Received: 14 August 2018 | Revised: 29 October 2018 | Accepted: 31 October 2018

DOI: 10.1111/anu.12860

ORIGINAL ARTICLE

WILEY Aquaculture Nutrition

Insect-based diets high in lauric acid reduce liver lipids in freshwater Atlantic salmon

Ikram Belghit | Rune Waagbø | Erik-Jan Lock | Nina S. Liland

Institute of Marine Research, Bergen, Norway

Correspondence
Ikram Belghit, Department of Requirement & Welfare, Institute of Marine Research, Bergen, Norway.
Email: Ikram.Belghit@hi.no

Funding information
Norwegian Research Council, Grant/Award Number: 238997, 244253 and 220634

Abstract

We evaluated the effect of a diet containing insect meal and insect oil on nutrient utilization, tissue fatty acid profile and lipid metabolism of freshwater Atlantic salmon (*Salmo salar*). Insect meal and insect oil from black soldier fly larvae (*Hermetia illucens*, L.; BSF), naturally high in lauric acid (12:0), were used to produce five experimental diets for an eight-week feeding trial. 85% of the dietary protein was replaced by insect meal and/or all the vegetable oil was replaced by one of two types of insect oil. A typical industrial diet, with protein from fishmeal and soy protein concentrate (50:50) and lipids from fish oil and vegetable oil (33:66), was fed to a control group. The dietary BSF larvae did not modify feed intake or whole body lipid content. Despite the high content of saturated fatty acids in the insect-based diets, the apparent digestibility coefficients of all fatty acids were high. There was a decrease in liver triacylglycerols of salmon fed the insect-based diets compared to the fish fed the control diet. This is likely due to the rapid oxidation and low deposition of the medium-chain fatty acid lauric acid.

KEYWORDS
Atlantic salmon, black soldier fly, fatty acid oxidation, lauric acid, lipid metabolism, medium-chain FA

1 | INTRODUCTION

Insects have been highlighted by the Food and Agricultural Organization of the United Nations as a potential feedstuff for animals (FAO, 2013). Depending on the species, stage of development (larval, pupa, nympha or imago) and their growth media, insects can be rich sources of protein, fat, minerals, vitamins and energy, making many insects optimal feedstuffs for animals, including fish. The suitability of the nutrient content but also the physiological effects of insects in fish feed has been widely reviewed (Borgogno et al., 2017; Gasco et al., 2016; Li et al., 2016; Li, Ji, Zhang, Zhou, & Yu, 2017; Lock, Arsiwalla, & Waagbø, 2016; Magalhães et al., 2017; Makkar, Tran, Heuzé, & Ankers, 2014; Tran, Heuzé, & Makkar, 2015; van Huis, 2013). In addition to their nutritive qualities, the utilization of insects as feed implies certain environmental benefits such as their low carbon footprint and high efficiency in the use of nutrients from the growth substrate. As of the recent EU commission regulation (2017/893–24/05/2017), the use of insect meal from seven different insect species (black soldier fly, common housefly, yellow mealworm, lesser mealworm, house cricket, banded cricket and field cricket) is allowed in aquafeeds, and a tremendous increase in investments in this sector is now seen.

The black soldier fly (BSF), *Hermetia illucens*, (Diptera, family of stratiomyidae) is original to the Americas but distributed throughout the tropics and warm temperate regions (Sheppard, Tomberlin, Joyce, Kiser, & Sumner, 2002). Its larvae are a scavenger and thrive on a variety of decomposing organic resources such as plants, algae, manure and food waste. When fed a high-quality substrate

such as food waste, the BSF larvae contain approximately 41% protein and 28% fat (on dry weight basis; Liland et al., 2017; Wang & Shelomi, 2017). Interestingly, compared to others insects such as yellow mealworm, house cricket or orange-spotted roach, the BSF larvae show a different fatty acid (FA) composition (Oonincx, van Broekhoven, van Huis, & van Loon, 2015). While the FA composition of most other commonly used insect species is dominated by mono- and polyunsaturated FA, the largest lipid fraction of BSF larvae is the medium-chain FA (MCFA, FA with an aliphatic tail of 6–12 carbon atoms), which is the principal constituent of the medium-chain triacylglycerides (MCT). Lauric acid (12:0) represents between 21% and 50% of total FA in BSF larvae, making its FA composition similar to coconut oil (Li et al., 2016; Liland et al., 2017; Tran et al., 2015). The role of MCT has been widely investigated in both livestock and human nutrition because of their rapid absorption and oxidation (St-Onge, Bosarge, Goree, & Darnell, 2008), and also due to their antimicrobial and antiviral properties (Dayrit, 2015; Sado-Kamdem, Vannini, & Guerzoni, 2009). In rodents and humans, consumption of MCT is associated with decreased feed intake and reduced fat deposition (Ooyama, Kojima, Aoyama, & Takeuchi, 2009; St-Onge et al., 2008). These effects have been attributed to the rapid metabolic utilization of MCFA. MCFAs are more polar than other lipids and thus less dependent on chylomicron and lipoproteins for transport, since they can be transported without the need for these systems. This results in an accelerated uptake by the enterocytes compared to longer-chained FA (Johnson, Young, Cotter, Lin, & Rowe, 1990; Stubbs & Harbron, 1996). Furthermore, MCFA do not require prior modifications by carnitine palmitoyltransferase-1 (CPT-1) for crossing the mitochondrial membrane and are therefore not dependent on this rate-limiting step of FA β-oxidation (Garlid, Orosz, Modrianský, Vassanelli, & Jezek, 1996).

The effects of dietary MCT as oil sources have been studied in various fish species, but the results regarding their suitability for use in fish feed are ambiguous (Craig & Gatlin, 1995; Figueiredo-Silva et al., 2012; Fontagné, Pruszynski, Corraze, & Bergot, 1999; Li et al., 2016; Nordrum, Krogdahl, Røsjø, Olli, & Holm, 2000; Røsjø et al., 2000). The use of dietary MCT has mostly been investigated in view of the replacement of marine ingredients (fish oil) and provided either as an MCT oil (8:0 and 10:0) (Nordrum et al., 2000) or from natural sources of 12:0 (BSF larvae oil (Li et al., 2016) or coconut oil

TABLE 1 Formulation and proximate composition of the experimental diets (DM basis) fed to Atlantic salmon (*Salmo salar*; previously published [Belghit et al., 2018])

Formulation	IM-0/VO	IM-0/IO1	IM-0/IO2	IM-85/VO	IM-85/IO1	IM-85/IO2
Ingredients (g/kg)						
Fishmeal LT94	350	350	350	60	60	60
Insect meal	0.0	0.0	0.0	600	600	600
Soy protein concentrate	296	295	295	50	50	50
Wheat gluten	143	143	143	144	144	144
Fish oil	46	46	46	69	69	69
Rapeseed oil	120	0.0	0.0	48	0.0	0.0
Insect oil-1	0.0	120	0.0	0.0	48	0.0
Insect oil-2	0.0	0.0	120	0.0	0.0	48
Vitamin &Mineral mix	3.0	3.0	3.0	3.0	3.0	3.0
Yttrium	2.0	2.0	2.0	2.0	2.0	2.0
Misc	42	42	42	26	26	26
Proximate analysis (g/kg)						
DM	940	930	930	960	940	940
Crude lipid	180	190	170	220	200	210
Crude protein	470	460	460	440	440	440
Carbohydrates	110	100	100	120	120	120
Ash	80	80	80	70	70	60
Gross energy (MJ/kg DM)	21.7	21.8	21.2	23.2	22.7	22.7
TBARS (nmol/g)	7.0	8.0	8.0	17	17	18

Note. IM-0/VO = diets without insect meal (IM) inclusion: protein from fishmeal (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); DM: dry matter; Misc: miscellaneous; TBARS: thiobarbituric acid-reactive substances.

(Williams, Williams, Smith, & Jones, 2006)). Dietary MCT resulted in increased absorption of protein, lipid and starch, while growth, feed intake and fat deposition correlate negatively with MCT intake in Atlantic salmon (Nordrum et al., 2000) and also in polka-dot grouper (*Cromileptes altivelis*) (Williams et al., 2006) Furthermore, the negative effect on lipid retention in polka-dot grouper has been suggested due to a high level of lauric acid (12:0) β-oxidation (Williams et al., 2006). Figueiredo-Silva et al. (2012), however, reported in rainbow trout (*Oncorhynchus mykiss* Walbaum) fed with a diet rich in MCT (as 12:0-rich coconut oil), a considerable accumulation of 12:0 in the whole body, representing more than 20% of total body FA (Figueiredo-Silva et al., 2012).

In the current study, we aimed to evaluate the effect of diets containing BSF larvae, high in lauric acid, on nutrient utilization, tissue FA profile and lipid metabolism of freshwater Atlantic salmon.

2 | MATERIAL AND METHODS

2.1 | Feed ingredients and experimental diet

2.1.1 | Diets

The practical details of the feed ingredients and the feeding trials with Atlantic salmon (*Salmo salar* L.) are reported in more detail elsewhere (Belghit et al., 2018). Briefly, black soldier fly larvae meal (IM) and oil (IO) reared on (1) media from organic waste streams, or on (2) media partially containing seaweeds (ground seaweed (*Ascophyllum nodosum*, mixed with media 1 (50:50)) were produced by Protix Biosystems BV (Amsterdam, The Netherlands). The experimental diets (Table 1) were formulated and produced by Cargill (Dirdal, Norway) and supplemented with 2% yttrium oxide as an inert digestibility marker. The control diet (IM-0/VO) represents a modern freshwater salmon diet, with protein from fishmeal and soy protein concentrate (50:50) and lipids from fish oil and vegetable oil (VO) (33:66). Five experimental diets containing insect ingredients 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). The diets were balanced to contain sufficient concentrations of essential AA (methionine and lysine were added) and additional fish oil was included in the diets without fishmeal to provide sufficient long-chained polyunsaturated FA.

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. Freshwater Atlantic salmon (mean weight 48.5 g) were randomly distributed into 24 tanks (n = 4), with 100 fish in each tank. Tanks (1 m in diameter) 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 four hours between the meals. Uneaten feed was collected, and pellets weighed and deducted from the total daily feed gift.

2.2 | Sampling

Fish were sampled at the start (day 0) and at the end of the trial (day 56). A total of ten randomly selected fish per tank were anaesthetized, killed by a blow to the head and individually weighed and body length measured. Faeces were collected by manual stripping from the same fish, pooled for each tank and frozen on dry ice for digestibility measurements. Blood (three fish per tank) was collected from the caudal vein by means of a heparinized medical syringe. For the analysis of nutrient composition, whole fish (10 fish per tank) and livers (six fish per tank) from each tank were pooled, homogenized and frozen in liquid N_2. Livers intended for lipid class analyses were pooled (three fish per tank), but not homogenized due to the lipolytic reactions occurring when breaking the tissue, and frozen on dry ice. Individual liver samples for qPCR analyses (three fish per tank) were frozen in liquid N_2. All samples were stored at −80°C.

