate (SPC) (50:50) and lipids from fish oil (FO) and vegetable oil (VO) (33:66). Five experimental diets were formulated, where 85% of the protein was replaced with IM (IM-85) and/or all the VO was replaced with IO, either produced from larvae grown on media 1 (IO1) or media 2 (IO2) (Table 1). Only the IM from the insect larvae grown on media 1 (organic waste streams) was used due to technical difficulties when producing the protein meal from insect larvae grown on media 2 (seaweeds). The IM contained relatively high concentrations of lipids (12%), and in the diets where both IM and IO were added, less IO was added so that the total insect lipid in the diet was not increased. Total IO added to the diets is presented in Table 1. The diets were balanced to contain sufficient essential AA (methionine and lysine were added) and some additional FO was included in the diets without FM to provide sufficient LC-PUFAs.

Table 1
Formulation and proximate composition of the six experimental diets fed to fresh-water Atlantic salmon (*Salmo salar*) for a period of 8 weeks.

Formulation	IM-0/VO	IM-0/IO1	IM-0/IO2	IM-85/VO	IM-85/IO1	IM-85/IO2
Ingredients (%)						
Fish meal LT94	35	35	35	6	6	6
Insect meal	0	0	0	60	60	60
Soy protein concentrate	29.6	29.5	29.5	5	5	5
Wheat gluten	14.3	14.3	14.3	14.4	14.4	14.4
Fish oil	4.6	4.6	4.6	6.9	6.9	6.9
Rapeseed oil	12	0	0	4.8	0	0
Insect oil-1	0	12	0	0	4.8	0
Insect oil-2	0	0	12	0	0	4.8
Vitamin &mineral mix	0.3	0.3	0.3	0.3	0.3	0.3
Yttrium	2	2	2	2	2	2
Misc	4.2	4.2	4.2	2.6	2.6	2.6
Sum	100	100	100	100	100	100
Total insect lipid added*	0	12	12	7.2	12	12
Proximate analysis						
DM (%)	94	93	93	96	94	94
Crude lipid (%)	18	19	17	22	20	21
Crude protein (%)	47	46	46	44	44	44
Carbohydrates (%)	11	10	10	12	12	12
Ash (%)	8	8	8	7	7	6
Gross energy (MJ kg^{-1} DM)	21.7	21.8	21.2	23.2	22.7	22.7
TBARS (nmol g^{-1})	7	8	8	17	17	18

IM-0/VO = diets without insect meal (IM) inclusion: protein from fish meal (FM) and soy protein concentrate (SPC)/lipids from vegetable oil (VO); IM-0/IO1 and IM-0/IO2 = protein from FM and SPC/VO replaced with insect oil 1 (IO1) or insect oil 2 (IO2); IM-85/VO = 85% of protein sources replaced with IM/lipids from VO; IM-85/IO1 and IM-85/IO2 = 85% of protein sources replaced with IM/VO replaced with IO1 or IO2. DM: dry matter; Misc: miscellaneous; TBARS: Thiobarbituric acid-reactive substances. *Calculated as IO + lipids originating from the IM (12% lipid).

2.1.2. Feeding trial and facilities

The feeding trial was conducted at Cargill Innovation's experimental facility in Dirdal (Norway) during February–April 2016, following the institutional and national guidelines for the care and use of animal, and approved by the National Animal Research Authority in Norway. Fresh water Atlantic salmon were randomly distributed into 24 tanks ($n = 4$), with 100 fish in each tank. One meter tanks contained 450 L filtered running freshwater with a temperature of 12 °C. The fish were fed one of the six diets (Table 1) during 8 weeks. Each diet was distributed by hand until visual satiation. Two daily meals were provided with a minimum of 4 h between the meals. Uneaten feed was collected and pellets counted, thus deduced from the total daily feeding.

2.2. Sampling

Fish were collected at the start (day 0) and at the end of the trial (day 56). At both samplings, a total of ten fish per tank (randomly selected) were anaesthetized, individually weighed and body length measured. The fish were examined externally to check for possible abnormalities. Liver and viscera were removed and weighed for calculation of organosomatic indices. Faeces were collected by manual stripping from the same fish, pooled for each tank and frozen on dry ice for digestibility measurements.

For analysis of proximate composition, ten whole fish were pooled

for each tank, frozen on dry ice and stored at −80 °C until analysis. From additional 6 fish per tank, the whole digestive tract was dissected, cleaned of attached adipose tissue and divided into proximal (PI), mid (MI) and distal (DI) intestine. Digesta from the PI1 (the proximal half of PI), PI2 (the distal half of PI), MI, DI1 (the proximal half of DI) and DI2 (the distal half of DI) of fish from the same tank was pooled and frozen immediately for the analysis of trypsin activity and total bile acids level. The empty intestinal segments (PI, MI, and DI) were frozen for the brush border enzyme activity analysis.

2.3. Analysis of chemical composition

Total nitrogen was analysed on freeze-dried, ground samples (feed, whole fish and faeces) using a CHNS elemental analyser (Vario Macro Cube, Elementar Analysensysteme GmbH, Langenselbold, Germany) and quantified according to Dumas (Dumas, 1831). The instrument was calibrated with EDTA (Leco Corporation, Saint Joseph, MI, USA). Sulfanilamide (Alfa Aesar GmbH & Co, Karlsruhe, Germany) and a standard meat reference material (SMRD 2000, LGC Standards, Teddington, UK) were used as control samples.

Analysis of total amino acids (not including cysteine and tryptophan) of the feed, whole fish and faeces was carried out by ultra-performance liquid chromatography (UPLC, Waters Acquity UPLC system) coupled with a UV detector (Espe et al., 2014; Liland et al., 2017). Wet, ground samples (feed, whole fish and faeces) equivalent of 30–40 mg of protein were hydrolysed in 6 M HCl at 110 °C, the residue was diluted in MilliQ-Plus water and filtered through a syringe-driven filter. Prior to the instrumental analysis, a derivatisation agent (AccQ.Tag™, Waters, Milford, MA, USA) was added to each sample. Finally, amino acids were separated by UPLC (column: Aquity UPLC BEH C18 1.7 μM, Waters, flowrate 0.7 mL min^{-1}) and results integrated by Empower 3 (Waters). Amino acids were quantified using standards from Thermo Fisher Scientific (Product number; 20088 Rockford, IL 61105 USA).

Starch in the feeds was quantified using an enzymatic method according to Hemre et al. (1989). Starch in 0.5 g freeze-dried, ground material was hydrolyzed with the heat-stable enzymes amylase (Termamyl-120L; Novo-Industries, Bagsværd, Denmark) for 30 min at 80 °C and amyloglucosidase (EC 3.2.1.3.; Boehringer, Ingelheim, Germany) for 30 min at 60 °C. Glucose was subsequently measured spectrophotometrically as nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) at 340 nm after a hexokinase/glucose-6-phosphate dehydrogenase reaction using a Maxmat PL multianalyser (Montpellier, France). Starch concentration was calculated as the difference in glucose concentration before and after enzymatic breakdown. Dextrin was used as reference material.

Apolar lipid (storage fat) content of the whole fish was determined gravimetrically after ethyl-acetate extraction, while the fat in feed and faeces after acid hydrolysis and extraction with diethyl ether. Energy density (kJ g^{-1} WW) was calculated by assuming caloric values of 39.7 J mg^{-1} for lipids, 18.2 J mg^{-1} for starch and 17.1 J mg^{-1} for proteins.

