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Insects in Atlantic salmon (*Salmo salar*) diets – comparison between full-fat, defatted, and de-chitinised meals, and oil and exoskeleton fractions

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RESEARCH ARTICLE

Abstract

The present study investigated the effect of meals and fractions of black soldier fly larvae (BSFL; *Hermetia illucens*) in diets for Atlantic salmon (*Salmo salar*) on the physical quality of feed pellets, nutrient utilisation, and growth performance. Six extruded diets were produced: control diet (CD); full-fat BSFL meal diet (IM); defatted BSFL meal diet (DFIM); de-chitinised BSFL meal diet (DCIM); BSFL oil diet (IO) and BSFL exoskeleton diet (EX). The full-fat, defatted and de-chitinised meals replaced 15% of protein in the control diet. An eight-week study was conducted using salmon with average 28 g initial weight. The full-fat and de-chitinised meals in the diets numerically reduced pellet hardness, expansion, and water stability. The full-fat and de-chitinised meals improved growth rate of salmon, whilst defatted meal, oil and exoskeleton supported similar growth performance as the control. Feed intake and growth rate of fish fed full-fat meal diet were higher than those fed the other insect diets, but defatted meal gave a better feed conversion ratio than full-fat meal. Defatted meal, de-chitinised meal and exoskeleton reduced protein digestibility in fish, however; defatted meal increased the digested protein retention. In conclusion, use of full-fat BSFL meal improved feed intake and growth rate of salmon when replacing 15% of dietary protein. The present results suggest that less processed full-fat form of BSFL is more optimal in diets for salmon and further processing to remove lipid or exoskeleton fractions would only lead to an additional cost.

Keywords: black soldier fly larvae, insect fractions, pellet quality, fish growth performance, nutrient utilisation

1. Introduction

Black soldier fly larvae (BSFL) (*Hermetia illucens*) have a great potential as a sustainable novel feed ingredient in fish feed due to its high nutrient value (Barroso *et al.*, 2014; Makkar *et al.*, 2014; Nogales-Mérida *et al.*, 2019) and its ability to utilise wide variety of organic materials efficiently (Wang and Shelomi, 2017). Numerous studies reported the effect of dietary inclusion of BSFL on nutrient utilisation, growth performance, and health of various fish species including Atlantic salmon (*Salmo salar*) (Belghit *et al.*, 2018, 2019a; Fisher *et al.*, 2020; Lock *et al.*, 2016; Weththasinghe *et al.*, 2021a,b).

BSFL contain the three major fractions; protein, lipid and exoskeleton (Müller *et al.*, 2017; Ravi *et al.*, 2020). The results of previous studies suggest that these fractions might differently affect the nutrient utilisation and growth performance of fish. Dietary inclusion of moderate levels of BSFL meal (<20%) did not compromise growth performance in salmon (Belghit *et al.*, 2019a; Fisher *et al.*, 2020; Weththasinghe *et al.*, 2021a), whereas higher inclusion levels (>20%) reduced growth rate (Fisher *et al.*, 2020; Weththasinghe *et al.*, 2021a). In contrast, Belghit *et al.* (2018) reported that de-chitinised BSFL protein meal in diets did not compromise the growth performance of salmon even at 60% inclusion level. The reduction of growth rate at higher BSFL levels was thus suggested to

be attributed to the presence of chitin in the exoskeleton of BSFL (Dumas *et al.*, 2018; Weththasinghe *et al.*, 2021a). Dietary inclusion of BSFL meal has also shown to reduce the nutrient digestibility in salmon (Belghit *et al.*, 2018; Weththasinghe *et al.*, 2021a). This might also be due to chitin (Hansen *et al.*, 2010; Karlsen *et al.*, 2017; Shiau and Yu, 1999) or high level of saturated fatty acids (Hua and Bureau, 2009) present in BSFL. Further, Weththasinghe *et al.* (2021a) reported that the lipid retention decreased at high dietary inclusion of BSFL meal in salmon. It was hypothesised that this was caused by high level of lauric acid (C12:0) in BSFL, which is preferred as a substrate for oxidation in salmon (Belghit *et al.*, 2019b; Renna *et al.*, 2017). On the other hand, bioactive compounds present in BSFL, such as antimicrobial peptides (Müller *et al.*, 2017; Park *et al.*, 2014, 2015), chitin, as well as lauric acid possess antimicrobial properties (Askarian *et al.*, 2012; Skřivanová *et al.*, 2006; Spranghers *et al.*, 2018), which could have a positive effect on gut microbiota, gut health and subsequently the growth performance in fish.

The quality and antimicrobial peptides in the protein fraction, fatty acid composition in the lipid fraction and the chitin in the exoskeleton fraction of BSFL might have contrasting effects on the nutrient utilisation and growth performance in fish. However, to the best of our knowledge, such effects of different fractions of BSFL have still not been evaluated in a single study. Further, limited information exists on the BSFL fractions on technical quality of extruded feed pellets. Therefore, the present study investigated the effects of meals (full-fat, defatted and de-chitinised) and fractions (oil and exoskeleton) of BSFL in extruded diets on physical pellet quality, nutrient utilisation, and growth performance in Atlantic salmon pre-smolts.