2.3 | Haemoglobin and plasma metabolite assays

Haemoglobin was measured on a Cell-Dyn 400 (Sequoia-Turner, California, USA) according to the manufacturer's instructions, using Para 12 control blood (Streck) for calibration. Plasma was analysed using a clinical bioanalyser (Maxmat PL analyser, Montpellier, France) and controls to determine the fraction cholesterol and triacylglycerol (TAG) concentrations.

2.4 | Fatty acid composition

Fatty acid analysis was performed on diets, homogenized whole fish, faeces and livers. Lipids from the samples were extracted using chloroform/methanol (2: 1, v/v). The extracted lipids were filtered and the remaining sample saponified and methylated using 12% BF3 in methanol. FA composition was analysed as previously described by Torstensen (Torstensen, Frøyland, & Lie, 2004). Briefly, the FAs were identified by retention time using standard mixtures of methyl esters (Nu-Chek Prep, Elyain, MN, USA), and the FA composition (area %) was determined. All samples were integrated using the software Chromeleon® version 7 connected to the gas–liquid chromatography (Thermo Scientific, Waltham, MA, USA). The amount of FA per gram sample was calculated using 19:0 methyl ester as internal standard.

2.5 | Lipid class composition

Lipid from the liver samples was extracted by adding chloroform-methanol (2:1, v/v, 20x the amount of the samples (v/w)) to samples immediately after homogenization on liquid N_2. The samples were then filtered, and the quantification of lipid classes was carried out by high-performance thin-layer chromatography as described by Torstensen et al. (2004).

2.6 | Sterol content

The analysis of phytosterols and cholesterol in liver and feeds was performed with gas chromatography as described by Laakso (Laakso, 2005). Each test tube was added 0.3 mg internal standard, 5-β-cholestan-3-α-ol, before weighing in the sample to contain 0.5–1 g sterols per test tube. The control material used was phytosterol-enriched margarine of the brand Vita pro-aktiv (Mills DA). After sample extraction and derivatization by silylation as described previously (Laakso, 2005), the samples were diluted 20x in hexane before GC analysis. The following instrumentation was used: Thermoquest trace GC 2000 with an auto-sampler AS2000 (Thermo Scientific), an on-column injector, a flame ionization detector and the column Equity-5® of length 30 m and 0.25 mm inner diameter (Supelco, Bellefonte, PA, USA). Helium gas was used as a carrier at 0.9 ml/min, and hydrogen and air were used as the detector gases at 35 and 350 ml/min, respectively. The initial temperature was 100°C, which was increased by 50°C/min to 300°C and maintained for 12 min. The peaks were identified with the software Chromeleon® version 7.

2.7 | Gene expression analysis: quantitative real-time PCR

Total RNA was extracted from liver samples using EZ1 RNA Universal Tissue Kit (Qiagen, Crawley, UK) according to the manufacturer's instructions and frozen at −80°C. Quantity and quality of the RNA were assessed by spectrophotometry and the Agilent 2100 Bioanalyzer (Agilent Technologies) as described previously (Sanden et al., 2016). The samples used in this experiment had 260/280 nm absorbance ratios that varied between 1.80 and 2.07. RNA integrity number (RIN) was tested for a subset of samples that all had RIN values between 8 and 10 indicating RNA samples suitable for RT-PCR. A two-step qPCR was used to measure the mRNA levels of the target and the reference genes. Quantitative PCR primers sequences, GenBank accession numbers and the efficiency of the RT reactions are presented in Supporting Information Table S1. RT reactions were performed on a GeneAmp PCR 9700 (Applied Biosystems) using the TaqMan® reverse transcriptase kit with oligo primers (Applied Biosystems). Samples were run in duplicate (500 ng ± 5%) in addition to a six-point dilution curve in triplicate (1,000–31.25 ng), non-template and non-amplification controls. Real-time PCR amplification and analysis were performed on a LightCycler 480 Real-time PCR system (Roche Applied Science) with SYBR® Green I Mastermix (Roche Applied Science). Pipetting of cDNA plates was done using a Biomek® 3000 Laboratory automation workstation (Beckman Coulter, Fullerton, California). Thermal cycling was done for forty-five cycles of 10 s at each at 95°C, 60°C and 72°C, followed by a melting curve analysis to confirm that only one product was present. The geNorm tool was used to determine a normalization factor from the two reference genes, *β-actin* and $ef1a_B$, which in turn was used to calculate mean normalized expressions of the target genes.

2.8 | Statistical analysis and calculations

All statistical analyses were performed using the free software environment R (R Development Core Team, 2014). Differences due to dietary treatments were detected by nested one-way ANOVA (random effect factor: tank) and Tukey's post hoc test (packages *nlme*; Pinheiro, Bates, DebRoy, Sarkar, & Core Team, 2010) and *multcomp* (Hothorn, Bretz, & Westfall, 2008). All data were tested for homogeneity of variance by Levene's test. If the data were identified as having non-homogeneous variance or non-normal distribution, they were subjected to a non-parametric analysis (Kruskal–Wallis test; Giraudoux, 2011). A significance level of $p < 0.05$ was used. All data are presented as means and standard deviations. Figures were made using GraphPad Prism version 7.01 for Windows (GraphPad Software, La Jolla, CA, USA).

Apparent digestibility (ADC) was calculated as the ratio between the inert marker, yttrium, and the nutrient within diet and faeces calculated based on wet weight:

$$\text{Apparent digestibility coefficient (ADC)} = 100 - [100*(Yd/Yf)*(CXf/CXd)],$$

where d is diet, f is faeces, Y is yttrium concentration, and CX is nutrient concentration.

Fatty acid productive value (FAPV):

$$\text{FAPV} = \frac{\text{g FA per tank at end of trial} - \text{g FA per tank at start of trial}}{\text{g FA eaten in total per tank during 8 week feeding trial}}$$

3 | RESULTS

3.1 | Dietary composition

The six experimental diets were similar in proximate composition, apart from the three diets containing IM that had slightly higher fat content and thus a higher energy content than the other three diets. This was due to a higher lipid content of the IM than estimated when formulating the feeds (based on earlier analyses of IM samples from the same production plant; Table 1). The TBARS, indicating lipid oxidation, were also slightly higher in the IM diets (Table 1), but were still below levels that are expected to cause any negative effects on the fish (Hamre, Kolås, Sandnes, Julshamn, & Kiessling, 2001). The diets varied widely in FA composition, with the most pronounced difference being the high concentrations of lauric acid (12:0, 19%–29% of total FA) in all five diets containing insect ingredients (Table 2). Lauric acid was below the quantification limit (0.01 mg/kg sample) in the control diet without insect ingredients (IM-0/VO). The dietary content of the saturated FA (SFA) 14:0 and 16:0 was also increased by the inclusion of insect ingredients, leading to a ~3.5x increase in total SFA in the diets with insect ingredients compared to the diet with ingredients of only marine and plant origin (Table 2). Total monounsaturated FA (MUFA) content was reduced to around the half by including insect ingredients in the diets (Table 2). Total n-3 polyunsaturated FA (PUFA) decreased with the use of insect ingredients, while total n-6 PUFA content was close to equal in all the diets (Table 2). The diet

TABLE 2 Fatty acid composition (g/100 g) and total fatty acids (FA, mg/g of wet weight) of the experimental diets fed to Atlantic salmon

	IM-0/VO	IM-0/IO1	IM-0/IO2	IM-85/VO	IM-85/IO1	IM-85/IO2
12:0	<LOQ	26	22	19	29	28
14:0	2.0	8.0	8.0	6.0	8.0	8.0
16:0	8.0	14	14	12	14	15
18:0	2.0	2.0	2.0	2.0	2.0	2.0
18:1n-9	38	9.0	10	19	10	10
18:1n-7	3.0	1.0	1.0	1.0	1.0	1.0
18:2n-6	14	12	10	13	14	13
18:3n-3	6.0	1.0	1.0	3.0	1.0	1.0
20:1n-9	5.0	4.0	4.0	4.0	4.0	4.0
18:4n-3	1.0	1.0	1.0	1.0	1.0	1.0
20:4n-6 ARA	0.2	0.2	0.8	0.1	0.1	0.3
22:1n-11	7.0	7.0	7.0	6.0	6.0	6.0
20:5n-3 EPA	3.0	3.0	4.0	3.0	2.0	3.0
22:5n-3 DPA	0.3	0.3	0.3	0.3	0.2	0.2
22:6n-3 DHA	4.0	4.0	4.0	3.0	3.0	3.0
Saturated FA	13	49	48	38	51	51
Sum 16:1	2.0	3.0	3.0	3.0	3.0	3.0
Sum 18:1	41	10	11	21	11	11
Sum 20:1	5.0	5.0	5.0	5.0	4.0	4.0
Sum 22:1	7.0	7.0	7.0	7.0	6.0	6.0
Sum MUFA	55	25	27	35	24	25
Sum EPA+DHA	7.0	7.0	8.0	6.0	5.0	5.0
Sum n-3	15	11	11	11	8.0	8.0
Sum n-6	14	13	11	13	14	13
Sum PUFA	29	23	22	24	22	22
n-3/n-6	1.0	0.8	1.0	0.8	0.6	0.6
n-6/n-3	1.0	1.2	1.0	1.2	1.7	1.6
Total FA (mg/g)	157	161	144	202	182	188

Note. IM-0/VO = diets without insect meal (IM) inclusion: protein from fishmeal (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); LOQ: limit of quantification (0.01 mg/kg sample); ARA: arachidonic acid; EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid; DHA: docosahexaenoic acid; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

containing the highest concentrations of lipid from the insects fed on seaweed (IM-0/IO-2) had higher concentrations of arachidonic acid (20:4n-6, ARA) and eicosapentaenoic acid (20:5n-3, EPA) than the other dietary groups (Table 2). Dietary inclusion of IM also resulted in higher concentrations of phytosterols (1,428–1,614 mg/kg) compared to diets devoid of IM (411–939 mg/kg), while the cholesterol content was lower in the IM diets (710–866 mg/kg) than in the diets without IM (1,602–1,721 mg/kg) (Table 3).

3.2 | Growth and digestibility

The fish grew to similar final weights (~137 g; Table 4) and there were no significant differences in growth observed between the six dietary groups, as described in more detail by Belghit et al. (2018). Daily feed intake, corrected for the metabolic weight of the fish, was also similar between the dietary groups (Table 4).

The apparent digestibility of most FA was significantly reduced with dietary IM and/or IO inclusion (Table 5). The diets causing the largest decrease in FA digestibility were the diets containing both IM and IO (IM-85/IO1 and IM-85/IO2). Interestingly, the digestibility of most omega-3 FA (18:4n-3, 20:5n-3, 22:5n-3, 22:6n-3 and total n-3 FA) was unaffected by the dietary changes (Table 5).