Mineral concentration in freeze-dried, ground material (feed, whole homogenized fish and faeces) was analysed by inductively coupled plasma massspectrometry (ICP-MS) after wet digestion in a microwave oven, as described by Julshamn et al. (2013) with some modifications. Shortly, the samples were digested in 69% nitric acid (2 mL) and 30% hydrogen peroxide (0.5 mL) using a microwave digestion system (UltraWAVE, Milestone, Sorisole, Italy). The solutions were diluted to 25 mL with deionized water (MilliQ, Merck Millipore, Billerica, MA, USA). Mineral concentrations in the samples were quantified by ICP-MS (iCapQ ICPMS, ThermoFisher Scientific, Waltham, MA, USA) equipped with an autosampler (FAST SC-4Q DX, Elemental Scientific, Omaha, NE, USA). Data were collected and processed using the Qtegra ICPMS Software (ThermoFisher Scientific).

Yttrium oxide concentrations in freeze-dried feed and faeces were analysed according to Otterå et al. (2003). Briefly, yttrium oxide was quantified by ICP-MS after wet digestion in a microwave oven (Otterå et al., 2003).

Thiobarbituric acid-reactive substances (TBARS) were determined in the feed by a method modified from Scmedes and Hølmer (1989). Homogenized samples (0.2 g) were weighed into screw-capped glass tubes and 4.0 mL of chloroform: methanol 2: 1 with 0.2 mL butylated hydroxytoluene was added. Samples were purged with N_2, and tubes were closed and incubated with constant shaking for 30 min at room temperature. Thereafter, 2.0 mL of a saturated EDTA solution was added and the tubes were centrifuged for 20 min at 1500 × *g*. A 2.0 mL aliquot of the methanol: water layer was transferred to clean screw-capped glass tubes, mixed with 2.0 mL TBA reagent (1% thiobarbituric acid in 5% trichloroacetic acid) and heated for 30 min at 100 °C. Absorption was measured at 532 nm and TBARS quantified by reference to an external standard (Malondialdehyde, MDA).

2.4. Digestive enzyme activity and total bile acids level determination

Freeze-dried digesta from PI1, PI2, MI, DI1 and DI2 was mixed thoroughly with cold distilled H_2O (1:10, *w/v*) on a rotating shaker at 4 °C for 10 min. After centrifugation (13,000*g*, 4 °C, 10 min), the supernatants were collected into 2 mL Eppendorf tubes, frozen in liquid N_2 and stored at −80 °C. Prior to fast freezing, supernatants for total bile acids determination were subjected to sonication for 60 s at 4 °C. Trypsin activity was measured using benzoyl arginine *p*-nitroanilide (Sigma no. B-4875, Sigma Chemical Co., St. Louis, MO, USA) as substrate modified from Kakade et al. (1973). As bovine trypsin shows a very different activity than that of salmon, the standard curve was not used for the calculation but to check if the assay worked. The trypsin activity is expressed as the difference in absorbance between the test and blank tube per mg dry matter (ΔOD/mg dry matter). Total bile acids were determined using the Enzabile test kit (catalog no. 550101, BioStat Diagnostic Systems, Cheshire, U.K.) and a curve derived from standardized taurocholic acid solution.

The PI, MI, and DI tissue was homogenized in cold tris-mannitol buffer (1:20 *w/v*) containing the serine protease inhibitor (24 μg/ml), 4-(2-aminoethyl)benzenesulfonyl fluoride HCl (Pefabloc® SC; Pentapharm Limited, Basel, Switzerland), using an Ultra Turrax® homogenizer (IKA, Staufen, Germany) followed by sonication at 4 °C for 15 s. The homogenates were frozen in liquid N_2 in aliquots and stored at −80 °C awaiting analysis. The leucine aminopeptidase (LAP) activity was determined using L-leucine-β-naphthylamide as substrate (Krogdahl et al., 2003). The enzyme activity is expressed as specific activity, normalized by the tissue protein. The protein concentration of homogenates was determined using the BioRad® Protein Assay kit based on the Bradford dye-binding method (BioRad Laboratories, Munich, Germany).

2.5. Calculations

Growth and nutritional indices were calculated as followed:

Apparent digestibility (AD) = $100 - (Y_d * CX_f) * (Y_f * CX_d)^{-1} * 100$, where d is diet, f is faeces, *Y* is yttrium concentration, and CX is nutrient concentration.

Condition factor (CF) = 100 × body weight (g)/length^3 (cm)

Daily growth index (DGI) = 100 × ((final body weight)$^{1/3}$ − (initial body weight)$^{1/3}$)/day

Feed conversion ratio (FCR) = feed intake (g)/fish weight gain (g)

Hepatic Somatic Index (HSI) = 100 ´ liver weight (g)/body weight (g)

Visceral Somatic Index (VSI) = 100 ´ viscera weight (g)/body weight (g)

Specific growth rate (SGR) = 100 × [ln final body weight (g) − ln initial body weight (g)]/days

Food intake (FI) = 100 × quantity of food taken/[day × ((initial

weight + final weight)/2)]
Protein/lipid efficiency ratio (PER/LER) = [final body weight (g) − initial body weight (g)]/protein/lipid intake (g)]

2.6. Statistical analysis

All statistical analyses were performed using the free software environment R (R Development Core Team, 2011). The experiment was designed to use a 2 × 3-way factorial ANOVA design with lipid (VO, IO1 and IO2) and protein (FM + SPC or IM) as varying factors. Differences due to dietary treatments were detected by nested two-way ANOVA (variables: protein and lipid source; random effect factor: tank) and Tukey's post hoc test using the packages *nlme* (Pinheiro et al., 2010) and *multcomp* (Hothorn et al., 2008). All data were tested for homogeneity of variance by Levene's test. Data, which were identified as non-homogeneous, were subjected to a non-parametric analysis (Kruskal Wallis test) (Giraudoux, 2011). Differences were regarded as significant when $P < 0.05$. All data are presented as means and pooled standard error (SE).

3. Results

3.1. Diet composition

Inclusion of IM in the experimental diet resulted in lower crude protein and ash content (44 and 6, respectively) compared to the diets devoid of IM (46 and 8, respectively), while the content of crude lipid and carbohydrates increased in IM diets (21 and 12, respectively) compared to the IM-0 diets (18 and 10, respectively) (Table 1). The composition of essential amino acids (AAs) were approximatively similar in all feeds (Table 2). The diets with insect meal (IM-85 diets) had lower concentrations of the non-essential AAs glutamic acid (Glu), hydroxoproline (Hyp) and taurine (Tau), while having a higher tyrosine (Tyr) concentration than the diets not containing insect meal.

The concentrations of essential minerals were similar between the diets and covered the requirements of Atlantic salmon (NRC, 2011) (Table 3). Replacing the FM and SPC with IM reduced dietary arsenic (As) and phosphorus (P), whereas manganese (Mn) and iron (Fe) concentrations increased in the diets with IM (Table 3). The level of peroxidation product (TBARS) was higher in the IM-85 diets (18 mmol/g) compared to in the IM-0 diets (7 mmol/g) (Table 1), but all these TBARS values are considered low and should not have any negative effect on the fish (Hamre et al., 2001).

IM-0/VO = diets without insect meal (IM) inclusion: protein from fish meal (FM) and soy protein concentrate (SPC)/lipids from vegetable oil (VO); IM-0/IO1 and IM-0/IO2 = protein from FM and SPC/VO replaced with insect oil 1 (IO1) or insect oil 2 (IO2); IM-85/VO = 85% of protein sources replaced with IM/lipids from VO; IM-85/IO1 and IM-85/IO2 = 85% of protein sources replaced with IM/VO replaced with IO1 or IO2.

Table 2
Total amino acid composition ($g\,kg^{-1}$ wet weight) of the six experimental diets fed to fresh-water Atlantic salmon (*Salmo salar*) for a period of 8 weeks.