2. Materials and methods

Rearing and processing of black soldier fly larvae

BSFL were reared and processed into meals or fractions at HiProMine S.A., Robakowo, Poland. The BSFL were fed *ad libitum*. The dry matter (DM) content of BSFL feed was normalised to 22% by adding wheat middlings (17%) to fresh vegetables and fruit pre-consumer waste mix, consisting of apples (15%), carrots (50%), potatoes (15%), and cabbage (20%). The feed mixture was ground (2,000 rpm, 55 kW; HPM milling system, HiProMine S.A.) to sieve through a 2 mm screen. In accordance with EC regulation (no 1069/09), the BSFL feed did not contain any animal products.

The larvae were harvested on the seventh day of rearing, sieved using a 3 mm screen, and washed with water on drum separator at 90 °C for 10 min (HPM cleaning system, HiProMine S.A.). A batch of BSFL was divided into two parts for further processing. The first part was dried at 110 °C for 1 h and then at 80 °C for 23 h until a constant weight was reached using a chamber air flow dryer (HiProMine S.A.) for full-fat BSFL meal production, then a part of it was defatted to obtain partially defatted meal and oil with use of oil press (Reinartz, model AP14/22, Neuss, Germany). The second part of BSFL was used to obtain partially de-chitinised meal and exoskeleton fraction. For the separation of partially de-chitinised BSFL and exoskeleton fraction, the mechanical de-chitinisation was applied using food press twin-screw processor with 0.3 mm screen diameter (Angel Juicer, model 7500, Busan, Korea). The de-chitinised BSFL and exoskeleton were dried at 110 °C for 1 h and then at 80 °C for 23 h until a constant weight was reached using a chamber air flow dryer (HiProMine S.A.). All the products were stored at 4 °C before use for feed production. The chemical compositions of BSFL ingredients are shown in Table 1 and Table S1.

Experimental diets

Six experimental diets were formulated to have similar amino acid profiles and lipid contents, and to meet NRC (2011) requirements for all essential amino acids and other nutrients for Atlantic salmon. The experimental diets comprised of a control diet (CD) containing fishmeal, soy protein concentrate (SPC) and corn gluten as protein sources, and fish oil as the main lipid source; three diets with full-fat BSFL meal (IM), defatted BSFL meal (DFIM) or de-chitinised BSFL meal (DCIM), replacing 15% of protein from fishmeal, SPC and corn gluten in the control diet; a diet containing BSFL oil (IO) and a diet containing BSFL exoskeleton (EX). The BSFL oil and exoskeleton were added to the diets to match the BSFL oil and chitin contents in full-fat BSFL meal diet, respectively. The internal marker, Yttrium oxide was added in all the diets for the determination of apparent digestibility coefficient (ADC) of nutrients. Crystalline methionine and monocalcium phosphate were added to all the diets to ensure that the diets met or exceeded the methionine (0.7%, DM basis (NRC, 2011)) and phosphorous (P) (0.8%, DM basis (NRC, 2011)) requirements of Atlantic salmon, respectively. Table 2 shows the ingredient and chemical composition of the six experimental diets.

Production of experimental diets

The extruded experimental diets were produced at the Norwegian University of Life Sciences (NMBU) Centre for Feed Technology (Fôrtek), Ås, Norway. First, all the weighed ingredients (except micro ingredients, fish oil and BSFL oil) were mixed for 3 min in an ISDECA mixer (60-l paddle-mixer, prototype, Fôrtek, Forberg, Norway). The material mixture was then ground in a Hammer mill (Bill bliss, horizontal, 18.5 kW, USA) with a 1 mm sieve, and mixed with micro-ingredients. The feed mash of full-fat and de-chitinised meal diets were ground again

Table 1. Chemical composition (%, as is) of meals and fractions of black soldier fly larvae (BSFL).

Nutrient	Full-fat BSFL meal	Defatted BSFL meal	De-chitinised BSFL meal	BSFL oil	BSFL exoskeleton
Dry matter	88.1	86.8	90.7	99.0	91.7
Crude protein	37.6	51.4	31.2	2	59.9
Crude lipid	29.6	11.7	43.5	96.0[1]	11.1
Ash	5.82	8.03	6.97	1.03	5.59
Chitin	7.05	9.65	2.15		19.8
Amino acids[2]					
Essential amino acids					
Methionine	0.41	0.56	0.42	NA	0.29
Threonine	1.16	1.58	1.10	NA	1.71
Valine	1.46	1.99	1.33	NA	2.75
Isoleucine	1.23	1.68	1.17	NA	1.91
Leucine	2.02	2.75	1.91	NA	3.11
Phenylalanine	1.24	1.69	1.19	NA	1.73
Histidine	0.95	1.30	0.88	NA	1.70
Lysine	1.92	2.62	1.90	NA	2.14
Arginine	1.48	2.03	1.42	NA	2.09
Tryptophan	0.22	0.29	0.23	NA	0.10
Non-essential amino acids					
Cysteine	0.23	0.32	0.23	NA	0.24
Aspartic acid	2.48	3.39	2.40	NA	3.34
Serine	1.27	1.73	1.17	NA	2.30
Glutamic acid	3.97	5.42	3.78	NA	5.89
Proline	1.96	2.68	1.78	NA	3.87
Glycine	1.52	2.08	1.37	NA	3.07
Alanine	2.25	3.07	2.07	NA	4.01
Tyrosine	2.56	3.50	2.25	NA	5.74
Total amino acid	28.33	38.68	26.61	NA	45.97

[1] Calculated as: Crude lipid = Dry matter – (Crude protein + Ash).