3.3 | Composition of whole-body and tissues

The whole body FA composition of the fish (Table 6) generally reflected that of the diets (Table 2). The concentration of SFA was ~2x higher in the fish given diets with insect ingredients (31%–36% of

TABLE 3 Sterol composition (mg/kg) of the experimental diets fed to Atlantic salmon

	IM-0/VO	IM-0/IO1	IM-0/IO2	IM-85/VO	IM-85/IO1	IM-85/IO2
Cholesterol (mg/kg)	1,694	1,721	1,602	866	710	711
Phytosterols (mg/Kg)						
Brassicasterol	97	14	15	38	8.0	6.0
Campesterol	281	130	71	407	343	320
Campestanol	14	30	17	58	63	60
Stigmasterol	10	15	8.0	27	29	26
Sitosterol/fucosterol[a]	470	343	259	933	895	870
Sitostanol	53	49	31	128	117	114
Total phyto	939	598	411	1,614	1,495	1,428
Phyto:chol ratio	0.55	0.35	0.26	1.86	2.10	2.01

Note. IM-0/VO = diets without insect meal (IM) inclusion: protein from fishmeal (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). FM/FO = 100% fishmeal and 100% fish oil; PP/VO = 80% plant protein and 70% vegetable oil.

[a]Sitosterol and fucosterol co-elute in the current method (assumed that some of the sitosterol in the diets containing IO2 is fucosterol due to the macroalgae used as a growth substrate for the larvae, but mostly being sitosterol).

TABLE 4 Growth, feed intake (previously published in Belghit et al., 2018), haemoglobin and plasma clinical chemistry including aspartate aminotransferase, alanine aminotransferase, cholesterol, total protein, TAG and osmolality of Atlantic salmon fed a control diet (IM-0/VO) or diets containing IM and/or IO1 or IO2 for a period of 8 weeks

	IM-0						IM-85					
	VO		IO1		IO2		VO		IO1		IO2	
	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*
IW (g)	49.0	1.5	49.0	1.9	47.0	0.7	48.0	2.5	48.5	2.7	49.7	1.7
FW (g)	143	3.0	133.5	7.0	135	5.0	140	5.0	133	10	137	8.0
FI (g/fish/day)	1.31	0.1	1.19	0.1	1.30	0.1	1.31	0.1	1.30	0.1	1.28	0.1
FI_{MBW}	1.38	0.1	1.29	0.1	1.26	0.1	1.32	0.1	1.28	0.1	1.34	0.1
Hg (g/100 ml)	10.0	1.0	10.5	1.0	10.5	1.0	11.0	0.5	11.0	0.7	11.2	0.7
ALT (IU/L)	35.0^{a}	6.0	25.0^{b}	2.5	35.0^{a}	7.0	26.0^{b}	1.0	21.0^{b}	4.0	29.0^{ab}	1.0
AST (IU/L)	754^{b}	76	765^{b}	135	$1{,}004^{a}$	51	758^{b}	61	617^{b}	121	694^{b}	118
Chol (mM)	10.0^{b}	1.0	11.0^{b}	0.6	12.0^{b}	0.4	14.0^{a}	0.5	14.0^{a}	1.3	14.0^{a}	0.2
T_{prot} (g/L)	42.0	4.0	47.0	4.0	50.0	2.0	49.0	3.0	48.0	4.0	48.0	1.0
TAG (mM)	3.5	0.5	3.0	0.6	3.0	0.3	3.5	0.4	4.0	0.5	4.0	0.6
Osmolality (mOsm/k)	324	3.2	323	2.5	323	2.6	321	1.0	326	2.2	322	3.6

Note. IM-0 = diets without insect meal (IM) inclusion: protein from fishmeal (FM) and soy protein concentrate (SPC); VO = lipids from vegetable oil (VO); IO1 and IO2 = VO replaced with insect oil 1 (IO1) or insect oil 2 (IO2); IM-85 = 85% of protein sources replaced with IM; IO1: insect oil from insects reared on organic side streams; IO2: insect oil from insects reared on organic side streams and seaweed (50,50); IW: initial weight; FW: final weight; FI: feed intake = 100 x quantity of feed eaten/[day x ((initial weight + final weight)/2)]; FI_{MBW}: feed intake expressed relative to metabolic body weight: (√(initial body weight x final body weight)$^{0.8}$) (√(initial body weight x final body weight)$^{0.8}$); Hg: haemoglobin; ALT: alanine aminotransferase; AST: aspartate aminotransferase; chol: cholesterol; T_{prot}: total protein; TAG: triacylglycerol.

Values are means and standard deviations with different superscripts letters next to values are significantly different, $p < 0.05$; (nested one-way ANOVA).

total FA) than in the fish fed the control diet (IM-0/VO) (17% of total FA) (Table 6). The increase in saturated FA in the insect-fed fish was accompanied by a decrease in MUFA and n-3 PUFA. There was also an increase in total FA in the whole-body of the fish fed insect protein (IM-85) compared to fish the fed IM-0 (Table 6).

In the insect-fed fish, lauric acid (12:0) made up around 10% of the whole body total FA, while this FA was below the quantification limit in both whole fish and liver of fish fed the diet without insect ingredients (Tables 6 and 7). The hepatic concentration of this same FA was, however, very low in all insect-fed fish (~1.5% of total FA,

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

	IM-0						IM-85					
	VO		IO1		IO2		VO		IO1		IO2	
	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*
12:0	nc		96.0	0.3	98.1	0.4	98.3	0.3	95.4	1.0	96.3	1.0
14:0	99.2^{a}	0.1	96.6^{b}	0.3	98.2^{a}	0.4	98.0^{ab}	0.3	94.3^{c}	1.0	95.4^{c}	1.0
16:0	98.1^{a}	0.1	96.5^{b}	0.2	97.5^{ab}	0.4	97.0^{ab}	0.4	94.1^{c}	1.0	95.0^{c}	0.9
18:0	97.3^{a}	0.1	95.7^{a}	0.4	96.5^{a}	0.6	95.5^{ab}	0.5	92.6^{c}	1.0	93.5^{c}	1.0
18:1n-9	99.7^{a}	0.2	98.9^{a}	0.7	99.0^{a}	0.1	99.0^{a}	0.7	96.9^{b}	0.4	98.0^{ab}	1.4
18:1n-7	99.5^{a}	0.1	97.8^{bd}	0.0	98.5^{ab}	0.1	98.5^{ab}	0.2	96.0^{c}	0.8	97.0^{cd}	0.7
18:2n-6	99.1^{a}	0.1	98.6^{ab}	0.1	98.8^{ab}	0.2	98.7^{ab}	0.1	97.9^{c}	0.3	98.1^{bc}	0.3
18:3n-3	99.7^{a}	0.1	98.7^{b}	0.2	99.0^{ab}	0.1	99.3^{a}	0.1	98.2^{b}	0.3	98.8^{b}	0.6
20:1n-9	99.5^{a}	0.4	98.3^{a}	1.0	98.5^{a}	0.3	98.4^{a}	0.2	95.6^{b}	0.8	96.7^{b}	0.7
18:4n-3	99.4	0.0	99.4	0.0	99.5	0.0	99.4	0.0	99.3	0.0	99.3	0.0
20:4n-6 ARA	97.3^{a}	0.2	97.7^{a}	0.0	99.2^{a}	0.0	97.0^{a}	0.2	94.0^{b}	2.0	98.8^{a}	1.0
22:1n-11	99.1^{a}	0.1	97.1^{b}	0.2	98.1^{ab}	0.2	97.9^{ab}	0.3	94.1^{c}	1.0	95.6^{c}	1.0
20:5n-3 EPA	99.4	0.1	99.1	0.1	99.4	0.1	99.6	0.0	99.2	0.0	99.3	0.0
22:5n-3 DPA	98.0	0.1	98.0	0.0	98.5	0.1	98.0	0.0	98.0	0.0	98.0	0.0
22:6n-3 DHA	99.0	0.1	99.0	0.9	98.0	0.1	99.0	0.1	98.0	0.0	99.0	0.6
Saturated FA	98.0^{a}	0.2	96.0^{b}	0.3	98.0^{a}	0.4	98.0^{a}	0.3	94.5^{c}	1.0	95.5^{bc}	1.0
Sum 16:1	99.5^{a}	0.1	99.0^{ab}	0.0	99.0^{ab}	0.1	99.0^{ab}	0.1	98.0^{b}	0.3	98.5^{b}	0.3
Sum 18:1	99.0^{a}	0.2	99.0^{a}	0.6	99.0^{a}	0.1	99.0^{a}	0.6	97.0^{b}	0.4	98.0^{b}	1.0
Sum 20:1	99.5^{a}	0.4	98.5^{a}	1.0	98.5^{a}	0.2	98.5^{a}	0.3	96.0^{b}	0.8	97.0^{b}	0.8
Sum 22:1	99.0^{a}	0.1	97.0^{b}	0.3	98.0^{ab}	0.2	98.0^{ab}	0.3	94.0^{c}	1.0	95.5^{c}	1.0
Sum MUFA	99.5^{a}	0.2	98.0^{bc}	0.3	99.0^{ab}	0.2	99.0^{ab}	0.5	96.0^{c}	0.5	97.0^{c}	1.0
Sum EPA+DHA	99.0	0.1	99.0	0.2	99.0	0.1	99.0	0.0	99.0	0.1	99.0	0.3
Sum n-3	99.0	0.1	99.0	0.2	99.0	0.1	99.0	0.1	99.0	0.1	99.0	0.3
Sum n-6	99.0^{a}	0.1	98.5^{ab}	0.1	99.0^{a}	0.1	98.5^{ab}	0.1	98.0^{bc}	0.3	98.0^{bc}	0.3
Sum PUFA	99.0^{a}	0.1	99.0^{a}	0.1	99.0^{a}	0.1	99.0^{a}	0.1	98.0^{b}	0.2	98.5^{b}	0.3

Note. IM-0 = diets without insect meal (IM) inclusion: protein from fishmeal (FM) and soy protein concentrate (SPC); VO = lipids from vegetable oil (VO); IO1 and IO2 = VO replaced with insect oil 1 (IO1) or insect oil 2 (IO2); IM-85 = 85% of protein sources replaced with IM; IO1: insect oil from insects reared on organic side streams; IO2: insect oil from insects reared on organic side streams and seaweed (50,50); IO2: insect oil from insects reared on organic side streams and seaweed (50:50). n.c: not calculated due to very low concentrations in either feed or faeces; ARA: arachidonic acid; EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid; DHA: docosahexaenoic acid; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

Values are means and standard deviations with different superscripts letters next to values are significantly different, $p < 0.05$; (nested one-way ANOVA).

Table 7). Both total hepatic FA and the hepatic content of neutral lipids, including TAG, decreased significantly when insect ingredients were included in the diets (Tables 7 and 8).