	IM-0/VO	IM-0/IO1	IM-0/IO2	IM-85/VO	IM-85/IO1	IM-85/IO2
Ala	23	22	23	25	25	25
Arg	26	25	26	20	20	20
Asp	44	42	43	39	39	37
Glu	80	77	80	57	57	54
Gly	22	22	22	20	21	21
His	11	11	11	10	11	11
Hyp	2.0	2.0	2.0	0.7	0.7	0.7
Ile	18	18	19	17	17	17
Leu	33	33	33	30	30	30
Lys	31	29	31	31	32	30
Met	10	10	10	11	11	11
Phe	20	21	21	19	19	19
Pro	24	24	24	25	25	25
Ser	21	21	20	18	19	18
Tau	3.0	2.5	2.5	0.6	0.6	0.5
Thr	18	18	18	16	17	16
Tyr	14	14	14	22	23	24
Val	22	22	23	24	25	25

Table 3
Mineral composition ($mg\,kg^{-1}$) of the six experimental diets fed to fresh-water Atlantic salmon for a period of 8 weeks.

	IM-0/VO	IM-0/IO1	IM-0/IO2	IM-85/VO	IM-85/IO1	IM-85/IO2
As	4.0	4.0	4.0	2.0	1.0	1.0
Ag	0.03	0.02	0.01	0.01	0.01	0.01
Ca	13,927	13,878	14,308	13,956	13,953	14,142
Cd	0.3	0.3	0.3	0.4	0.4	0.4
Co	0.09	0.08	0.1	0.1	0.1	0.1
Cr	3.0	3.0	3.0	3.0	3.0	3.0
Cu	12	11	11	15	16	15
Fe	177	170	270	337	366	436
Hg	0.03	0.03	0.03	0.01	0.01	0.01
K	11,664	11,649	11,630	9030	8817	8167
Mg	2029	1995	2013	1910	1928	1876
Mn	62	62	65	220	228	227
Mo	2.0	2.0	2.0	0.7	0.7	0.7
Na	4249	4169	4191	1612	1480	1357
Ni	0.6	0.7	0.7	1.0	1.0	1.0
P	14,045	14,098	14,360	11,605	11,325	11,148
Pb	0.07	0.09	0.07	0.15	0.14	0.14
Se	1.0	1.0	1.0	0.5	0.5	0.5
V	3.0	3.0	3.0	3.0	3.0	3.0
Zn	200	187	198	219	220	224

IM-0/VO = diets without insect meal (IM) inclusion: protein from fish meal (FM) and soy protein concentrate (SPC)/lipids from vegetable oil (VO); IM-0/IO1 and IM-0/IO2 = protein from FM and SPC/VO replaced with insect oil 1 (IO1) or insect oil 2 (IO2); IM-85/VO = 85% of protein sources replaced with IM/lipids from VO; IM-85/IO1 and IM-85/IO2 = 85% of protein sources replaced with IM/VO replaced with IO1 or IO2.

3.2. Growth performance and somatic index

At start of the trial, the fish had a mean weight of 49 g (Table 4). After 56 days, the fish had grown to approximatively three fold of the initial body weight (137 g). The oil source in the diet had a significant effect on growth; fish fed diets with IO1 had significantly lower SGR and DGI (1.79 and 2.60, respectively) compared with fish fed VO (1.90 and 2.80, respectively) and IO2 (1.85 and 2.75, respectively). There was no significant effects on growth due to the protein source and no significant interactions were shown. Hepatosomatic index (HSI) and viscerosomatic index (VSI) of fish fed with IM-85 diets (1.40 and 11.36, respectively) were significantly higher than in the fish fed with the IM-0 diets (1.06 and 10.43, respectively). Daily FI, FCR, CF and PER were not significantly affected by dietary treatments (Table 4).

3.3. Apparent nutrient digestibility

The digestibility of CP, CL and ash was significantly reduced by replacing dietary plant and fish protein with IM (3, 1 and 3%, respectively) (Fig. 1). The replacement of plant- and fish protein with IM also reduced the digestibility of all AAs calculated (1–2%), except for asparagine (Table 5). The oil source had a significant effect on CL digestibility; fish fed IO1 had a lower lipid digestibility than the fish fed VO and IO2 (Fig. 1). A significant interaction between protein and lipid sources was observed for ash digestibility (Fig. 1).

Table 4
Mean growth performance and feed utilization of fresh-water Atlantic salmon fed a control diet (IM-0/VO) or diets containing IM and/or IO1 or IO2 for a period of 8 weeks.

	Diets							P		
	IM-0/VO	IM-0/IO1	IM-0/IO2	IM-85/VO	IM-85/IO1	IM-85/IO2	Pooled SE	p	o	p x o
IW (g)	49.0	49.0	46.7	47.9	48.6	49.7	0.40	NS	NS	NS
FW (g)	143.0	133.4	135.0	139.5	133.0	137.0	1.42	NS	NS	NS
DGI §	2.84	2.64	2.78	2.82	2.64	2.69	0.02	NS	*	NS
SGR §	1.91	1.80	1.88	1.89	1.78	1.83	0.01	NS	*	NS
HSI	1.02	1.13	1.12	1.38	1.41	1.43	0.03	**	NS	NS
VSI	10.69	10.41	10.24	11.34	11.18	11.62	0.12	**	NS	NS
FCR	0.77	0.79	0.82	0.79	0.85	0.81	0.01	NS	NS	NS
FI	1.31	1.19	1.30	1.31	1.30	1.28	0.01	NS	NS	NS
CF	1.40	1.40	1.43	1.45	1.43	1.45	0.005	NS	NS	NS
PER	2.77	2.73	2.67	2.86	2.67	2.80	0.02	NS	NS	NS
LER	7.24	6.61	7.22	5.72	5.88	5.86	0.14	*	NS	NS

IW: initial weight; FW: final weight; DGI (%/fish/day): daily growth increase; SGR (%/fish/day): specific growth rate; HSI: hepatosomatic index; VSI: viscerosomatic index; FCR: food conversion ratio; FI (g/fish/day): feed intake; CF: condition factor; PER: protein efficiency ratio; LER: lipid efficiency ratio.

P value for p (protein source), o (oil source) and interaction between protein and oil (p $_v$. o): $*P < 0.05$; $**P < 0.01$ (two-way ANOVA).

§DGI = significant effect of oil source: IO1 − VO = *0.001*; IO2 − VO = 0.09; IO2 − IO1 = 0.15

§SGR = significant effect of oil source: IO1 − VO = *0.03*; IO2 − VO = 0.24; IO2 − IO1 = 0.12

3.4. Digestive enzyme activity and total bile acids level

The trypsin activity and total bile acids concentration in different intestinal segments were not affected by the inclusion of insect ingredients (Table 6). For LAP activity, fish fed the IM-85 diets showed markedly lower activity in the PI and MI, in contrast to a minor but significant increase of the enzyme activity in the DI (Table 6). The oil source also changed LAP activity in the DI, with lower activities observed in fish fed on IO-based diets.

3.5. Whole fish composition

The inclusion of IM in the diets (IM-85 diets) resulted in significant increases in whole fish DM compared to IM-0 fed fish (Fig. 2). No dietary effects on whole fish CP, CL or ash were observed.

The concentration of some AAs (Ala, Asp, Glu, Leu, Lys and Val) were significantly higher in the fish fed insect lipid (IO1 or IO2) compared with fish fed with dietary VO (Table 7). Most AAs, however, remained unaffected by the diets.

Feeding the IM-85 diets led to significantly reduced whole body arsenic (As), copper (Cu), mercury (Hg) and selenium (Se) content, while iron (Fe) and manganese (Mn) concentrations increased significantly compared with IM-0 diets (Table 8).

IM-0/VO = diets without insect meal (IM) inclusion: protein from fish meal (FM) and soy protein concentrate (SPC)/lipids from vegetable oil (VO); IM-0/IO1 and IM-0/IO2 = protein from FM and SPC/VO replaced with insect oil 1 (IO1) or insect oil 2 (IO2); IM-85/VO = 85% of protein sources replaced with IM/lipids from VO; IM-85/IO1 and IM-85/IO2 = 85% of protein sources replaced with IM/VO replaced with IO1 or IO2. IO1: insect oil from insects reared on organic side streams, IO2: insect oil from insects reared on organic side streams and seaweed (50,50).