[2] Water corrected values. The amino acids compositions of full-fat and de-chitinised BSFL meals are calculated values based on the analysed amino acid compositions of defatted BSFL meal and BSFL exoskeleton. NA = not analysed.

in the Hammer mill (Bill bliss, horizontal, 18.5 kW, USA) with a 0.5 mm sieve to prevent the production of coarse particles and less integrated pellets after the extrusion. The diets were extruded in a five-section Bühler twin-screw extruder (BCTG 62/20 D, Uzwil, Switzerland) fitted with four 2.5 mm die holes. The extruder operated without a pre-conditioner and the screw configuration shown previously by Weththasinghe *et al.* (2021a) was used during the extrusion of diets. The feed mash was fed into the first section of the extruder using a small K-tron feeder. The screw speed was increased when the extrusion of insect ingredients containing diets. The extruded pellets were dried at 60 °C for 1 h using fan heaters (15KW, Inelco heaters, Dania-heater 15 kW, Fjerritslev, Denmark). The pellets were then cooled at room temperature and vacuum coated with fish oil and/or BSFL oil in Gentle Vacuum Coater – 80 prototype (Fôrtek, Amandus-Kahl).

Fish study, rearing facilities, and sampling

The fish study was conducted at the Centre for Fish Research, NMBU, Ås, Norway. The study consisted of 900 Atlantic salmon (Aqua Gen Atlantic QLT-innOva SHIELD) with an average 28 g initial weight. The fish were randomly distributed into 18 fiberglass tanks (50 fish per tank) with recirculated freshwater (average temperature of 14.4±0.4 °C). The tanks were supplied with water at 6 l/min and dissolved oxygen levels were kept above 7.0 mg/l in the outlet water. The study lasted for eight weeks and triplicate tanks of salmon were fed one of the six experimental diets. The fish were kept under continuous light and fed *ad libitum* (i.e. 10% excess) with electrically driven belt feeders according to a six-hours feeding program per day. The uneaten feed was collected daily using the wedge wire screens fitted to the outlet of tanks as explained by Shomorin *et al.* (2019) and daily feed intake in each tank

Table 2. Ingredient and chemical composition of experimental diets[1] with meals or fractions of black soldier fly larvae (BSFL).

Ingredients (%)[2]	CD	IM	DFIM	DCIM	IO	EX
Fishmeal[a]	22.50	18.57	18.57	18.57	22.50	21.78
Soy protein concentrate[b]	34.50	28.48	28.48	28.48	34.50	33.39
Corn gluten[c]	5.50	4.54	4.54	4.54	5.50	5.32
Full-fat BSFL meal[d]	0.00	20.36	0.00	0.00	0.00	0.00
Defatted BSFL meal[e]	0.00	0.00	14.89	0.00	0.00	0.00
De-chitinised BSFL meal[f]	0.00	0.00	0.00	24.53	0.00	0.00
BSFL oil[g]	0.00	0.00	0.00	0.00	6.24	0.00
BSFL exoskeleton[h]	0.00	0.00	0.00	0.00	0.00	7.20
Wheat flour[i]	14.65	14.65	14.65	14.65	14.65	14.65
Fish oil[j]	16.00	10.47	14.75	5.82	10.05	15.36
Methionine[k]	0.20	0.20	0.20	0.20	0.20	0.20
Choline chloride[l]	0.15	0.15	0.15	0.15	0.15	0.15
Yttrium[m]	0.01	0.01	0.01	0.01	0.01	0.01
Vit/min premix[n]	0.65	0.65	0.65	0.65	0.65	0.65
Monocalcium Phosphate[o]	0.80	0.80	0.80	0.80	0.80	0.80
Wheat bran[p]	5.04	1.12	2.31	1.60	4.75	0.49
Chemical composition (%, as is)						
Dry matter	91.6	91.9	93.0	92.9	93.3	91.7
Crude protein	46.6	44.4	46.0	46.6	46.6	47.3
Crude lipid	19.6	20.3	17.8	12.9	18.3	17.0
Starch	13.1	12.2	12.4	12.4	12.6	11.7
Ash	6.70	6.60	6.77	7.23	6.70	6.61
Chitin[q]		1.44	1.44	0.53		1.43
Macro mineral composition (%, as is)						
Total phosphorous	1.13	1.10	1.10	1.24	1.11	1.10
Calcium	1.11	1.19	1.29	1.28	1.28	1.19
Magnesium	0.18	0.19	0.20	0.23	0.17	0.17
Amino acid composition[r] (%, as is)						
Essential amino acids						
Methionine	0.98	0.90	0.90	0.94	0.90	0.94
Threonine	1.59	1.48	1.51	1.56	1.58	1.59
Valine	1.55	1.52	1.54	1.51	1.56	1.64
Isoleucine	1.78	1.65	1.70	1.75	1.80	1.79
Leucine	3.40	3.05	3.12	3.23	3.40	3.36
Phenylalanine	1.99	1.76	1.81	1.90	1.97	1.88
Histidine	1.07	1.04	1.05	1.06	1.07	1.09
Lysine	2.62	2.47	2.49	2.59	2.60	2.58
Arginine	2.72	2.42	2.46	2.57	2.71	2.63
Non-essential amino acids						
Cysteine	0.45	0.41	0.41	0.43	0.44	0.43
Aspartic acid	4.13	3.68	3.82	3.99	4.12	4.05
Serine	1.84	1.66	1.71	1.71	1.80	1.85
Glutamic acid	8.05	7.08	7.32	7.55	8.00	7.90
Proline	2.12	2.05	2.17	2.07	2.16	2.32
Glycine	1.70	1.64	1.67	1.63	1.69	1.78
Alanine	1.96	1.99	2.02	1.91	1.96	2.11
Tyrosine	1.21	1.41	1.43	1.24	1.25	1.48
Total amino acid	39.2	36.2	37.1	37.6	39.0	39.4

[1] CD = Control diet; DCIM = De-chitinised BSFL meal diet; DFIM = Defatted BSFL meal diet; EX = BSFL exoskeleton diet; IM = Full-fat BSFL meal diet; IO = BSFL oil diet.