The hepatic concentrations of total phytosterols reflected the dietary phytosterol concentrations and were higher in the fish given fish oil and rapeseed oil as only lipid source compared to the fish given diets containing insect lipids (Tables 3 and 9). The content of hepatic cholesterol was significantly lower in the fish fed the IM-85 diets (mean value of 2,256 mg/kg) than in the fish fed the IM-0 diets (mean value of 2,680 mg/kg) (Table 9). The concentration of plasma cholesterol, however, was significantly higher in the fish fed diets containing IM-85 (14 mM) compared to fish fed the IM-0 diets (10–12 mM; Table 4).

3.4 | Fatty acid productive values (FAPVs)

The calculated FAPV reflects the efficiency of the use of specific FA and groups of FA; values below 1 reflect a net loss of a FA during the 8 weeks of the trial, while values above 1 indicate a net production of a FA. Figure 1 presents the FAPV for selected FA (FAPVs for all FA are presented in Supporting Information Table S2). The total use of the FA (FAPV of total FA) was ~0.80, meaning

TABLE 6 Fatty acid composition (% of total FA) and total fatty acids (FA, mg/g of wet weight) of the whole-body of Atlantic salmon fed a control diet (IM-0/VO) or diets containing IM and/or IO1 or IO2 for a period of 8 weeks

	IM-0						IM-85					
	VO		IO1		IO2		VO		IO1		IO2	
	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*
12:0	<LOQ		9.0	0.7	9.0	0.3	8.0	0.3	11.5	0.3	11.5	0.6
14:0	2.6^{c}	0.1	5.0^{b}	0.2	6.0^{a}	0.1	4.5^{b}	0.1	6.0^{a}	0.1	6.0^{a}	0.1
16:0	11.0^{b}	0.1	14.0^{a}	0.2	14.0^{a}	0.1	13.5^{ab}	0.1	14.5^{a}	0.1	14.5^{a}	0.1
18:0	3.0^{b}	0.1	3.3^{ab}	0.1	3.4^{ab}	0.1	3.6^{a}	0.1	3.7^{a}	0.1	3.6^{a}	0.1
18:1n-9	33.0^{a}	0.3	19.0^{c}	0.7	19.0^{c}	0.3	24.0^{b}	0.5	19.0^{c}	0.1	19.0^{c}	0.4
18:1n-7	2.8^{a}	0.1	2.0^{b}	0.1	2.0^{b}	0.1	2.0^{b}	0.1	1.8^{c}	0.1	1.8^{c}	0.1
18:2n-6	10.7^{b}	0.1	10.6^{b}	0.1	9.4^{c}	0.1	11.5^{a}	0.1	11.5^{a}	0.1	11.0^{a}	0.1
18:3n-3	3.5^{a}	0.0	1.7^{c}	0.1	1.6^{c}	0.1	2.3^{b}	0.0	1.6^{c}	0.0	1.7^{c}	0.0
20:1n-9	5.0^{a}	0.1	4.4^{b}	0.1	4.3^{b}	0.1	4.2^{bc}	0.1	4.0^{c}	0.1	4.1^{c}	0.1
18:4n-3	1.1^{a}	0.0	0.9^{b}	0.0	0.8^{b}	0.0	0.8^{b}	0.0	0.8^{b}	0.0	0.8^{b}	0.0
20:4n-6 ARA	0.7^{a}	0.1	0.5^{c}	0.0	0.7^{a}	0.1	0.6^{b}	0.1	0.6^{b}	0.1	0.6^{b}	0.1
22:1n-11	4.6^{a}	0.1	4.5^{a}	0.1	4.5^{a}	0.1	4.1^{b}	0.1	4.0^{b}	0.1	4.0^{b}	0.1
20:5n-3 EPA	2.0^{a}	0.1	1.8^{a}	0.1	1.8^{a}	0.0	1.5^{b}	0.1	1.4^{b}	0.1	1.5^{b}	0.1
22:5n-3 DPA	0.9^{a}	0.0	0.9^{a}	0.0	0.9^{a}	0.0	0.7^{b}	0.0	0.7^{b}	0.0	0.7^{b}	0.0
22:6n-3 DHA	8.3^{a}	0.1	8.7^{a}	0.3	8.7^{a}	0.3	6.6^{b}	0.1	6.7^{b}	0.1	6.8^{b}	0.4
Saturated FA	17^{c}	0.2	33^{b}	1.0	33^{b}	0.5	31^{b}	0.5	36^{a}	0.6	36^{a}	0.1
Sum 16:1	2.8^{c}	0.1	4.0^{a}	0.0	4.0^{a}	0.1	3.4^{b}	0.0	4.0^{a}	0.0	4.0^{a}	0.0
Sum 18:1	37^{a}	0.5	22^{c}	0.1	22^{c}	0.4	27^{b}	0.5	22^{c}	0.1	22^{c}	0.4
Sum 20:1	5.5^{a}	0.1	5.2^{ab}	0.1	5.0^{bc}	0.1	4.9^{c}	0.1	4.7^{c}	0.1	4.8^{c}	0.1
Sum 22:1	5.0^{a}	0.1	4.9^{a}	0.1	5.0^{a}	0.1	4.4^{b}	0.1	4.3^{b}	0.1	4.4^{b}	0.1
Sum MUFA	51^{a}	0.3	37^{c}	0.8	37^{c}	0.5	40^{b}	0.8	35^{c}	0.2	35^{c}	0.6
Sum EPA+DHA	10.0^{a}	0.2	11.0^{a}	0.5	10.5^{a}	0.2	8.0^{b}	0.2	8.0^{b}	0.2	8.0^{b}	0.5
Sum n-3	17.0^{a}	0.2	15.0^{a}	0.6	15.0^{a}	0.4	13.0^{b}	0.1	12.0^{b}	0.3	12.0^{b}	0.5
Sum n-6	13.0^{b}	0.1	13.0^{b}	0.1	11.5^{c}	0.1	14.0^{a}	0.2	14.0^{a}	0.1	13.5^{a}	0.1
Sum PUFA	30^{a}	0.2	29^{b}	0.7	27^{c}	0.5	27^{c}	0.3	26^{c}	0.4	26^{c}	0.4
n-3/n-6	1.3^{a}	0.0	1.2^{a}	0.1	1.3^{a}	0.0	0.9^{b}	0.0	0.9^{b}	0.0	0.9^{b}	0.0
n-6/n-3	0.7^{b}	0.0	0.8^{b}	0.1	0.7^{b}	0.0	1.0^{a}	0.0	1.1^{a}	0.1	1.0^{a}	0.1
Total FA	107^{a}	9.0	110^{a}	10	110^{a}	8.0	122^{b}	6.0	122^{b}	2.0	118^{b}	11

Note. IM-0 = diets without insect meal (IM) inclusion: protein from fishmeal (FM) and soy protein concentrate (SPC); VO = lipids from vegetable oil (VO); IO1 and IO2 = VO replaced with insect oil 1 (IO1) or insect oil 2 (IO2); IM-85 = 85% of protein sources replaced with IM; IO1: insect oil from insects reared on organic side streams; IO2: insect oil from insects reared on organic side streams and seaweed (50,50); IO2: insect oil from insects reared on organic side streams and seaweed (50:50); LOQ: limit of quantification (0.01 mg/kg sample); ARA: arachidonic acid; EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid; DHA: docosahexaenoic acid; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.
Values are means and standard deviations with different superscripts letters next to values are significantly different, $p < 0.05$; (nested one-way ANOVA).

that 80% of the FA eaten during the eight-week trial were recuperated in the fish tissues. Lauric acid FAPV was ~0.5 for all the fish given diets with insect ingredients, while docosahexaenoic acid (22:6n-3, DHA) had a FAPV of ~1.5, reflecting a net oxidation and production of lauric acid and DHA, respectively. FAPV of DHA was significantly lower in the fish fed IM-85 diets than the ones fed the IM-0 diets (Figure 1). The fish fed the IM-0/IO2 diet had the lowest FAPV of ARA, being significantly lower than all the other dietary groups, while the IM-0/IO1 and the IM-85/IO2 fed fish had intermediate values (Figure 1).

3.5 | Hepatic gene expression

Few and small dietary effects were detected on the hepatic gene expression of markers of lipid metabolism and liver health. The expression of *cpt-1a* and *ppar-γ* was significantly higher in the fish fed protein

TABLE 7 Hepatic fatty acid composition ((% of total FA) and total fatty acids (FA, mg/g of wet weight) of the Atlantic salmon fed a control diet (IM-0/VO) or diets containing IM and/or IO1 or IO2 for a period of 8 weeks

	IM-0						IM-85					
	VO		IO1		IO2		VO		IO1		IO2	
	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*
12:0	<LOQ		1.7	0.5	1.2	0.1	1.5	0.2	1.5	1.0	2.0	0.3
14:0	1.4^{c}	0.1	3.2^{a}	0.2	3.2^{a}	0.1	2.9^{ab}	0.1	3.5^{a}	0.1	3.4^{a}	0.1
16:0	12.1^{c}	1.0	17.5^{ab}	0.3	14.0^{b}	0.1	16.6^{b}	0.1	18.0^{a}	0.8	18.2^{a}	0.2
18:0	5.2^{c}	0.2	6.0^{b}	0.2	6.0^{c}	0.3	6.4^{ab}	0.4	6.9^{a}	0.3	6.8^{a}	0.4
18:1n-9	27.4^{a}	2.0	14.2^{c}	0.5	13.5^{c}	1.0	18.8^{b}	1.0	16.6^{bc}	1.1	15.6^{c}	0.5
18:1n-7	2.9	0.4	2.1	0.6	2.4	0.5	2.7	0.5	2.4	0.3	1.7	0.0
18:2n-6	6.7^{b}	0.2	6.4^{b}	0.2	4.8^{c}	0.3	7.0^{ab}	0.2	7.0^{ab}	0.5	7.2^{a}	0.2
18:3n-3	1.3^{a}	0.2	0.4^{b}	0.1	0.3^{b}	0.1	0.5^{b}	0.1	0.2^{b}	0.1	0.3^{b}	0.1
20:1n-9	4.4^{a}	0.4	3.1^{b}	0.3	2.8^{b}	0.5	3.1^{b}	0.3	2.6^{b}	0.4	2.8^{b}	0.1
18:4n-3	0.2	0.0	0.2	0.1	0.2	0.1	0.2	0.1	0.1	0.1	0.04	0.1
20:4n-6 ARA	2.0^{c}	0.1	2.1^{c}	0.1	3.7^{a}	0.2	2.5^{b}	0.3	2.4^{b}	0.2	2.7^{b}	0.1
22:1n-11	1.0	0.1	1.0	0.4	0.8	0.1	0.8	0.1	0.8	0.1	0.9	0.1
20:5n-3 EPA	2.7^{a}	0.3	2.8^{a}	0.2	2.6^{a}	0.1	2.2^{b}	0.1	2.1^{b}	0.1	2.1^{b}	0.1
22:6n-3 DHA	21.6^{c}	2.0	27.1^{b}	2.0	30.0^{ab}	1.5	23.3^{c}	1.4	23.5^{c}	1.5	23.4^{c}	1.0
Saturated FA	19.0^{c}	1.0	28.6^{b}	0.4	27.3^{b}	0.2	27.5^{b}	0.7	30.1^{a}	0.5	30.8^{a}	0.8
Sum 18:1	31.4^{a}	2.0	17.2^{c}	1.0	17.0^{c}	1.0	22.5^{b}	1.0	20.0^{bc}	1.0	18.5^{bc}	0.3
Sum 20:1	4.7^{a}	0.9	3.6^{ab}	0.3	3.0^{b}	0.6	3.4^{ab}	0.5	3.0^{b}	0.5	3.3^{b}	0.1
Sum 22:1	1.3	0.2	1.3	0.5	1.1	0.2	1.1	0.1	1.1	0.1	1.2	0.1
Sum MUFA	40.0^{a}	2.0	25.0^{c}	2.0	24.0^{c}	2.0	29.0^{b}	1.0	27.0^{bc}	1.0	25.0^{c}	0.1
Sum EPA+DHA	24.0^{b}	2.0	30.0^{a}	2.0	32.5^{a}	2.0	25.5^{b}	2.0	25.5^{b}	1.0	25.5^{b}	1.0
Sum n-3	27.5^{b}	2.0	32.0^{a}	2.0	34.5^{a}	2.0	27.5^{b}	2.0	27.3^{b}	1.0	27.3^{b}	1.0
Sum n-6	12.2	0.1	13.0	1.0	12.7	0.1	14.5	1.0	14.5	1.0	15.0	1.0
Sum PUFA	40.0^{b}	2.0	45.0^{a}	2.0	47.3^{a}	2.0	42.2^{b}	2.0	41.8^{b}	1.0	42.5^{b}	1.0
n-3/n-6	2.3^{bc}	0.2	2.5^{b}	0.3	2.7^{a}	0.2	1.9^{c}	0.2	1.9^{c}	0.2	1.8^{c}	0.1
n-6/n-3	0.4^{b}	0.1	0.4^{b}	0.1	0.4^{b}	0.1	0.5^{a}	0.1	0.5^{a}	0.1	0.6^{a}	0.1
Total FA	37.0^{a}	4.0	27.6^{b}	1.0	27.4^{b}	2.0	25.7^{b}	2.0	25.4^{b}	1.0	24.0^{b}	1.0