4. Discussion

Using insect ingredients in the fish feeds did not affect the voluntary feed intake, indicating no negative effect on palatability of the insects-based diets for the Atlantic salmon. Even at inclusion levels of 600 g IM per kg diet, no negative effects on growth performances or feed conversion ratios were observed. This is in accordance with other trials using BSF protein meal or whole insect meal to replace fish meal in diets for salmonids, where no effects on growth were seen (Renna et al., 2017; Sealey et al., 2011; St-Hilaire et al., 2007). The fish fed diets with insect oil from BSF larvae grown on substrate enriched with marine macroalgae (IO2) grew as fast as the control group (fed dietary VO), while the fish fed diets with insect oil from larvae grown on media containing only terrestrial organic waste (IO1) grew slightly less. Interestingly, a positive effect on growth has previously been reported in rainbow trout fed diets containing BSF raised on cow manure and fish offal compared to BSF raised on a diet of cow manure alone (Sealey et al., 2011; St-Hilaire et al., 2007). Indeed, the partial inclusion of the

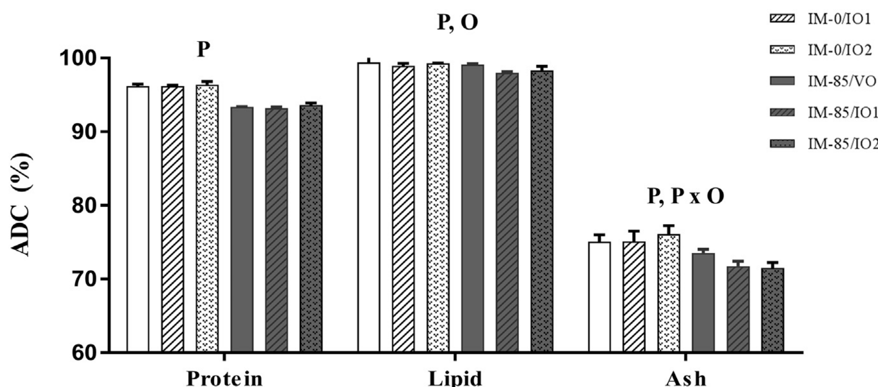

Fig. 1. Apparent digestibility coefficients (ADC) (%) of crude protein, crude lipid and ash in fish fed a control diet (IM-0/VO) or diets containing IM and/or IO1 or IO2 for a period of 8 weeks. Values are means, with their standard deviation represented by vertical bars. P, significant effect of dietary protein source. O, significant effect of dietary lipid source. P × O, interaction between the main effects of the two factors ($P < 0.05$, two-way ANOVA).
ADC% protein; $P \leq 0.01$, O = 0.08, P × O = 0.7
ADC% lipid; P ≤ 0.01, O ≤ 0.01 (IO1-VO = 0.01; IO2-VO = 0.1; IO2-IO1 = 0.39), P × O = 0.26
ADC% ash; P ≤ 0.01, O = 0.2, P × O = 0.01.

Table 5
Apparent digestibility coefficients (ADC %) of amino acids in fresh-water Atlantic salmon fed a control diet (IM-0/VO) and diets containing IM and/or IO1 or IO2 for a period of 8 weeks.

	Diets							P		
	IM-0/VO	IM-0/IO1	IM-0/IO2	IM-85/VO	IM-85/IO1	IM-85/IO2	Pooled SE	p	o	p x o
Ala	97.6	97.6	97.9	96.5	96.5	96.6	0.12	*	NS	NS
Arg §	97.8	98.1	98.1	97.5	97.4	97.7	0.05	*	*	NS
Asp	94.5	94.2	94.9	95.4	95.6	95.5	0.12	*	NS	NS
Glu	98.3	98.2	98.5	97.2	97.2	97.2	0.12	*	NS	NS
Gly	96.0	96.1	96.3	94.8	94.7	94.8	0.15	*	NS	NS
His	n.c.	n.c.	n.c.	96.0	96.0	96.3	0.06	–	–	–
Hyp	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.				
Ile	97.2	97.9	98.0	96.8	96.7	96.9	0.11	*	NS	NS
Leu	98.0	98.0	98.1	97.1	97.0	97.0	0.10	*	NS	NS
Lys	97.9	97.9	98.1	96.5	96.6	96.5	0.15	*	NS	NS
Met	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.				
Phe	n.c.	n.c.	n.c.	97.2	97.1	97.3	0.04	–	–	–
Pro §	97.9	98.0	98.1	97.2	97.2	97.5	0.08	*	*	NS
Ser	97.0	97.2	97.3	96.0	96.1	96.3	0.11	*	NS	NS
Thr	96.7	96.9	97.1	95.5	95.8	95.8	0.13	*	NS	NS
Tyr	n.c.	n.c.	n.c.	97.2	97.3	97.5	0.04	–	–	–
Val	97.5	97.6	97.9	96.7	96.8	96.8	0.10	*	NS	NS

IM-0/VO = diets without insect meal (IM) inclusion: protein from fish meal (FM) and soy protein concentrate (SPC)/lipids from vegetable oil (VO); IM-0/IO1 and IM-0/IO2 = protein from FM and SPC/VO replaced with insect oil 1 (IO1) or insect oil 2 (IO2); IM-85/VO = 85% of protein sources replaced with IM/lipids from VO; IM-85/IO1 and IM-85/IO2 = 85% of protein sources replaced with IM/VO replaced with IO1 or IO2. IO1: insect oil from insects reared on organic side streams, IO2: insect oil from insects reared on organic side streams and seaweed (50,50).
P value for p (protein source), o (oil source) and interaction between the main effects of the two factors (p $_v$. o): *$P < 0.05$ (two-way ANOVA).
§Arg = significant effect of oil source: IO1-VO = 0.56; IO2-VO = *0.04*; IO2-IO1 = 0.40
§Pro = significant effect of oil source: IO1-VO = 0.71; IO2-VO = *0.04*; IO2-IO1 = 0.33
n.c., not calculated due to very low concentrations in either feed or faeces.

Table 6
Digestive enzyme activity and total bile acids level in the intestine of fresh-water Atlantic salmon fed a control diet (IM-0/VO) and diets containing IM and/or IO1 or IO2 for a period of 8 weeks.

Diet								P		
	IM-0/VO	IM-0/IO1	IM-0/IO2	IM-85/VO	IM-85/IO1	IM-85/IO2	Pooled SE	p	o	p × o
Trypsin/PI1	231	271	209	198	244	196	27.5	NS	NS	NS
Trypsin/PI2	178	243	200	158	218	140	25.2	NS	NS	NS
Trypsin/MI	135	153	100	132	133	108	18.4	NS	NS	NS
Trypsin/DI1	65	63	88	41	59	42	15.9	NS	NS	NS
Trypsin/DI2	7.0	8.0	7.0	8.0	11	9.0	1.7	NS	NS	NS
Bile acids/PI1	142	163	150	120	138	124	15.0	NS	NS	NS
Bile acids/PI2	120	142	128	116	116	95	13.9	NS	NS	NS
Bile acids/MI	92	100	81	89	88	83	9.4	NS	NS	NS
Bile acids/DI1	35	37	47	25	29	26	6.7	NS	NS	NS
Bile acids/DI2	4.0	7.0	6.0	4.0	6.0	5.0	1.1	NS	NS	NS
LAP/PI	472	516	483	361	346	365	24.6	***	NS	NS
LAP/MI	312	266	247	199	198	192	10.0	***	NS	NS
LAP/DI §	363	324	322	377	347	354	10.9	*	*	NS