[2] [a] = LT fishmeal, Norsildmel AS, Bergen, Norway; [b] = soy protein concentrate, Tradkon SPC HC-200, Sojaprotein, Becej, Serbia; [c] = corn gluten meal, Baolingbao Biology, Shangdong Yucheng, China; [d] = full-fat BSFL meal, HiProMine S.A., Poznań, Poland; [e] = defatted BSFL meal, HiProMine S.A., Poznań, Poland; [f] = de-chitinised BSFL meal, HiProMine S.A., Poznań, Poland; [g] = BSFL oil, HiProMine S.A., Poznań, Poland; [h] = BSFL exoskeleton, HiProMine S.A., Poznań, Poland; [i] = wheat flour 78%, batch number: 5093060546, Norgesmøllene, Bergen, Norway; [j] = fish oil, Norsildmel AS, Bergen, Norway; [k] = L-methionine, Bestamino™ Cj Cheiljedang, Seoul, Korea; [l] = choline chloride 70%, $C_5H_{14}ClNO$, 139.6 g/mol, Vilomix, Hønefoss, Norway; [m] = yttrium oxide (Y_2O_3) Metal Rare Earth Limited, Shenzhen, China; [n] = vit/min premix, Farmix, Trouw Nutrition, LA Putten, the Netherlands. Per kg of feed; Vitamin A 3,250 IU, Vitamin D3 1,950 IU, Vitamin E 260 IU, Vitamin K3 13 mg, Vitamin B1 20 mg, Vitamin B2 33 mg, d-Ca-pantothenate 52 mg, Niacinamide 98 mg, Vitamin B6 20 mg, Folic acid 6.5 mg, Vitamin B12 33 µg, Vitamin C 163 mg, Biotin 358 µg, Calcium iodate, anhydrous, Iodine 3.9 mg, Manganese (II) oxide, Manganese 20 mg, Zinc oxide, Zinc 137 mg; [o] = monocalcium phosphate, Monohydrate, BOLIFOR® MCP-F, Yara Phosphates Oy, Animal Nutrition, Sweden; [p] = wheat bran, Norgesmøllene, Bergen, Norway; [q] = calculated based on the chitin content of the respective BSFL ingredient and its inclusion level in the diet; [r] = water corrected values.

was quantified according to Helland *et al.* (1996). The fish mortality was checked daily. Initial and final body weights of fish were measured at the start and end of the eight-week study period. Fifteen fish at the start and five fish per tank at end of the study were sampled, pooled, homogenised and freeze dried for whole body composition analysis. In addition, at the end of the study, another six fish from each tank were randomly sampled, anaesthetised, euthanised by a sharp blow to the head and weighed individually. The fish were dissected, and the liver was removed. The attached adipose tissue and fat around liver were removed and the liver weight was measured to calculate hepatosomatic index (HSI). After the eight-week study period, fish were fed with experimental diets for two additional weeks for faeces collection. Fish were carefully stripped three times with seven days interval for faecal collection from the posterior intestine according to Austreng (1978). The stripped faeces were stored immediately at -20 °C prior to freeze drying. Tricaine methanesulfonate (MS-222) (80 mg/l) was used to anaesthetise fish during weighing, sampling, and stripping. All the experimental procedures were conducted adhering to the guidelines for the care and use of animals in Norway (The Norwegian Animal Welfare Act and the Norwegian Regulation and Animal Experimentation).

Physical pellet quality analysis

The bulk density of the uncoated pellets was measured after the extrusion. The other physical quality parameters were measured in oil-coated pellets. As explained by Hansen *et al.* (2010), Doris pellet tester (AKVAsmart, Bryne, Norway) was used to estimate pellet durability. The durability of the pellets was measured in triplicates using a 2 mm screen. Hardness was measured using 15 pellets with average length and diameter from each diet with a Texture analyser with a 5 kg load cell (Tinius Olsen, H5KT, Salfords, UK) according to Øverland *et al.* (2009). The expansion of the extruded pellets was determined by measuring the width of 30 randomly selected pellets per diet using the Texture analyser (Tinius Olsen, H5KT). The mean value of the time required for 10 randomly picked pellets to sink 1 m in 17 °C tap water was recorded to determine the sinking velocity of the pellets. The method explained by Baeverfjord *et al.* (2006) was used to measure the water stability of pellets within 30 and 60 min.