Note. IM-0 = diets without insect meal (IM) inclusion: protein from fishmeal (FM) and soy protein concentrate (SPC); VO = lipids from vegetable oil (VO); IO1 and IO2 = VO replaced with insect oil 1 (IO1) or insect oil 2 (IO2); IM-85 = 85% of protein sources replaced with IM; IO1: insect oil from insects reared on organic side streams; IO2: insect oil from insects reared on organic side streams and seaweed (50,50); LOQ.: limit of quantification (0.01 mg/kg sample); ARA: arachidonic acid; EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid; DHA: docosahexaenoic acid; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

Values are means and standard deviations with different superscripts letters next to values are significantly different, $p < 0.05$; (nested one-way ANOVA).

of plant and marine origin in combination with IO1 (IM-0/IO1) compared to the fish that had eaten IM in combination with VO, IO1 and IO2 (IM-85/VO, IM-85/IO1 and IM-85/IO2, (Supporting Information Figure S1). The expression of *acox* was significantly higher in the fish fed protein of plant and marine origin in combination with IO1 (IM-0/IO1) than in the fish that had eaten IM in combination with IO2 (IM-85/IO2) (Supporting Information Figure S1). The expression of *fas*, *srebp1*, *lxr*, *ppar-α* and *apob100* (Supporting Information Figure S1), as well as *hsp70* and *sod* (Supporting Information Figure S2), was also measured, but no dietary effects were detected on their expression.

4 | DISCUSSION

The use of BSF protein meal as a feed ingredients in aquaculture has gotten increased investments, but most of the attention has been focused on the insect protein and its suitability for use in fish feeds (Barroso et al., 2014; Gasco et al., 2016; Magalhães et al., 2017). The high lipid content of BSF and a FA composition differing largely from most commonly used plant oils makes the metabolic effects of the lipid fraction of this insect species an important topic.

TABLE 8 Liver lipid class composition (mg/g) of Atlantic salmon fed a control diet (IM-0/VO) or diets containing IM and/or IO1 or IO2 for a period of 8 weeks

	IM-0						IM-85					
	VO		IO1		IO2		VO		IO1		IO2	
	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*
Sphingomyelin	1.9	0.3	1.8	0.2	1.7	0.1	2.1	0.3	1.8	0.3	1.9	0.1
Phosphatidylcholine	22^{a}	2.0	22^{a}	1.6	23^{a}	2.5	21^{ab}	2.5	21^{ab}	2.0	17^{b}	1.0
Phosphatidylserine	2.0	0.5	2.2	0.3	2.0	0.3	2.0	0.4	1.6	0.2	1.7	0.2
Phosphatidylinositol	2.8^{a}	0.3	3.0^{a}	0.1	3.0^{a}	0.1	2.5^{b}	0.2	2.3^{b}	0.3	2.4^{b}	0.1
Cardiolipin	0.7	0.2	0.8	0.1	0.7	0.0	0.5	0.1	0.5	0.1	0.5	0.1
Phosphatidylethanolamine	4.3	0.7	5.0	0.3	5.0	1.7	4.8	1.5	4.7	0.3	3.3	0.8
Diacylglycerol	0.7^{a}	0.1	0.6^{ab}	0.1	0.5^{ab}	0.0	0.5^{ab}	0.1	0.5^{ab}	0.1	0.4^{b}	0.0
Cholesterol	3.6	0.6	4.0	0.4	3.7	0.4	3.6	0.3	3.4	0.4	3.5	0.1
Free fatty acids	1.8	0.4	1.2	0.6	1.5	0.5	1.4	0.9	0.9	0.3	0.8	0.6
Triacylglycerol	17^{a}	6.0	9.0^{b}	2.0	7.0^{b}	2.0	10^{b}	2.0	9.0^{b}	3.0	9.0^{b}	1.0
Sum polar lipids	34^{a}	3.0	34^{a}	2.0	36^{a}	5.0	33^{ab}	4.0	32^{ab}	2.0	27^{b}	2.0
Sum neutral lipids	23^{a}	6.0	14^{b}	2.0	13^{b}	2.0	15^{b}	2.0	14^{b}	3.0	13^{b}	1.0
Sum lipids	53^{a}	4.0	49^{b}	2.0	49^{b}	6.0	48^{b}	6.0	46^{b}	4.0	40^{b}	3.0
Neutral/polar lipids	1.5^{b}	0.3	2.4^{a}	0.4	2.7^{a}	0.4	2.2^{a}	0.1	2.3^{a}	0.5	2.0^{a}	0.1

Note. IM-0 = diets without insect meal (IM) inclusion: protein from fishmeal (FM) and soy protein concentrate (SPC); VO = lipids from vegetable oil (VO); IO1 and IO2 = VO replaced with insect oil 1 (IO1) or insect oil 2 (IO2); IM-85 = 85% of protein sources replaced with IM; IO1: insect oil from insects reared on organic side streams; IO2: insect oil from insects reared on organic side streams and seaweed (50,50).
Values are means and standard deviations with different superscripts letters next to values are significantly different, $p < 0.05$; (nested one-way ANOVA).

The majority of the FA in BSF larvae are SFA (60% of total FA), and more than half of this SFA is made up of the medium-chained FA 12:0 lauric acid (Liland et al., 2017; Ramos-Bueno, González-Fernández, Sánchez-Muros-Lozano, García-Barroso, & Guil-Guerrero, 2016; Surendra, Olivier, Tomberlin, Jha, & Khanal, 2016). Of the insects commonly used in animal or human nutrition, BSF is the only one which contains this FA in high concentrations (Oonincx et al., 2015). In the present trial, lauric acid made up around a third of the total FA supply in the insect-based diets, and contributed, together with 14:0 and 16:0, to a high concentration of total SFA at ~50% of total FA. The upper recommended value for SFA in salmonid feeds varies between 12% and 25% of total FA (Caballero et al., 2002; Torstensen, Lie, & Frøyland, 2000) due to an often-observed reduction in digestibility in diets high in SFA, especially at low temperatures (Ng, Sigholt, & Gordon Bell, 2004). The current trial did, despite the high content of SFA, show high lipid digestibility in all groups and only very small effects on digestibility of crude lipid (Belghit et al., 2018) or individual FA were seen. There were also no detrimental health effects and no negative effects on growth performance due to the high load of SFA (Belghit et al., 2018). Similar results have been obtained in Jian carp, rainbow trout and seawater stage Atlantic salmon fed BSF larvae ingredients and similar concentrations of SFA (Li et al., 2016; Lock et al., 2016; Renna et al., 2017). Overall, the lipid from the insect-based diets of the current study was highly digestible and seemingly efficiently utilized by the Atlantic salmon.

A decreased feed intake has been observed in some fish feeding trials with diets containing MCFA (Nordrum, Olli, Rosjo, Holm, & Krogdahl, 2003; Williams et al., 2006), but not in others (Figueiredo-Silva et al., 2012; Hamre et al., 2001; Simó-Mirabet et al., 2017). An increase in dietary MCT (as 8:0 and 10:0 (50:50) up to 46% of total fat) decreased the feed intake with up to 40% in Atlantic salmon, also severely affecting growth performance (Nordrum et al., 2000). Similarly, in polka-dot grouper, feed intake dropped by 50% when fed diets containing coconut oil (dietary 12:0 ~ 40% of total FA) compared to a control diet containing olive oil, leading to negative effects on growth performance (Williams et al., 2006). Dietary MCFA are rapidly oxidized, rather than stored as fat, and it has been shown that this rapid oxidation of MCFA can reduce appetite, leading to a reduced feed intake in humans and rats (Ooyama et al., 2009; St-Onge & Jones, 2002; St-Onge et al., 2008). The feed intake of rainbow trout fed a diet rich in coconut oil, and similar levels of dietary lauric acid as in the current trial (31% of total FA, current trial: 19%–29% of total FA) were, however, unaffected (Figueiredo-Silva et al., 2012). The same lack of effects on feed intake was reported in hybrid tilapia (*Oreochromis* sp.) fed crude palm kernel oil (lauric acid 46% of total FA) (Ng, Lim, & Sidek, 2001). Similarly, in the present trial, the feed intake of Atlantic salmon fed the insect-based diets rich in lauric acid was decreased only by 7% compared to fish fed the control diet, with no negative effects on growth performance. Thus, the inhibitory effect of

TABLE 9 Liver sterol composition (mg/kg) of Atlantic salmon fed a control diet (IM-0/VO) or diets containing IM and/or IO1 or IO2 for a period of 8 weeks