IM-0/VO = diets without insect meal (IM) inclusion: protein from fish meal (FM) and soy protein concentrate (SPC)/lipids from vegetable oil (VO); IM-0/IO1 and IM-0/IO2 = protein from FM and SPC/VO replaced with insect oil 1 (IO1) or insect oil 2 (IO2); IM-85/VO = 85% of protein sources replaced with IM/lipids from VO; IM-85/IO1 and IM-85/IO2 = 85% of protein sources replaced with IM/VO replaced with IO1 or IO2. IO1: insect oil from insects reared on organic side streams, IO2: insect oil from insects reared on organic side streams and seaweed (50,50).
PI: proximal intestine; MI: mid intestine; DI: distal intestine; LAP: leucine aminopeptidase.
P value for p (protein source), o (oil source) and interaction between the main effects of the two factors (p $_v$. o): *$P < 0.05$; ***$P < 0.001$ (two-way ANOVA).
§ LAP/DI = significant effect of oil source: IO1-VO = 0.02; IO2-VO = *0.04*; IO2-IO1 = 0.97.

brown algae, *Ascophyllum nodosum,* in the media improved the nutritional composition of the BSF by introducing marine nutrients into the larvae such as EPA, iodine and vitamin E (Liland et al., 2017). Therefore, the nutritional composition of BSF larvae grown on feeding media enriched with marine nutrients may therefore, not surprisingly, be especially beneficial for the growth of marine carnivorous fish species.

Most previous trials where BSF protein meal or whole insect meal has replaced FM at different inclusion levels (between 6 and 400 g kg^{-1} diet) have shown no negative effects on growth for rainbow trout, channel catfish, tilapia, yellow catfish, gilthead seabream and European seabass (Bondari and Sheppard, 1987; Karapanagiotidis et al., 2014; Magalhães et al., 2017; Renna et al., 2017; Li et al., 2017). However, Kroeckel et al. (2012) reported a negative effect on growth parameters for juvenile turbot when dietary BSF meal increased from 330 to 756 g kg^{-1} in the diet (Kroeckel et al., 2012). The authors attributed this negative effect to a decrease of feed intake due to the low palatability of insect-based diets. The altered growth of the fish might be caused by the presence of chitin, which could influence feed intake, bioavailability and digestibility of the nutrients and therefore resulting in reduced fish growth (Kroeckel et al., 2012). The insect protein meal used in the current trial should not contain very high concentrations of chitin as the mantle of the larvae was separated from the biomass before separating the insects into protein meal and oil. This is likely why the current trial shows no negative effects of IM on growth parameters.

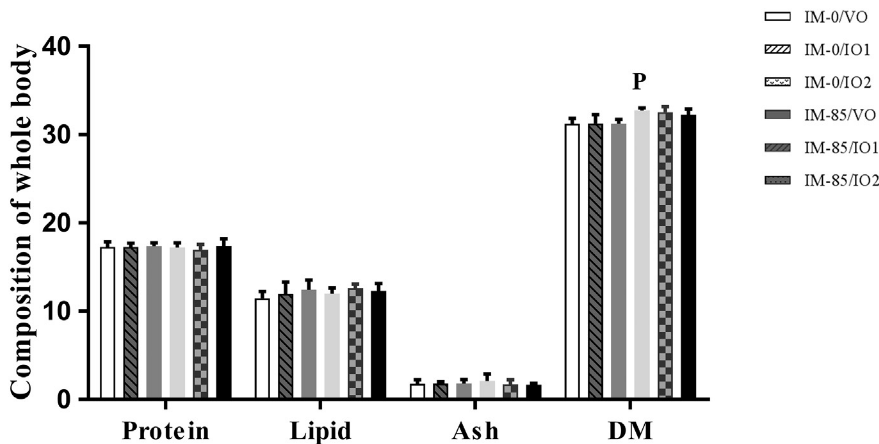

Fig. 2. Composition of whole body (% of wet weight) of fresh-water Atlantic salmon fed a control diet (IM-0/VO) or diets containing IM and/or IO1 or IO2 for a period of 8 weeks. Values are means, with their standard deviation represented by vertical bars. P, significant effect of dietary protein source. O, significant effect of dietary lipid source. P × O, interaction between the main effects of the two factors ($P < 0.05$, two-way ANOVA). DM; P ≤0.01, O = 0.75, P × O = 0.75.

In accordance with previous findings in European seabass (Magalhães et al., 2017) and Jian carp (Li et al., 2017), the inclusion of BSF larvae meal in Atlantic salmon's diet did not affect proteinase (trypsin) activity in the intestine. Nonetheless, fish fed the IM-based diets showed markedly lower activity of LAP, a brush border enzyme breaking down peptides into AAs, in the proximal and mid intestine, where the majority of proteins are digested and absorbed. The reduced activity of this enzyme might be due to the content of chitin in IM-based diets, which is a hard polysaccharide that could interfere with intestinal homeostasis, causing for example changes in intestinal turnover or sloughing. Concomitantly, the apparent digestibility of crude protein, as well as most amino acids, was significantly lower when IM was included in the diets, compared to diets with fishmeal and SPC. Despite the decreased digestibility of crude protein, feed intake and feed conversion ratio were unaffected by inclusion of IM in the diets, probably indicating an increased utilization of digestible proteins in the fish fed the IM diets. On the other hand, a nitrogen-to protein conversion ratio factor of 6.25 (Kjeldahl, 1883) was used to estimate the protein content, overestimating the non-protein nitrogen in insect meal diets, as chitin is in the outer layers and might affected the protein digestibility values in insect-meal groups.

However, the digestibility values were, in general high and comparable to, or even higher than, the digestibility obtained for salmonids fed with other alternative protein sources, such as wheat gluten meal, bacterial protein meal or poultry by-product meal (Aas et al., 2006; Burr et al., 2012; Storebakken et al., 2000). Additionally, the protein efficiency ratio and the whole body crude protein were unaffected by dietary inclusion of insect meal, indicating that insect meal can be utilized efficiently as protein sources for fresh-water Atlantic salmon. These results are consistent with those observed in other studies with European seabass using BSF larvae meal at 195 g kg^{-1} feed (Magalhães et al., 2017) or mealworm beetle meal at 250 g kg^{-1} feed (Gasco et al.,

Table 7
Whole-fish amino acid concentration (mg g^{-1}) of fresh-water Atlantic salmon fed a control diet (IM-0/VO) or diets containing IM and/or IO1 or IO2 for a period of 8 weeks.

	Diets							*P*		
	IM-0/VO	IM-0/IO1	IM-0/IO2	IM-85/VO	IM-85/IO1	IM-85/IO2	Pooled SE	p	o	p × o
Ala §	9.8	10.0	10.2	9.6	10.3	10.3	0.07	NS	*	NS
Arg	9.3	8.8	9.3	9.1	9.2	9.4	0.08	NS	NS	NS
Asp §	16.8	17.4	17.6	16.7	17.8	17.6	0.1	NS	*	NS
Glu §	22.6	22.9	23.7	22.0	23.3	23.5	0.1	NS	*	NS
Gly	9.1	8.9	9.7	9.0	9.3	9.2	0.1	NS	NS	NS
His	4.2	4.0	4.2	4.1	4.2	4.2	0.03	NS	NS	NS
Hyp	0.8	0.9	0.9	0.8	0.8	0.8	0.04	NS	NS	NS
Ile	7.3	7.4	7.5	7.2	7.6	7.7	0.05	NS	NS	NS
Leu ¥	12.8	12.8	13.0	12.6	13.2	13.2	0.07	NS	*	NS
Lys §	15.2	15.8	15.9	15.1	16.3	16.0	0.1	NS	*	NS
Met	5.0	4.9	5.1	5.0	5.1	5.1	0.03	NS	NS	NS
Phe	7.0	6.6	6.9	6.9	6.9	7.1	0.06	NS	NS	NS
Pro	6.1	6.0	6.5	6.0	6.3	6.4	0.07	NS	NS	NS
Ser	6.9	6.8	7.0	6.9	7.1	7.1	0.04	NS	NS	NS
Tau	1.0	1.0	1.0	0.8	0.7	0.7	0.03	*	NS	NS
Thr	7.8	7.7	7.9	7.7	7.9	8.0	0.04	NS	NS	NS
Tyr	5.6	5.2	5.5	5.6	5.6	5.7	0.05	NS	NS	NS
Val¥	9.2	9.2	9.3	9.0	9.4	9.5	0.05	NS	*	NS