Chemical analysis

The feed and freeze-dried faeces and fish were ground. The samples were oven dried at 104 °C until a constant weight was reached to measure DM content. Ash contents were determined by combustion at 550 °C. The nitrogen (N) contents of BSFL ingredients and fish were estimated by Kjeldahl method according to Commission Regulation (EC) No 152/ 2009. The N contents of faeces were analysed by CHNS Elemental Analyzer (Vario El Cube elemental analyser system GmbH, Hanau, Germany). The crude protein content was determined as N×6.25. The N content in diets was measured by both methods and the values obtained by CHNS Elemental Analyzer were used for protein digestibility estimates, whereas the values obtained by Kjeldahl method were used for protein retention estimates. The chitin contents of BSFL ingredients were measured as explained by Finke (2007). In brief, acid detergent fibre (ADF) content and the percentage of amino acids in the ADF rest fraction was measured. The ADF content was corrected for the sum of amino acids and the remainder of the ADF fraction was considered as chitin. The crude lipid contents of BSFL ingredients, faeces and fish were determined after extraction with petroleum ether and acetone (70/30) using an Accelerated Solvent Extractor (ASE200; Dionex Corp., Sunnyvale, CA, USA). The crude lipid contents of diets were measured by acid hydrolysis and ether extraction according to NMKL 160 (modified) at Eurofins Agro Testing Norway AS, Moss, Norway. The method explained by McCleary *et al.* (1994) was used with some modifications to measure starch content. Briefly, the starch in the samples were converted into glucose using heat-stable α-amylase and amyl glucosidase-enzymes, and glucose content was measured by a spectrometer (RX4041 Randox Daytona+, Randox Laboratories, Antrim, UK). The Biochrom 30 Amino Acid Analyser (Biochrom Ltd., Cambridge, UK) was used to analyse amino acid contents according to Commission Regulation (EC) No 152/2009. The fatty acid content of BSFL oil was determined by synthesising the fatty acid to fatty acid methyl esters using Trace GC Ultra gas chromatograph (Thermo Fisher Scientific, Waltham, MA, USA) as explained by O'Fallon *et al.* (2007). Yttrium (Y), calcium and magnesium contents were measured after acid decomposition in a microwave digestion system (Start D, Milestone Srl, Sorisole, Italy) using a microwave plasma atomic emission spectrometer (MP-AES 4200, Agilent Technologies, Santa Clara, CA, USA). Total P contents were analysed using a commercial spectrophotometric kit (PH8328, Randox Laboratories) after combustion and acid digestion according to Commission Regulation (EC) No 152/2009.

Calculations

The pellet expansion (%) was calculated as ((Width of pellet – Die diameter) / Die diameter) × 100. Specific mechanical energy (Wh/kg) was calculated as $(2 \times \pi \times 60^{-1}) \times$ (Screw speed × Torque × Throughput). Specific growth rate (SGR) (%) was calculated as [(ln (Fish final body weight (g)) – ln (Fish initial body weight (g))) / Study period (days)] × 100. Feed conversion ratio (FCR) was calculated as Feed intake of fish (g DM) / Fish body weight gain (g). HSI (%) was calculated as Weight of liver (g) /Fish body weight (g) × 100. ADC of nutrients (%) was calculated as (1 – [(Y concentration in diet / Y concentration in faeces) × (Nutrient concentration in faeces / Nutrient concentration in diet)])

× 100. Faecal excretion of nutrients (%) was calculated as (100 – ADC of nutrients). The dissolved nutrient fraction (g/kg) was calculated as [Nutrient digested (g) – (Final nutrient content in fish (g) – Initial nutrient content in fish (g))] / Fish body weight gain (kg). Protein and lipid efficiency ratios were calculated as Fish body weight gain (g) / Protein or lipid intake (g). Apparent nutrient retention (% intake) was calculated as [(Final nutrient content in fish (g) – Initial nutrient content in fish (g)) / Nutrient intake (g)] × 100. Nutrient retention (% digested nutrient) was calculated as [(Final nutrient content in fish (g) – Initial nutrient content in fish (g)) / Nutrient digested (g)] × 100.

Statistical analysis

The data were analysed using one-way ANOVA and Tukey's multiple comparison test was used to compare the means. Differences at $P<0.05$ were regarded as significant. The analyses were performed using IBM SPSS Statistics 27 software (IBM Corp., Armonk, NY, USA).

3. Results

Feed production and physical pellet quality

The lipid-rich full-fat and de-chitinised insect meals increased the lipid content of the feed mash prior to extrusion. To compensate for this, the throughput and water addition to the extruder were increased to obtain pellets with desirable physical quality in the full-fat and de-chitinised insect meals included diets. In addition, the fifth barrel of the extruder was cooled to obtain the desired bulk density in these two diets, and this led to reduced temperature in the fifth barrel and the die. Further, decreased die pressure and torque were observed during the extrusion process of these two diets (Table 3).

The pellet durability measured by the Doris pellet tester showed an overall high physical quality of the pellets. However, the full-fat and de-chitinised insect meal included diets showed numerically lower pellet hardness, expansion, and water stability as well as numerically higher bulk density after the extrusion (Table 4 and Figure 1).

Growth performance

Only four fish died throughout the study period. The fish fed full-fat insect meal showed higher feed intake, accompanied by higher final body weight and SGR compared with the fish fed the control and the other insect diets. In addition, the inclusion of de-chitinised insect meal in the diet also increased final body weight and SGR of fish compared to the control diet but decreased compared to the full-fat meal diet. The defatted insect meal, oil and exoskeleton supported similar final body weight and SGR as the control diet fed fish. Although the FCR of the fish fed insect diets did not differ from the fish fed control diet, defatted meal and exoskeleton included diets gave lower FCR in fish than the full-fat meal diet. The HSI was not affected by the dietary treatments (Table 5).

Table 3. Extruder parameters during the production of experimental diets[1] with meals or fractions of black soldier fly larvae (BSFL).