	IM-0						IM-85					
	VO		IO1		IO2		VO		IO1		IO2	
	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*
Cholesterol (mg/kg)	2,731^{a}	161	2,626^{a}	108	2,685^{a}	35	2,355^{b}	96	2,277^{b}	169	2,137^{b}	57
Phytosterols (mg/kg)												
Brassicasterol	10	0.7	9.2	1.0	8.0	0.5	8.0	2.2	7.0	0.9	8.0	0.9
Campesterol	41^{a}	7.0	14^{c}	2.0	11^{c}	0.4	42^{a}	4.0	32^{b}	3.0	29^{b}	4.0
Campestanol	1.0^{a}	0.1	0.9^{a}	0.2	1.0^{a}	0.1	0.4^{b}	0.1	0.4^{b}	0.0	0.3^{b}	0.1
Stigmasterol	4.1^{a}	0.8	4.4^{a}	2.3	3.0^{ab}	0.7	1.5^{b}	0.7	1.7^{b}	0.8	3.0^{ab}	0.9
Sitosterol/fucosterol*	12^{ab}	4.0	10^{bc}	1.5	7.0^{c}	3.0	19^{a}	7.0	15^{ab}	3.0	18^{a}	3.0
Sitostanol	4.0^{a}	0.6	4.0^{a}	0.7	3.0^{a}	0.3	6.0^{b}	0.5	6.0^{b}	0.5	7.0^{b}	1.0
Total phytosterols	73^{a}	13	44^{b}	4.0	33^{c}	2.0	77^{a}	10	63^{a}	6.0	66^{a}	6.0
Phyto:chol ratio	0.03^{a}	0.0	0.02^{b}	0.0	0.01^{b}	0.0	0.03^{a}	0.0	0.03^{a}	0.0	0.03^{a}	0.0

Note. IM-0/VO = diets without insect meal (IM) inclusion: protein from fishmeal (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).

Values are means and standard deviations with different superscripts letters next to values are significantly different, $p < 0.05$; (nested one-way ANOVA).

*Sitosterol and fucosterol co-elute in the current method (assumed that some of the sitosterol in the diets containing IO2 is fucosterol due to the macroalgae used as a growth substrate for the larvae, but mostly being sitosterol).

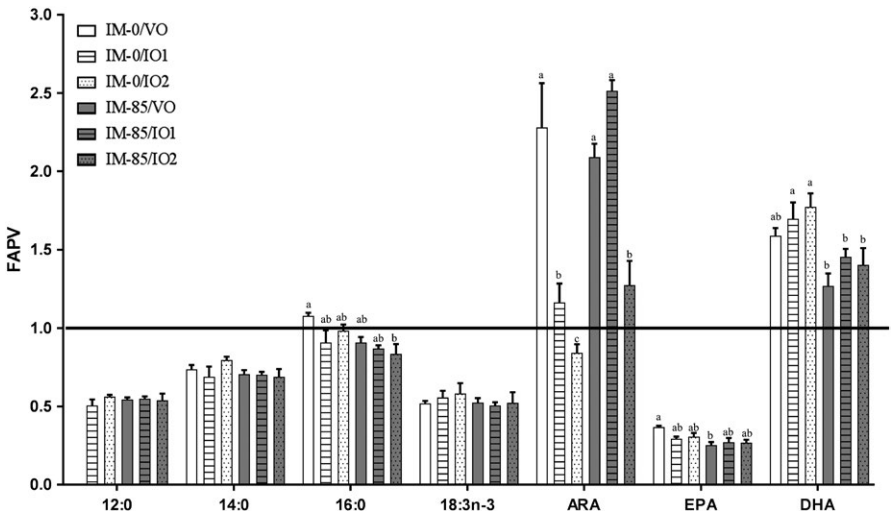

FIGURE 1 Fatty acid productive values (FAPVs) of selected fatty acids (12:0, 14:0, 16:0, 18:3n-3, arachidonic acid (ARA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)) of fish fed diets containing IM and/or IO1 or IO2 for a period of 8 weeks. Values are means of 4 values per dietary treatment, with their standard deviation represented by vertical bars. Values below 1 reflect a net loss of a fatty acid during the 8 weeks of the trial, while values above 1 indicate a net production of a fatty acid. Different superscripts letters indicate significantly different values ($p < 0.05$, nested one-way ANOVA). FAPVs for remaining fatty acids are presented in Supporting Information Table S2

dietary MCFA on feed intake seems to vary among fish species and source of MCFA.

A change in the FA composition in the tissues can have a profound impact on the metabolism, as the FA, both in storage depots and in membranes, act as substrates for lipid-derived components, affect gene expression through effects on nuclear receptors and influence membrane stability and fluidity. Increased 12:0 in extrahepatic tissues has been shown to affect macrophage activity (Yaqoob & Calder, 1995) as well as genes involved in adipocyte differentiation and growth (Bueno et al., 2007). In the current trial, ~50% of the lauric acid ingested during the whole feeding trial was recuperated in the tissues, meaning that ~50% was either oxidized or not absorbed

by the intestine. The high digestibility of lauric acid suggests that most of the lauric acid ingested must have been oxidized rather than not taken up. The storage of lauric acid was lower than for all other FA (except for eicosapentaenoic acid); with as much as ~70% of the 14:0 and ~90% of the 16:0 ingested being recuperated in the tissues. Studies on humans have shown that lauric acid is highly oxidized compared to other FA, as in a study where 40% of ingested lauric acid was oxidized after 9 hr compared to only 13% of stearic acid (18:0) (DeLany, Windhauser, Champagne, & Bray, 2000). Similar results are seen also in fish, where a diet high in MCFA (lauric acid from coconut oil) had a higher lipid oxidation than a diet based on long-chained FA (LCFA) (18 carbon-rich olive oil as lipid source) in polka-dot grouper (Smith, Williams, Williams, Barclay, & Venables, 2005). As such, the fat from the insect-based diets, containing high levels of the rapidly oxidized MCFA, should be used less for storage, possibly leading to a loss of adiposity, as shown in mammals (St-Onge et al., 2008) and also in fish (Smith et al., 2005). In the present trial, however, feeding fish diets high in lauric acid did not reduce the whole body lipid compared to fish fed a diet without MCFA (Belghit et al., 2018). Similarly, Jian carp and rainbow trout fed diets high in MCFA did not have a reduction in the whole body lipids (Figueiredo-Silva et al., 2012; Li et al., 2016). Due to an apparent high oxidization of lauric acid, the concentration of this FA was substantially lower in the whole body of salmon fed insect-based diets (lauric acid ≈ 8%–12% and SFA ≈ 31%–36% of total FA) than in the insect-based diets (lauric acid ≈ 19%–29% and saturated FA ≈ 38%–51% of total FA). An increase in dietary SFA beyond 38% did not further increase the total SFA in the tissues. Other fish trials got similar results, like Jian carp or rainbow trout fed BSF larvae ingredients (dietary lauric acid 17% and 28% of total FA, respectively), where lower concentrations of lauric acid were found in the whole-body (10% of total FA) and in the muscle (14% of total FA), respectively (Renna et al., 2017; Zhou, Liu, Ji, & Yu, 2018). The high oxidation and low storage of lauric acid in the current trial indicate that lauric acid is a good source of energy for Atlantic salmon. The accumulation of lauric acid in the fish also implies that fish fed BSF would be a source of MCFA for human consumption, although studies with fish up to slaughter weight (for Atlantic salmon ~3–6 kg) would have to be performed to verify that this accumulation of MCFA also happens in fish of a size relevant for human consumption.

In the current trial, an increase in lauric acid was found in the whole-body of the fish, being absent in the control fed fish and lying around 8%–12% of total FA in the fish fed the insect-based diets. However, these FAs were found only in very low concentrations in the liver of salmon fed insect-based ingredients (hepatic lauric acid was ~1%–2% of total FA). Additionally, in spite of a higher fat content in the insect meal containing diets as well as in the whole-body of the IM fed fish compared to the IM-0 groups, the hepatic lipid decreased with ~35% in the salmon fed insect-based diets compared to the control group. The hepatic concentration of TAG was reduced to around half in all insect-fed fish compared to the IM-0/VO control group. This could be related to the properties of lauric acid, which as a MCFA is characterized by a limited potential for storage as triglycerides. In contrast to our results, Røsjø *et al.* reported a 20% increased TAG in the liver of Atlantic salmon fed dietary MCT (8:0 and 10:0; Røsjø et al., 2000). The authors found an accumulation of 18:0 and a reduced mitochondrial β-oxidation of 16:0 in the liver of salmon fed dietary MCFA compared to fish fed LCFA (Røsjø et al., 2000). Lauric acid has, however, been considered to be more efficiently used by fish than 8:0 and 10:0 (Craig & Gatlin, 1995; Fontagné, Robin, Corraze, & Bergot, 2000), which could explain the different results obtained by Røsjø *et al.* and the current findings. When ingested, MCFA are able to pass directly from the intestine to the liver via the portal vein, where they are rapidly oxidized to acetyl-coenzyme and thus driving ketone body production (Kinderlerer, 1994; McCarty & DiNicolantonio, 2016). Lipid hydrolysis increased peroxisome proliferator-activated receptors-α transcription, which plays an important role in hepatic lipid catabolism by inducing the expression of genes involved in the mitochondrial and peroxisomal fatty acid oxidation (Henderson & Sargent, 1985). In the current trial, there were no differences in the gene expression of markers of peroxisomal and mitochondrial β-oxidation (*acyl-Coa oxidase and carnitine palmitoyl transferase 1 a*, respectively) between fish fed diets containing insects and the control diet. The results from the gene expression do thus not support the hypothesis of an increased hepatic β-oxidation in the insect-fed fish. Most studies investigating the metabolic use of MCFA, do, however, compare MCFA with LCFA, like fish oil or olive oil (Figueiredo-Silva et al., 2012; Nordrum et al., 2003; Williams et al., 2006). In the current study, all the six dietary treatments, although differing in content of MCFA, contained the same amounts of LCFA. This could have led to less visible effects on the gene expression of markers in the pathways of lipid oxidation.

The activities of serum aspartate aminotransferase and alanine aminotransferase, used as markers for liver damage, were only lightly affected by dietary inclusion of insect ingredients, and found in levels indicating no liver damage in any of the dietary groups. To look for more discrete changes in hepatic health, the gene expression of two markers of stress response, *heat-shock protein-70* and *superoxide dismutase*, was measured in the liver. In Jian carp, a dietary substitution of fishmeal with defatted BSF larvae meal led to an increased expression of hepatic *heat-shock protein*, suggesting that an inclusion of BSF larvae in the diets induced a stress response (Li et al., 2017). Also, a dietary inclusion of BSF larvae, cricket and maggot meal resulted in higher activity and gene expression of the antioxidant system of carp and African catfish (superoxide dismutase and catalase in serum and liver) (Li et al., 2017; Ogunji, Nimptsch, Wiegand, & Schulz, 2007; Taufek et al., 2016). The enhanced antioxidant response might be related to the content of chitin and its derivatives found in the insect exoskeleton (Li et al., 2017; Ogunji et al., 2007; Taufek et al., 2016). Additionally, the medium-chain FA lauric acid of the BSF larvae has been shown to have an antibacterial activity in pigs (Spranghers et al., 2018). The expression of *heat-shock protein-70* and *superoxide dismutase* was, however, not affected by using the insect ingredients in the current trial. There were thus no signs of the insect ingredients causing any

negative or positive effect on hepatic health. Additionally, the values of blood haemoglobin observed in the dietary trial were within reference values determined for Atlantic salmon (Sandnes, Lie, & Waagbø, 1988).