IM-0/VO = diets without insect meal (IM) inclusion: protein from fish meal (FM) and soy protein concentrate (SPC)/lipids from vegetable oil (VO); IM-0/IO1 and IM-0/IO2 = protein from FM and SPC/VO replaced with insect oil 1 (IO1) or insect oil 2 (IO2); IM-85/VO = 85% of protein sources replaced with IM/lipids from VO; IM-85/IO1 and IM-85/IO2 = 85% of protein sources replaced with IM/VO replaced with IO1 or IO2. IO1: insect oil from insects reared on organic side streams, IO2: insect oil from insects reared on organic side streams and seaweed (50,50).
P value for p (protein source), o (oil source) and interaction between the main effects of the two factors (p_v. o): *$P < 0.05$ (two-way ANOVA).
§ALA = significant effect of oil source: IO1-VO = *0.01*; IO2-VO ≤*0.01*; IO2-IO1 = 0.38.
§ASP = significant effect of oil source: IO1-VO = *0.02*; IO2-VO ≤*0.01*; IO2-IO1 = 0.96.
§GLU = significant effect of oil source: IO1-VO = *0.04*; IO2-VO ≤*0.01*; IO2-IO1 = 0.13.
§LYS = significant effect of oil source: IO1-VO = *0.03*; IO2-VO = *0.01*; IO2-IO1 = 0.84.
¥LEU = significant effect of oil source: IO1-VO = 0.15; IO2-VO ≤*0.01*; IO2-IO1 = 0.46.
¥VAL = significant effect of oil source: IO1-VO = 0.22; IO2-VO = *0.01*; IO2-IO1 = 0.27.

Table 8
Whole-fish mineral concentrations (mg kg^{-1}) of fresh-water Atlantic salmon fed a control diet (IM-0/VO) or diets containing IM and/or IO1 or IO2 for a period of 8 weeks.

	Diets							P		
	IM-0/VO	IM-0/IO1	IM-0/IO2	IM-85/VO	IM-85/IO1	IM-85/IO2	Pooled SE	p	o	p × o
As	2.0	2.0	2.0	0.3	0.3	0.3	0.16	**	NS	NS
Ag	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.				
Ca	2975	2600	2600	3050	2975	2650	162	NS	NS	NS
Cd	0.01	0.01	0.01	0.01	0.01	0.01	0.001	NS	NS	NS
Co	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.				
Cr	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.				
Cu	1.0	1.0	1.0	0.7	0.7	0.7	0.2	**	NS	NS
Fe	8.0	8.0	8.0	11	12	12	0.3	**	NS	NS
Hg	0.02	0.02	0.02	0.01	0.01	0.01	0.001	**	NS	NS
K	4075	4075	4125	3925	3975	3950	25	*	NS	NS
Mg	310	305	305	310	313	313	2	NS	NS	NS
Mn	1.0	1.0	1.0	2.0	2.0	2.0	0.1	**	NS	NS
Mo	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.				
Na	688	698	693	668	673	695	7	NS	NS	NS
Ni	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		NS	NS	NS
P	4200	3975	4000	4175	4150	3925	81	NS	NS	NS
Pb	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.				
Se	0.2	0.2	0.3	0.1	0.1	0.1	0.01	**	NS	NS
V	0.1	0.1	0.1	0.04	0.05	0.05	0.01	**	NS	NS
Zn	37	23	29	33	33	33	2	NS	NS	NS

IM-0/VO = diets without insect meal (IM) inclusion: protein from fish meal (FM) and soy protein concentrate (SPC)/lipids from vegetable oil (VO); IM-0/IO1 and IM-0/IO2 = protein from FM and SPC/VO replaced with insect oil 1 (IO1) or insect oil 2 (IO2); IM-85/VO = 85% of protein sources replaced with IM/lipids from VO; IM-85/IO1 and IM-85/IO2 = 85% of protein sources replaced with IM/VO replaced with IO1 or IO2. IO1: insect oil from insects reared on organic side streams, IO2: insect oil from insects reared on organic side streams and seaweed (50,50).
P value for p (protein source), o (oil source) and interaction between the two factors (p_v. o): **P* < 0.05; ***P* < 0.01 (two-way ANOVA).
n.d.: not detectable.

2016).

Essential amino acid content of BSF larvae has been described in several reviews (Henry et al., 2015; Makkar et al., 2014). Generally, BSF larvae meal has a favorable AA profile, similar to that of FM and SPC, however it is considered low in tryptophan and sulfur amino acids, like methionine (Makkar et al., 2014). Our own studies did confirm the low level of methionine in the BSF produced at the same installations as used to produce insects for the current feeds (Liland et al., 2017). The calculated digestibility of all AAs was high and ranged between 95 and 98%. To our knowledge, only one study on the ADC of AAs of insect based diets in fish has been conducted (Magalhães et al., 2017), showing high digestibility of the AAs of BSF larvae meal also in European seabass (Magalhães et al., 2017). Therefore, BSF larvae meal seem to be a valuable source of easily digestible AAs for different fish species.

A significant increase in both hepatosomatic index (HSI) and visceral somatic index (VSI) was seen due to inclusion of insect meal in the diets. The same effects were reported in a study with sea-water phase Atlantic salmon, in which fish meal was partially replaced with BSF insect meal (Lock et al., 2016). In the trial performed by Lock et al. (2016) as well as in the current trial, the diets leading to changes in VSI and HSI were low in taurine content. Taurine is a non-essential metabolite involved in maintaining the osmotic balance in cells and regulating lipid metabolism (Ripps and Shen, 2012). In the case of low dietary supply of taurine, the fish can produce it using precursor sulfur AAs, such as methionine or cysteine. However, when such sulfur AAs are not supplied in sufficient concentrations in diets, this can lead to lower production and tissue concentrations of taurine, as probably seen in the current trial reduction in whole-body taurine in the IM-fed fish. The essential AA methionine was added to the diets in the current trial in order to fulfil the requirements of Atlantic salmon (≈10–11 g kg^{-1}) (Espe et al., 2008; Espe et al., 2007; NRC, 2011). However, due to the low taurine concentrations in the IM-diets, an addition of methionine beyond the normal requirements of the fish could be beneficial. Indeed, addition of taurine to a diet low in taurine (due to high plant protein) had a positive effect on lipid metabolism and reduced lipid depositions in juvenile Atlantic salmon (Espe et al., 2012). In addition, Atlantic salmon fed diets low in methionine show decreased hepatic taurine and a higher liver weight compared to fish fed diets with adequate methionine levels (Espe et al., 2008, 2010). As methionine was not added in surplus in the current diets, it is likely that less methionine was metabolized to taurine and the reduced taurine tissues concentration might have influenced the lipid metabolism and –storage.

High phosphorus (P) content in aquaculture diets can have a negative impact the environment via an excessive eutrophication of the aquatic ecosystem. The BSF insect meal used in the current trial had a low P content and thus lowered the dietary phosphorus load when included in the diets. BSF insect meal is generally always lower in P than typical fish meals (Liland et al., 2017), so this change in dietary composition was expected when replacing fish meal with IM. This decreased P did not affect the fish, as whole fish P was unaffected by the diets, but the lower concentration of P in insect-based diets could be used to reduce P pollution from Atlantic salmon farms.