Extruder parameter[2]	CD	IM	DFIM	DCIM	IO	EX
Throughput (kg/h)	35	40	30	40	30	30
Barrel 1 (°C)	42	22	32	26	37	40
Barrel 2 (°C)	86	33	49	37	60	77
Barrel 3 (°C)	107	67	108	84	114	113
Barrel 4 (°C)	113	107	123	105	127	123
Barrel 5 (°C)	111	84	122	92	127	122
Die temperature (°C)	105	91	122	99	127	122
Die pressure (Bar)	23	4	12.3	2.8	25.2	22.4
Screw speed (rpm)	270	400	400	425	385	400
Torque (%)	53	43	48	38	60	49
Drive power (kW)	6.5	7.6	8.8	7.3	10.7	9
SME[a] (Wh/kg)	449	510	616	440	771	630
Water addition[b] (kg/h)	14	15.5	14	16.5	14	14
Lipid[c] (%)	2.50	7.97	3.75	12.64	2.48	3.01

[1] CD = Control diet; DCIM = De-chitinised BSFL meal diet; DFIM = Defatted BSFL meal diet; EX = BSFL exoskeleton diet; IM = Full-fat BSFL meal diet; IO = BSFL oil diet.

[2] [a] = specific mechanical energy; [b] = water added into the extruder; [c] = percentage of lipid in the feed mash prior to extrusion.

Table 4. Physical pellet quality of experimental diets[1] with meals or fractions of black soldier fly larvae (BSFL).

Pellet quality parameter[2]	CD	IM	DFIM	DCIM	IO	EX	SEM[3]
Bulk density (g/l)[a]	566.7	586.3	540.3	580	528.7	560.7	5.36
Durability (%)[a]	97.7	96.4	98.0	93.7	98.6	98.3	0.42
Hardness (N)[b]	18.2	12.3	13.4	11.1	23.6	14.0	0.82
Expansion (%)[c]	18.4	1.67	17.4	-0.81	32.3	21.8	0.89
Sinking velocity (m/s)[d]	0.10	0.08	0.09	0.08	0.08	0.09	0.002

[1] CD = Control diet; DCIM = De-chitinised BSFL meal diet; DFIM = Defatted BSFL meal diet; EX = BSFL exoskeleton diet; IM = Full-fat BSFL meal diet; IO = BSFL oil diet.

[2] [a] = mean of 3 observations; [b] = mean of 15 observations; [c] = mean of 30 observations; [d] = mean of 10 observations.

[3] Standard error mean.

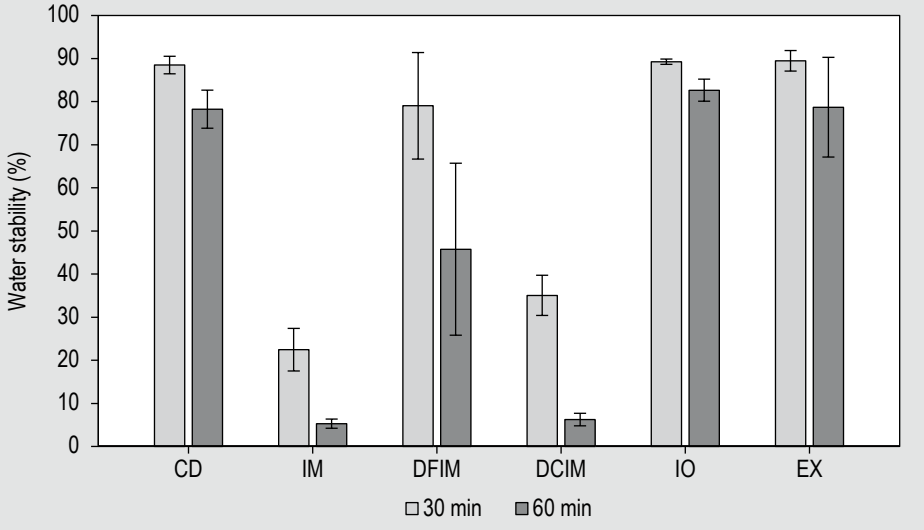

Figure 1. Water stability (dry matter retention %) of pellets of experimental diets with meals or fractions of black soldier fly larvae (BSFL) within 30 min and 60 min (mean of 3 observations). Error bars indicate standard deviation. CD: Control diet. IM: Full-fat BSFL meal diet. DFIM: Defatted BSFL meal diet. DCIM: De-chitinised BSFL meal diet. IO: BSFL oil diet. EX: BSFL exoskeleton diet.

Table 5. Performance of fish fed experimental diets[1] with meals or fractions of black soldier fly larvae (BSFL).[2]

	CD	IM	DFIM	DCIM	IO	EX	SEM[3]	*P*-value
Initial body weight (g)	28.3	28.3	28.3	28.3	28.3	28.3	0.007	0.14
Final body weight (g)	98.4[c]	114.8[a]	104.2[bc]	107.1[b]	99.8[bc]	104.7[bc]	1.41	<0.001
Specific growth rate (%)	2.01[c]	2.26[a]	2.10[bc]	2.14[b]	2.03[bc]	2.11[bc]	0.02	<0.001
Feed intake (g DM/fish)	53.1[b]	68.8[a]	55.9[b]	59.4[b]	55.7[b]	57.1[b]	1.31	<0.001
Feed conversion ratio	0.76[ab]	0.80[a]	0.74[b]	0.76[ab]	0.78[ab]	0.75[b]	0.006	0.013
Hepatosomatic index (%)[4]	1.32	1.35	1.42	1.36	1.34	1.37	0.01	0.48

[1] CD = Control diet; DCIM = De-chitinised BSFL meal diet; DFIM = Defatted BSFL meal diet; EX = BSFL exoskeleton diet; IM = Full-fat BSFL meal diet; IO = BSFL oil diet.