In the current trial, inclusion of IM in the diets increased the plasma cholesterol concentrations, despite the low levels of cholesterol in the IM-85 diets compared to the diets without IM. The IM-85 diets also had higher dietary phytosterol concentrations, leading to large changes in the ratio between phytosterols:cholesterol (IM-0 diets 0.26–0.55, IM-85 diets ~1.86–2.10). Phytosterols are known to inhibit the uptake of cholesterol and to be able to affect lipid metabolism through the activity of nuclear receptors such as *lxr* (Plat, Nichols, & Mensink, 2005). Based on previous research on Atlantic salmon, it is, however, not expected that phytosterol concentrations as in the current trial would have any large effect on plasma cholesterol (Sissener, Rosenlund, Stubhaug, & Liland, 2018). The study by Sissener *et al*. looked at similar concentrations and ratios of phytosterols and cholesterol as in the current trial, but was conducted on seawater stage Atlantic salmon (Sissener et al., 2018). It is not possible to pinpoint the cause of the increased plasma cholesterol of the IM fed fish in the current trial, but it is likely caused by either the changed sterol composition of the IM diets, or due to some other component found in IM not analysed in the current work. It could also possibly be connected to the higher lipid content in the IM-85 diets and the resulting higher whole body lipid in the fish fed these diets. MCFA-rich diets have been shown to lower plasma cholesterol in fish (Li et al., 2017), but no effects of MCFA were seen on plasma cholesterol in this study.

In conclusion, the present study shows that the lipid of BSF larvae, rich in the MCFA lauric acid (12:0) and total SFA, is highly digestible by Atlantic salmon (ADC >95%). Lauric acid seemed to have been oxidized to a larger extent than other dietary FA, but without affecting the feed intake or the whole body lipid content. Approximately 50% of the lauric acid ingested during the whole feeding trial was recuperated in the fish tissues. No health effects of this change in tissue FA composition could be detected. The high content of lauric acid in the insect-based diets led to a decreased liver lipid storage compared to when the fish were fed a control diet without insects. This is likely due to the rapid oxidation and low tissue deposition of this particular FA. It is not certain how, or if, this decrease in liver lipid would affect the metabolism and health of the fish, since no other signs of a change in hepatic health were seen.

ACKNOWLEDGEMENT

This study was supported by the following projects, all funded by the Norwegian Research Council: Aquafly, grant number 238997, Aquafly steroler, grant number: 244253 and RAFFPINN, grant number 220634.

CONFLICT OF INTEREST

No potential conflict of interest was reported by the author(s).

ORCID

Ikram Belghit https://orcid.org/0000-0002-5074-5559

REFERENCES

Barroso, F. G., de Haro, C., Sánchez-Muros, M.-J., Venegas, E., Martínez-Sánchez, A., & Pérez-Bañón, C. (2014). The potential of various insect species for use as food for fish. *Aquaculture*, *422–423*, 193–201. https://doi.org/10.1016/j.aquaculture.2013.12.024.

Belghit, I., Liland, N. S., Waagbø, R., Biancarosa, I., Pelusio, N., Li, Y., … Lock, E.-J. (2018). Potential of insect-based diets for Atlantic salmon (*Salmo salar*). *Aquaculture*, *491*, 72–81. https://doi.org/10.1016/j.aquaculture.2018.03.016.

Borgogno, M., Dinnella, C., Iaconisi, V., … G. (2017). Inclusion of Hermetia illucens larvae meal on rainbow trout (*Oncorhynchus mykiss*) feed: Effect on sensory profile according to static and dynamic evaluations. *Journal of the Science of Food and Agriculture*, *97*(10), 3402–3411. https://doi.org/10.1002/jsfa.8191.

Bueno, A. A., Oyama, L. M., de Oliveira, C., Pisani, L. P., Ribeiro, E. B., Silveira, V. L. F., & Oller do Nascimento, C. M. (2007). Effects of different fatty acids and dietary lipids on adiponectin gene expression in 3T3-L1 cells and C57BL/6J mice adipose tissue. *Pflügers Archiv - European Journal of Physiology*, *455*(4), 701–709. https://doi.org/10.1007/s00424-007-0330-3.

Caballero, M. J., Obach, A., Rosenlund, G., Montero, D., Gisvold, M., & Izquierdo, M. S. (2002). Impact of different dietary lipid sources on growth, lipid digestibility, tissue fatty acid composition and histology of rainbow trout. *Oncorhynchus Mykiss*. *Aquaculture*, *214*(1), 253–271. https://doi.org/10.1016/S0044-8486(01)00852-3.

Craig, S. R., & Gatlin, I. I. I. D. M. (1995). Coconut Oil and Beef Tallow, But Not Tricaprylin, Can Replace Menhaden Oil in the Diet of Red Drum (Sciaenops ocellatus) without Adversely Affecting Growth or Fatty Acid Composition. *The Journal of Nutrition*, *125*(12), 3041–3048. https://doi:10.1093/jn/125.12.3041

Dayrit, F. M. (2015). The properties of lauric acid and their significance in coconut oil. *Journal of the American Oil Chemists' Society*, *92*(1), 1–15. https://doi.org/10.1007/s11746-014-2562-7.

DeLany, J. P., Windhauser, M. M., Champagne, C. M., & Bray, G. A. (2000). Differential oxidation of individual dietary fatty acids in humans. *The American Journal of Clinical Nutrition*, *72*(4), 905–911. https://doi.org/10.1093/ajcn/72.4.905.

FAO (2013). *Forestry paper. Edible insects: Futur prospects for food and feed security*. Rome: FAO.

Figueiredo-Silva, A. C., Kaushik, S., Terrier, F., Schrama, J. W., Médale, F., & Geurden, I. (2012). Link between lipid metabolism and voluntary food intake in rainbow trout fed coconut oil rich in medium-chain TAG. *British Journal of Nutrition*, *107*(11), 1714–1725. https://doi.org/10.1017/s0007114511004739.

Fontagné, S., Pruszynski, T., Corraze, G., & Bergot, P. (1999). Effect of coconut oil and tricaprylin vs. triolein on survival, growth and fatty acid composition of common carp (*Cyprinus carpio L.*) larvae. *Aquaculture*, *179*(1), 241–251. https://doi.org/10.1016/S0044-8486(99)00193-3.

Fontagné, S., Robin, J., Corraze, G., & Bergot, P. (2000). Growth and survival of European sea bass (*Dicentrarchus Labrax*) larvae fed from first feeding on compound diets containing medium-chain triacylglycerols. *Aquaculture*, *190*(3), 261–271. https://doi.org/10.1016/S0044-8486(00)00401-4.

Garlid, K. D., Orosz, D. E., Modrianský, M., Vassanelli, S., & Jezek, P. (1996). On the mechanism of fatty acid-induced proton transport by mitochondrial uncoupling protein. *Journal of Biological Chemistry*, *271*(5), 2615–2620. https://doi.org/10.1074/jbc.271.5.2615.

Gasco, L., Henry, M., Piccolo, G., Marono, S., Gai, F., Renna, M., … Chatzifotis, S. (2016). Tenebrio molitor meal in diets for European

sea bass (*Dicentrarchus labrax L.*) juveniles: Growth performance, whole body composition and in vivo apparent digestibility. *Animal Feed Science and Technology*, *220*, 34–45. https://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, K., Kolås, K., Sandnes, K., Julshamn, K., & Kiessling, A. (2001). Feed intake and absorption of lipid oxidation products in Atlantic salmon (*Salmo salar*) fed diets coated with oxidised fish oil. *Fish Physiology and Biochemistry*, *25*(3), 209–219. https://doi.org/10.1023/a:1022257928437.

Henderson, R. J., & Sargent, J. R. (1985). Chain-length specificities of mitochondrial and peroxisomal beta-oxidation of fatty acids in livers of rainbow trout (*Salmo gairdneri*). *Comparative Biochemistry and Physiology: Part B*, *82*(1), 79–85.

Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous inference in general parametric models. *Biometrical Journal*, *50*(3), 346–363. https://doi.org/10.1002/bimj.200810425.

Johnson, R. C., Young, S. K., Cotter, R., Lin, L., & Rowe, W. B. (1990). Medium-chain-triglyceride lipid emulsion: Metabolism and tissue distribution. *American Journal of Clinical Nutrition*, *52*(3), 502–508. https://doi.org/10.1093/ajcn/52.3.502

Kinderlerer, J. L. (1994). Degradation of the Lauric acid oils. *International Biodeterioration & Biodegradation*, *33*(4), 345–354. https://doi.org/10.1016/0964-8305(94)90012-4.

Laakso, P. (2005). Analysis of sterols from various food matrices. *European Journal of Lipid Science and Technology*, *107*(6), 402–410. https://doi.org/10.1002/ejlt.200501134.

Li, S., Ji, H., Zhang, B., Tian, J., Zhou, J., & Yu, H. (2016). Influence of black soldier fly (*Hermetia illucens*) larvae oil on growth performance, body composition, tissue fatty acid composition and lipid deposition in juvenile Jian carp (*Cyprinus carpio var. Jian*). *Aquaculture*, *465*, 43–52. https://doi.org/10.1016/j.aquaculture.2016.08.020.

Li, S., Ji, H., Zhang, B., Zhou, J., & Yu, H. (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. https://doi.org/10.1016/j.aquaculture.2017.04.015.

Liland, N. S., Biancarosa, I., Araujo, P., Biemans, D., Bruckner, C. G., Waagbø, R., … Lock, E.-J. (2017). Modulation of nutrient composition of black soldier fly (*Hermetia illucens*) larvae by feeding seaweed-enriched media. *PLoS ONE*, *12*(8), e0183188. https://doi.org/10.1371/journal.pone.0183188.

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

Magalhães, R., Sánchez-López, A., Leal, R. S., Martínez-Llorens, S., Oliva-Teles, A., & Peres, H. (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. https://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. *Animal Feed Science and Technology*, *197*, 1–33. https://doi.org/10.1016/j.anifeedsci.2014.07.008.

McCarty, M. F., & DiNicolantonio, J. J. (2016). Lauric acid-rich medium-chain triglycerides can substitute for other oils in cooking applications and may have limited pathogenicity. *Open Heart*, *3*(2), e000467. https://doi.org/10.1136/openhrt-2016-000467.