The use of IM as a protein source led to decreased levels of arsenic (As) and mercury (Hg) in the diets which was visible as an 85% reduction of arsenic and a 50% reduction of mercury in the whole fish. This was expected, as these contaminants are typically associated with marine ingredients, such as fish meal (Berntssen et al., 2004). Cadmium (Cd) and lead (Pb), which are often present in farmed insect larvae (Charlton et al., 2015; Diener et al., 2015), increased in the diets with IM inclusion. However, the composition of the whole body was unaffected by the increase of these contaminants in the diets. Overall, the inclusion of insect-derived ingredients in the diets did not pose any challenges in terms of feed safety, and the levels of As, Hg, Cd and Pb in the diets did not exceed any of the current EU maximum levels set for these contaminants in fish feed (Directive 2002/32/EC and amendments).

5. Conclusion

In conclusion, this study demonstrated that is possible to add 600 g kg^{-1} of insect meal in combination with insect oil in the diets of

fresh-water Atlantic salmon without any adverse effect on growth performances, feed utilization, apparent digestibility and whole body composition. Furthermore, the BSF protein meal seems to be a good source of AA and has high bioavailability for AA in Atlantic salmon. Moreover, the whole body proximate composition was not influenced by the use of insects-based diets. One should, however, be aware of the low concentrations of taurine in BSF insect meal and the possible effects this could have on lipid metabolism and -deposition.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2018.03.016.

References

Aas, T.S., Hatlen, B., Grisdale-Helland, B., Terjesen, B.F., et al., 2006. Effects of diets containing a bacterial protein meal on growth and feed utilisation in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 261 (1), 357–368. http://dx.doi.org/10.1016/j.aquaculture.2006.07.033.

Alegbeleye, W.O., Obasa, S.O., Olude, O.O., Otubu, K., et al., 2012. Preliminary evaluation of the nutritive value of the variegated grasshopper (*Zonocerus variegatus* L.) for African catfish *Clarias gariepinus* (Burchell. 1822) fingerlings. Aquac. Res. 43 (3), 412–420. http://dx.doi.org/10.1111/j.1365-2109.2011.02844.x.

Barroso, F.G., De Haro, C., Sánchez-Muros, M.-J., Venegas, E., et al., 2014. The potential of various insect species for use as food for fish. Aquaculture 422–423, 193–201. http://dx.doi.org/10.1016/j.aquaculture.2013.12.024.

Berntssen, M.H.G., Hylland, K., Julshamn, K., Lundebye, A.K., et al., 2004. Maximum limits of organic and inorganic mercury in fish feed. Aquac. Nutr. 10 (2), 83–97. http://dx.doi.org/10.1046/j.1365-2095.2003.00282.x.

Bondari, K., Sheppard, D.C., 1987. Soldier fly, *Hermetia illucens L.*, larvae as feed for channel catfish, *Ictalurus punctatus* (Rafinesque), and blue tilapia, *Oreochromis aureus* (Steindachner). Aquac. Res. 18 (3), 209–220. http://dx.doi.org/10.1111/j.1365-2109.1987.tb00141.x.

Borgogno, M., Dinnella, C., Laconisi, V., Fusi, R., et al., 2017. Inclusion of Hermetia illucens larvae meal on rainbow trout (*Oncorhynchus mykiss)* feed: effect on sensory profile according to static and dynamic evaluations. J. Sci. Food Agric. http://dx.doi.org/10.1002/jsfa.8191.

Boyd, C.E., McNevin, A.A., 2015. Front matter. In: In Aquaculture, Resource Use, and the Environment. John Wiley & Sons, Inc., pp. i–xxv.

Burr, G.S., Wolters, W.R., Barrows, F.T., Hardy, R.W., 2012. Replacing fishmeal with blends of alternative proteins on growth performance of rainbow trout (*Oncorhynchus mykiss*), and early or late stage juvenile Atlantic salmon (*Salmo salar*). Aquaculture 334, 110–116. http://dx.doi.org/10.1016/j.aquaculture.2011.12.044.

Charlton, A.J., Dickinson, M., Wakefield, M.E., Fitches, E., et al., 2015. Exploring the chemical safety of fly larvae as a source of protein for animal feed. J. Insects Food Feed 1 (1), 7–16.

Čičková, H., Newton, G.L., Lacy, R.C., Kozánek, M., 2015. The use of fly larvae for organic waste treatment. Waste Manag. 35, 68–80. http://dx.doi.org/10.1016/j.wasman.2014.09.026.

De Jesus Raposo, M.F., De Morais, A.M.B., De Morais, R.M.S.C., 2015. Marine polysaccharides from algae with potential biomedical applications. Mar. Drugs 13 (5), 2967–3028. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4446615/.

Development Core Team, R., 2011. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria (ISBN 3-900051-07-0).

Diener, S., Zurbrügg, C., Tockner, K., 2015. Bioaccumulation of heavy metals in the black soldier fly, *Hermetia illucens* and effects on its life cycle. J. Insects Food Feed 1 (4), 261–270.

Dumas, J.P., 1831. Lettre de M. Dumas a M. Gay-Lussac, sur le procedes de l'analyse organique. Ann. Chim. Phys. 2 (47), 198–215.

Espe, M., Lemme, A., Petri, A., El-Mowafi, A., 2007. Assessment of lysine requirement for maximal protein accretion in Atlantic salmon using plant protein diets. Aquaculture 263 (1), 168–178.

Espe, M., Hevroy, E.M., Liaset, B., Lemme, A., et al., 2008. Methionine intake affect hepatic sulphur metabolism in Atlantic salmon, *Salmo salar*. Aquaculture 274 (1), 132–141. http://dx.doi.org/10.1016/j.aquaculture.2007.10.051.

Espe, M., Rathore, R.M., Du, Z.-Y., Liaset, B., et al., 2010. Methionine limitation results in increased hepatic FAS activity, higher liver 18:1 to 18:0 fatty acid ratio and hepatic TAG accumulation in Atlantic salmon, *Salmo salar*. Amino Acids 39 (2), 449–460. http://dx.doi.org/10.1007/s00726-009-0461-2.

Espe, M., Ruohonen, K., El-Mowafi, E., 2012. Effect of taurine supplementation on the metabolism and body lipid-to-protein ratio in juvenile Atlantic salmon (*Salmo salar*). Aquac. Res. 43 (3), 349–360. http://dx.doi.org/10.1111/j.1365-2109.2011.02837.x.

Espe, M., Andersen, S.M., Holen, E., Rønnestad, V., et al., 2014. Methionine deficiency does not increase polyamine turnover through depletion of hepatic S-adenosylmethionine in juvenile Atlantic salmon. Br. J. Nutr. 112 (8), 1274–1285.

Directive 2002/32/EC of the European Parliament and the Council of 7 May 2002 on Undesirable Substances in Animal Feed.

FAO, 2013. Forestry paper. In: Edible Insects: Futur Prospects for Food and Feed Security, Rome.

FAO, 2016. The State of Word Fisheries and Aquaculture 2016 Contributing to Food Security and Nutrition for All, Rome. (200 pp).

Gasco, L., Henry, M., Piccolo, G., Marono, S., et al., 2016. *Tenebrio molitor* meal in diets for European sea bass (*Dicentrarchus labrax* L.) juveniles: growth performance, whole body composition and in vivo apparent digestibility. Anim. Feed Sci. Technol. 220, 34–45. http://dx.doi.org/10.1016/j.anifeedsci.2016.07.003.

Giraudoux, P. (2011). 'kruskalmc' in Pgirmess: Data Analysis in Ecology, R package Version 1.5.2.: patrick.giraudoux@univ-fcomte.fr.

Hamre, Kristin., Kolås, K., Sandnes, K., Julshamn, K., et al., 2001. Feed intake and absorption of lipid oxidation products in Atlantic salmon (*Salmo salar*) fed diets coated with oxidised fish oil. Fish Physiol. Biochem. 25 (3), 209–219. http://dx.doi.org/10.1023/A:1022257928437.