[2] Values in the same row with different superscripts are significantly different at $P<0.05$.

[3] Standard error mean (n=3).

[4] Mean of 18 fish.

Digestibility, faecal excretion, and dissolved fraction of nutrients

ADC of DM, crude lipid and starch were not affected by the dietary treatments. The full-fat insect meal and insect oil diets fed fish showed similar ADC of protein as the control diet fed fish. The defatted meal, de-chitinised meal and exoskeleton containing diets fed fish had lower ADC of protein, accompanied by increased faecal N excretion compared to the control diet fed fish. However, ADC of protein did not differ between full-fat insect meal diet and other insect diets (Table 6).

The fish fed full-fat and de-chitinised insect meals containing diets showed lower faecal excretion of P, whereas fish fed full-fat meal, defatted meal and insect oil containing diets had lower faecal excretion of calcium, than the fish fed control diet. The dissolved fractions of N and P in insect meals or fractions containing diets did not differ from the control diet, except defatted meal diet, in which the dissolved fraction of N was lower than the control and full-fat meal diets. Further, the defatted insect meal fed fish showed a lower dissolved P fraction compared to de-chitinised insect meal diet fed fish (Table 6).

Nutrient retention

The retention of ingested protein was not affected by dietary treatments. The protein efficiency ratio of fish fed insect diets did not differ from the fish fed control diet. Further, the retentions of digested protein of fish fed insect diets, except defatted insect meal diet, were also similar as the fish fed control diet. In defatted insect meal diet, the retention of digested protein was higher compared to both control and full-fat insect meal diets. Fish fed the insect diets except full-fat meal diet showed higher lipid efficiency ratio (LER) than the control diet fed fish, where the fish fed de-chitinised meal showed the highest. The retention of both ingested and digested lipid in fish fed the insect diets did not differ from the control diet, except for those fed the de-chitinised meal diet, which had a higher retention of lipid. Both LER and lipid retentions were lower in the full-fat meal diet than the diets containing other insect meals or fractions. The apparent P retention was higher in defatted insect meal diet fed fish compared to those fed control and other insect diets (Table 7).

4. Discussion

The present study investigated the effect of dietary inclusion of meals and fractions of BSFL on physical feed pellet quality, nutrient utilisation, and growth performance in Atlantic salmon pre-smolts. The results provide important information to determine how the BSFL should be processed to optimise its use in extruded diets for salmon.

Feed production and physical pellet quality

The high level of lipid in full-fat and de-chitinised insect meals increased the lipid content in the feed mash during extrusion. As explained in Weththasinghe *et al.* (2021a) and Hansen *et al.* (2011), increase in lipid content in the

Table 6. Apparent digestibility coefficient (%), faecal excretion (%) and dissolved fraction of nutrients (g/kg of fish body weight gain) of fish fed experimental diets[1] with meals or fractions of black soldier fly larvae (BSFL).[2]

	CD	IM	DFIM	DCIM	IO	EX	SEM[3]	*P*-value
Apparent digestibility coefficients								
Dry matter	73.5	75.6	74.8	74.3	73.5	73.9	0.27	0.175
Crude protein	89.4^{a}	88.0^{abc}	87.4^{bc}	86.9^{c}	89.0^{ab}	86.7^{c}	0.28	0.001
Crude lipid	97.6	97.6	97.1	95.7	96.9	97.1	0.23	0.16
Starch	84.2	82.0	82.8	83.2	84.9	81.6	0.37	0.054
Ash	21.8^{b}	28.4^{ab}	31.0^{a}	28.0^{ab}	21.9^{b}	25.7^{ab}	1.02	0.016
Faecal excretions								
Phosphorous	49.0^{ab}	43.2^{c}	43.5^{bc}	42.2^{c}	52.9^{a}	47.8^{abc}	1.01	<0.001
Calcium	90.4^{a}	78.0^{b}	76.1^{b}	82.5^{ab}	79.2^{b}	83.3^{ab}	1.30	0.002
Magnesium	69.8	57.2	60.9	57.7	59.7	56.4	1.94	0.398
Nitrogen	10.6^{c}	12.0^{abc}	12.6^{ab}	13.2^{a}	11.0^{bc}	13.3^{a}	0.28	0.001
Dissolved fractions								
Nitrogen	25.3^{a}	25.0^{a}	21.5^{b}	23.2^{ab}	25.2^{a}	23.7^{ab}	0.42	0.020
Phosphorous	0.71^{ab}	0.91^{ab}	0.05^{b}	1.06^{a}	0.29^{ab}	0.57^{ab}	0.11	0.020

[1] CD = Control diet; DCIM = De-chitinised BSFL meal diet; DFIM = Defatted BSFL meal diet; EX = BSFL exoskeleton diet; IM = Full-fat BSFL meal diet; IO = BSFL oil diet.

[2] Values in the same row with different superscripts are significantly different at $P<0.05$.

[3] Standard error mean (n=3).