Ng, W.-K., Lim, P.-K., & Sidek, H. (2001). The influence of a dietary lipid source on growth, muscle fatty acid composition and erythrocyte osmotic fragility of hybrid tilapia. *Fish Physiology and Biochemistry*, *25*(4), 301–310. https://doi.org/10.1023/a:1023271901111

Ng, W.-K., Sigholt, T., & Gordon Bell, J. (2004). The influence of environmental temperature on the apparent nutrient and fatty acid digestibility in Atlantic salmon (Salmo salar L.) fed finishing diets containing different blends of fish oil, rapeseed oil and palm oil. *Aquaculture Research*, *35*(13), 1228–1237. https://doi.org/10.1111/j.1365-2109.2004.01131.x

Nordrum, S., Krogdahl, Å., Røsjø, C., Olli, J. J., & Holm, H. (2000). Effects of methionine, cysteine and medium chain triglycerides on nutrient digestibility, absorption of amino acids along the intestinal tract and nutrient retention in Atlantic salmon (*Salmo salar L.*) under pair-feeding regime. *Aquaculture*, *186*(3), 341–360. https://doi.org/10.1016/S0044-8486(99)00385-3.

Nordrum, S., Olli, J. j., Rosjo, C., Holm, H., & Krogdahl, A. (2003). Effects of graded levels of medium chain triglycerides and cysteine on growth, digestive processes and nutrient utilization in sea water reared Atlantic salmon (*Salmo salar, L.*) under ad libitum feeding regime. *Aquaculture Nutrition*, *9*(4), 263–274. https://doi.org/10.1046/j.1365-2095.2003.00252.x.

Ogunji, J. O., Nimptsch, J., Wiegand, C., & Schulz, C. (2007). Evaluation of the influence of housefly maggot meal (magmeal) diets on catalase, glutathione S-transferase and glycogen concentration in the liver of Oreochromis niloticus fingerling. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *147*(4), 942–947. https://doi.org/10.1016/j.cbpa.2007.02.028.

Oonincx, D. G. A. B., van Broekhoven, S., van Huis, A., & van Loon, J. J. A. (2015). Feed conversion, survival and development, and composition of four insect species on diets composed of food by-products. *PLoS ONE*, *10*(12), e0144601. https://doi.org/10.1371/journal.pone.0144601.

Ooyama, K., Kojima, K., Aoyama, T., & Takeuchi, H. (2009). Decrease of food intake in rats after ingestion of medium-chain triacylglycerol. *Journal of Nutritional Science and Vitaminology*, *55*(5), 423–427. https://doi.org/10.3177/jnsv.55.423.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D. & R Core Team. (2010). nlme: Linear and Nonlinear Mixed Effects Models. R package version, 3.1-137.

Plat, J., Nichols, J. A., & Mensink, R. P. (2005). Plant sterols and stanols: Effects on mixed micellar composition and LXR (target gene) activation. *Journal of Lipid Research*, *46*, 2468–2476.

R Development Core Team. (2014). *R Core Team*. Vienna, Austria: R A Language and Environment for Statistical computing.

Ramos-Bueno, R. P., González-Fernández, M. J., Sánchez-Muros-Lozano, M. J., García-Barroso, F., & Guil-Guerrero, J. L. (2016). Fatty acid profiles and cholesterol content of seven insect species assessed by several extraction systems. *European Food Research and Technology*, *242*(9), 1471–1477. https://doi.org/10.1007/s00217-016-2647-7.

Renna, M., Schiavone, A., Gai, F., Dabbou, S., Lussiana, C., Malfatto, V., & Gasco, L. (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. *Journal of Animal Science and Biotechnology*, *8*, 57. https://doi.org/10.1186/s40104-017-0191-3.

Røsjø, C., Nordrum, S., Olli, J. J., Krogdahl, Å., Ruyter, B., & Holm, H. (2000). Lipid digestibility and metabolism in Atlantic salmon (*Salmo salar*) fed medium-chain triglycerides. *Aquaculture*, *190*(1), 65–76. https://doi.org/10.1016/S0044-8486(00)00395-1.

Sado-Kamdem, S. L., Vannini, L., & Guerzoni, M. E. (2009). Effect of α-linolenic, capric and lauric acid on the fatty acid biosynthesis in *Staphylococcus aureus*. *International Journal of Food Microbiology*, *129*(3), 288–294. https://doi.org/10.1016/j.ijfoodmicro.2008.12.010.

Sanden, M., Liland, N. S., Sæle, Ø., Rosenlund, G., Du, S., Torstensen, B. E., & Sissener, N. H. (2016). Minor lipid metabolic perturbations in the liver of Atlantic salmon (*Salmo salar L.*) caused by suboptimal dietary content of nutrients from fish oil. *Fish Physiology and Biochemistry*, *42*(5), 1463–1480. https://doi.org/10.1007/s10695-016-0233-3.

Sandnes, K., Lie, Ø., & Waagbø, R. (1988). Normal ranges of some blood chemistry parameters in adult farmed Atlantic salmon,

Salmo salar. *Journal of Fish Biology*, *32*(1), 129–136. https://doi.org/10.1111/j.1095-8649.1988.tb05341.x.

Sheppard, D. C., Tomberlin, J. K., Joyce, J. A., Kiser, B. C., & Sumner, S. M. (2002). Rearing methods for the black soldier fly (Diptera: Stratiomyidae). *Journal of Medical Entomology*, *39*(4), 695–698. https://doi.org/10.1603/0022-2585-39.4.695.

Simó-Mirabet, P., Piazzon, M. C., Calduch-Giner, J. A., Ortiz, Á., Puyalto, M., Sitjà-Bobadilla, A., & Pérez-Sánchez, J. (2017). Sodium salt medium-chain fatty acids and Bacillus-based probiotic strategies to improve growth and intestinal health of gilthead sea bream (*Sparus aurata*). *PeerJ*, *5*, e4001. https://doi.org/10.7717/peerj.4001.

Sissener, N. H., Rosenlund, G., Stubhaug, I., & Liland, N. S. (2018). Tissue sterol composition in Atlantic salmon (*Salmo salar* L.) depends on the dietary cholesterol content and on the dietary phytosterol:Cholesterol ratio, but not on the dietary phytosterol content. *British Journal of Nutrition*, *119*, 599–609.

Smith, D. M., Williams, I. H., Williams, K. C., Barclay, M. C., & Venables, W. N. (2005). Oxidation of medium-chain and long-chain fatty acids by polka dot grouper *Cromileptes altivelis*. *Aquaculture Nutrition*, *11*(1), 41–48. https://doi.org/10.1111/j.1365-2095.2004.00321.

Spranghers, T., Michiels, J., Vrancx, J., Ovyn, A., Eechhout, M., & De Clercq, P. (2018). Gut antimicrobial effects and nutritional value of black soldier fly (*Hermetia illucens* L.) prepupae for weaned piglets. *Animal Feed Science Technology*, *235*, 33–42.

St-Onge, M.-P., Bosarge, A., Goree, L. L. T., & Darnell, B. (2008). Medium chain triglyceride oil consumption as part of a weight loss diet does not lead to an adverse metabolic profile when compared to olive oil. *Journal of the American College of Nutrition*, *27*(5), 547–552. https://doi.org/10.1080/07315724.2008.10719737

St-Onge, M.-P., & Jones, P. J. H. (2002). Physiological effects of medium-chain triglycerides: potential agents in the prevention of obesity. *The Journal of Nutrition*, *132*(3), 329–332. https://doi.org/10.1093/jn/132.3.329.

Stubbs, R. J., & Harbron, C. G. (1996). Covert manipulation of the ratio of medium- to long-chain triglycerides in isoenergetically dense diets: Effect on food intake in ad libitum feeding men. *International Journal of Obesity and Related Metabolic Disorders*, *20*(5), 435–444.

Surendra, K. C., Olivier, R., Tomberlin, J. K., Jha, R., & Khanal, S. K. (2016). Bioconversion of organic wastes into biodiesel and animal feed via insect farming. *Renewable Energy*, *98*, 197–202. https://doi.org/10.1016/j.renene.2016.03.022.

Taufek, N. M., Aspani, F., Muin, H., Raji, A. A., Razak, S. A., & Alias, Z. (2016). The effect of dietary cricket meal (*Gryllus bimaculatus*) on growth performance, antioxidant enzyme activities, and haematological response of African catfish (*Clarias gariepinus*). *Fish Physiology and Biochemistry*, *42*(4), 1143–1155. https://doi.org/10.1007/s10695-016-0204-8.

Torstensen, B. E., Frøyland, L., & Lie, Ø. (2004). Replacing dietary fish oil with increasing levels of rapeseed oil and olive oil – effects on Atlantic salmon (*Salmo salar* L.) tissue and lipoprotein lipid composition and lipogenic enzyme activities. *Aquaculture Nutrition*, *10*(3), 175–192. https://doi.org/10.1111/j.1365-2095.2004.00289.x.

Torstensen, B. E., Lie, Ø., & Frøyland, L. (2000). Lipid metabolism and tissue composition in Atlantic salmon (*Salmo salar L.*)—Effects of capelin oil, palm oil, and oleic acid-enriched sunflower oil as dietary lipid sources. *Lipids*, *35*(6), 653–664. https://doi.org/10.1007/s11745-000-0570-6.

Tran, G., Heuzé, V., & Makkar, H. P. S. (2015). Insects in fish diets. *Animal. Frontiers*, *5*(2), 37–44. https://doi.org/10.2527/af.2015-0018.

van Huis, A. (2013). Potential of insects as food and feed in assuring food security. *Annual Review of Entomology*, *58*(1), 563–583. https://doi.org/10.1146/annurev-ento-120811-153704.

Wang, Y.-S., & Shelomi, M. (2017). Review of black soldier fly (*Hermetia illucens*) as animal feed and human food. *Foods*, *6*(10), 91. https://doi.org/10.3390/foods6100091.

Williams, I., Williams, K. C., Smith, D. M., & Jones, M. (2006). Polka-dot grouper, *Cromileptes altivelis*, can utilize dietary fat efficiently. *Aquaculture Nutrition*, *12*(5), 379–387. https://doi.org/10.1111/j.1365-2095.2006.00437.x.

Yaqoob, P., & Calder, P. (1995). Effects of dietary lipid manipulation upon inflammatory mediator production by murine macrophages. *Cellular Immunology*, *163*(1), 120–128. https://doi.org/10.1006/cimm.1995.1106.

Zhou, J. S., Liu, S. S., Ji, H., & Yu, H. B. (2018). Effect of replacing dietary fish meal with black soldier fly larvae meal on growth and fatty acid composition of Jian carp (*Cyprinus carpio var. Jian*). *Aquaculture Nutrition*, *24*(1), 424–433. https://doi.org/10.1111/anu.12574.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Belghit I, Waagbø R, Lock E-J, Liland NS. Insect-based diets high in lauric acid reduce liver lipids in freshwater Atlantic salmon. *Aquacult Nutr.* 2019;25:343–357. https://doi.org/10.1111/anu.12860