Hemre, G.-I., Lie, Ø., Lied, E., Lambertsen, G., 1989. Starch as an energy source in feed for cod (*Gadus morhua*): digestibility and retention. Aquaculture 80, 261–270. http://dx.doi.org/10.1016/0044-8486(89)90174-9.

Henry, M., Gasco, L., Piccolo, G., Fountoulaki, E., 2015. Review on the use of insects in the diet of farmed fish: past and future. Anim. Feed Sci. Technol. 203, 1–22. http://dx.doi.org/10.1016/j.anifeedsci.2015.03.001.

Hothorn, T., Bretz, F., Westfall, P., 2008. Simultaneous inference in general parametric models. Biom. J. 50 (3), 346–363.

Iaconisi, V., Marono, S., Parisi, G., Gasco, L., et al., 2017. Dietary inclusion of Tenebrio molitor larvae meal: effects on growth performance and final quality treats of blackspot sea bream (*Pagellus bogaraveo*). Aquaculture 476, 49–58. http://dx.doi.org/10.1016/j.aquaculture.2017.04.007.

Julshamn, K., Maage, A., Norli, H.S., Grobecker, K.H., et al., 2013. Determination of arsenic, cadmium, mercury, and lead in foods by pressure digestion and inductively coupled plasma/mass spectrometry: first action 2013.06. J. AOAC Int. 96 (5).

Kakade, M., Hoffa, D.E., Liener, I.E., 1973. Contribution of trypsin inhibitors to the deleterious effects of unheated soybeans fed to rats. J. Nutr. 103, 1772–1778.

Karapanagiotidis, I., Daskalopoulou, E., Vogiatzis, I., Rumbos, C., et al., 2014. Substitution of Fishmeal by Fly *Hermetia illucens* Prepupae Meal in the Diet of Gilthead Seabream (*Sparus aurata*).

Kjeldahl, J., 1883. New method for the determination of nitrogen in organic substances. Z. Anal. Chem. 22, 366–382.

Kroeckel, S., Harjes, A.G.E., Roth, I., Katz, H., et al., 2012. When a turbot catches a fly: evaluation of a pre-pupae meal of the black soldier fly (*Hermetia illucens*) as fish meal substitute — growth performance and chitin degradation in juvenile turbot (*Psetta maxima*). Aquaculture 364–365, 345–352. http://dx.doi.org/10.1016/j.aquaculture.2012.08.041.

Krogdahl, A., 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 salar* L.). Aquac. Nutr. 9, 361–371. http://dx.doi.org/10.1046/j.1365-2095.2003.00264.x.

Li, S., Ji, H., Zhang, B., Zhou, J., et al., 2017. Defatted black soldier fly (*Hermetia illucens)* larvae meal in diets for juvenile Jian carp (*Cyprinus carpio* var. Jian): growth performance, antioxidant enzyme activities, digestive enzyme activities, intestine and hepatopancreas histological structure. Aquaculture 477, 62–70. http://dx.doi.org/10.1016/j.aquaculture.2017.04.015.

Liland, N.S., Biancarosa, I., Araujo, P., Biemans, D., et al., 2017. Modulation of nutrient composition of black soldier fly (*Hermetia illucens*) larvae by feeding seaweed-enriched media. PLoS One 12 (8), e0183188. http://dx.doi.org/10.1371/journal.pone.0183188.

Lock, E., Arsiwalla, T., Waagbø, R., 2016. Insect larvae meal as an alternative source of nutrients in the diet of Atlantic salmon (*Salmo salar*) postsmolt. Aquac. Nutr. 22 (6), 1202–1213. http://dx.doi.org/10.1111/anu.12343.

Magalhães, R., Sánchez-López, A., Leal, R.S., Martínez-Llorens, S., et al., 2017. Black soldier fly (*Hermetia illucens*) pre-pupae meal as a fish meal replacement in diets for European seabass (*Dicentrarchus labrax*). Aquaculture 476, 79–85. http://dx.doi.org/10.1016/j.aquaculture.2017.04.021.

Makkar, H.P.S., Tran, G., Heuzé, V., Ankers, P., 2014. State-of-the-art on use of insects as animal feed. Anim. Feed Sci. Technol. 197, 1–33. http://dx.doi.org/10.1016/j.anifeedsci.2014.07.008.

National Research Council (NRC), 2011. Nutrient Requirements of Fish and Shrimp. The National Academies Press, Washington, DC.

Nguyen, T.T.X., Tomberlin, J.K., Vanlaerhoven, S., 2015. Ability of black soldier fly (Diptera: Stratiomyidae) larvae to recycle food waste. Environ. Entomol. 44 (2), 406–410. http://dx.doi.org/10.1093/ee/nvv002.

Otterå, H., Garatun-Tjeldsto, O., Julshamn, K., Austreng, E., 2003. Feed preferences in juvenile cod estimated by inert lanthanide markers-effects of moisture content in the feed. Aquac. Int. 11, 217–224.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., et al., 2010. nlme: Linear and Nonlinear Mixed Effects Models, v 3.1-97. R Package.

Renna, M., Schiavone, A., Gai, F., Dabbou, S., et al., 2017. Evaluation of the suitability of a partially defatted black soldier fly (*Hermetia illucens L.*) larvae meal as ingredient for rainbow trout (*Oncorhynchus mykiss Walbaum*) diets. J. Anim. Sci. Biotechnol. 8, 57.

Ripps, Harris, Shen, Wen, 2012. Review: taurine: a “very essential” amino acid. Mol. Vis. 18, 2673–2686.

Sánchez-Muros, M.-J., Barroso, F.G., Manzano-Agugliaro, F., 2014. Insect meal as renewable source of food for animal feeding: a review. J. Cleaner Production 65, 16–27. http://dx.doi.org/10.1016/j.jclepro.2013.11.068.

Scmedes, A., Hølmer, G., 1989. A new thiobarbituric acid (TBA) method for determing free malondialdehyde (MDA) and hydroperoxides selectively as a measure of lipid peroxidation. J. Am. Oil Chem. Soc. 66, 813–817.

Sealey, W.M., Gaylord, T.G., Barrows, F.T., Tomberlin, J.K., et al., 2011. Sensory analysis of rainbow trout, *Oncorhynchus mykiss*, fed enriched black soldier fly prepupae, *Hermetia illucens*. J. World Aquac. Soc. 42 (1), 34–45. http://dx.doi.org/10.1111/j.1749-7345.2010.00441.x.

St-Hilaire, S., Sheppard, C., Tomberlin, J.K., Irving, S., et al., 2007. Fly prepupae as a feedstuff for rainbow trout, *Oncorhynchus mykiss*. J. World Aquac. Soc. 38 (1), 59–67. http://dx.doi.org/10.1111/j.1749-7345.2006.00073.x.

Storebakken, T., Shearer, K.D., Baeverfjord, G., Nielsen, B.G., et al., 2000. Digestibility of macronutrients, energy and amino acids, absorption of elements and absence of intestinal enteritis in Atlantic salmon, *Salmo salar*, fed diets with wheat gluten. Aquaculture 184 (1), 115–132. http://dx.doi.org/10.1016/S0044-8486(99)00316-6.

Tran, G., Heuzé, V., Makkar, H.P.S., 2015. Insects in fish diets. Anim. Front. 5 (2), 37–44. http://dx.doi.org/10.2527/af.2015-0018.

Van Huis, A., 2013. Potential of insects as food and feed in assuring food security. Ann. Rev. Entomol. 58 (1), 563–583. http://dx.doi.org/10.1146/annurev-ento-120811-153704.

Veldkamp, T., Duinkerken, G.V., Huis, A.V., Lakemond, C.M.M., et al., 2012. Insects as a Sustainable Feed Ingredient in Pig and Poultry Diets: A Feasibility Study. Wageningen UR Livestock Production, Wageningen, The Netherlands, Report No. 638. pp. 1–48.