Table 7. Nutrient retention parameters in fish fed experimental diets[1] with meals or fractions black soldier fly larvae (BSFL).[2]

	CD	IM	DFIM	DCIM	IO	EX	SEM[3]	*P*-value
Protein efficiency ratio	2.60^{ab}	2.61^{ab}	2.75^{a}	2.64^{ab}	2.57^{b}	2.59^{ab}	0.02	0.028
Lipid efficiency ratio	6.17^{d}	5.71^{e}	7.10^{b}	9.54^{a}	6.55^{c}	7.21^{b}	0.30	<0.001
Apparent protein retention (% intake)	48.4	47.4	50.5	48.6	48.6	48.3	0.32	0.087
Apparent protein retention (% digested protein)	54.1^{b}	53.8^{b}	57.8^{a}	56.0^{ab}	54.6^{ab}	55.7^{ab}	0.42	0.027
Apparent lipid retention (% intake)	88.3^{bc}	77.9^{c}	97.8^{b}	128.7^{a}	96.7^{b}	94.1^{b}	3.91	<0.001
Apparent lipid retention (% digested lipid)	90.4^{bc}	79.8^{c}	100.8^{b}	134.5^{a}	99.7^{b}	96.9^{b}	4.21	<0.001
Apparent phosphorous retention (% intake)	43.4^{b}	47.2^{b}	55.9^{a}	47.3^{b}	44.0^{b}	45.9^{b}	1.16	0.003

[1] CD = Control diet; DCIM = De-chitinised BSFL meal diet; DFIM = Defatted BSFL meal diet; EX = BSFL exoskeleton diet; IM = Full-fat BSFL meal diet; IO = BSFL oil diet.

[2] Values in the same row with different superscripts are significantly different at $P<0.05$.

[3] Standard error mean (n=3).

mash can result in reduced dough temperature and starch gelatinisation during extrusion, that further can lead to a lower physical quality of extruded pellets. This might explain the numerically lower hardness observed in full-fat and de-chitinised insect meals included diets in the present study. The higher bulk densities of full-fat and de-chitinised insect meal diets were accompanied by lower pellet expansion. The reduced pellet expansion in these two diets is in line with previous reports indicating that increased lipid content in feed mash decreased extrudate expansion (Hansen *et al.*, 2011; Ilo *et al.*, 2000; Weththasinghe *et al.*, 2021a). High lipid levels in the feed mash can reduce the pressure during the extrusion, resulting in poor expansion (Ottoboni *et al.*, 2018). In addition, the decreased pellet expansion in full-fat and de-chitinised insect meal diets might also be related to lower temperature in the fifth barrel of the extruder (Bandyopadhyay and Rout, 2001; Kothakota *et al.*, 2013; Pathania *et al.*, 2013). As shown by Hansen *et al.* (2011), the increased level of lipid content in the feed mash followed with reduced pellet expansion is also, most probably, explaining the reduced pellet water stability of full-fat and de-chitinised insect meal diets in the present study. Previous studies also showed reduced water stability of extruded fish feed pellets containing full-fat BSFL meal (Rawski *et al.*, 2020; Weththasinghe *et al.*, 2021a).

Nutrient utilisation and growth performance in salmon

In the present study, the replacement of 15% of dietary protein with full-fat insect meal, rather than with processed BSFL meal by separation of lipid or exoskeleton fractions supported higher growth rate, and is, thus, more resource efficient and less costly. The improvement in growth rate of fish fed the full-fat meal diet can be a result of the higher feed intake of fish fed this diet. The increased feed intake indicates that moderate level of full-fat BSFL meal in diets (20%) might increase palatability for salmon. Rawski *et al.* (2020) also reported that dietary inclusion of full-fat BSFL meal (10-30%) increased feed acceptance in Siberian sturgeon (*Acipenser baerii*). In contrast, our previous study showed that 8-32% full-fat BSFL meal in diets had no effect on feed intake of salmon (Weththasinghe *et al.*, 2021a). In the present study, the improved feed intake might be due to lower pellet water stability of full-fat meal diet as many water-soluble nutrients are known chemo-attractants and feed stimulants (Simon *et al.*, 2021). In addition to the improved feed intake, other processes may also be involved in increase in growth rate of salmon fed full-fat insect meal in the present study. BSFL are rich in bioactive compounds such as chitin (Caligiani *et al.*, 2018; Finke, 2013), antimicrobial peptides (Müller *et al.*, 2017; Park *et al.*, 2014, 2015) and medium-chain fatty acid, lauric acid (C12:0) (40% of the total fatty acid) (Table S1). These compounds are shown to possess antimicrobial properties (Askarian *et al.*, 2012; Skřivanová *et al.*, 2006; Spranghers *et al.*, 2018), which could have a positive effect on gut health and growth performance in fish. Thus, it is possible that the improved growth of fish fed full-fat meal diet might partially be due to the functional properties of BSFL protein, lipid, and exoskeleton fractions. However, in contrast to the present results, replacing 6.25-12.5% of dietary protein with full-fat BSFL meal had no effect on salmon growth rate, whereas replacing 25% of dietary protein had adverse effects on growth rate in our previous study (Weththasinghe *et al.*, 2021a).

Previous studies reported that feeding krill meal containing chitin or chitin from shrimp shells reduced growth in salmon (Hansen *et al.*, 2010; Karlsen *et al.*, 2017). In the present study, feeding the full-fat insect meal, defatted insect meal and insect exoskeleton diets containing 1.4% of chitin did not compromise growth performance in salmon, suggesting that this level of chitin may not be sufficient to cause negative effects on fish performance. Similarly, in previous studies, the presence of up to 1.2% and 2.1% of BSFL chitin in diets did not reduce growth

rate in salmon (Weththasinghe *et al.*, 2021a) and rainbow trout (*Oncorhynchus mykiss*) (Renna *et al.*, 2017; Terova *et al.*, 2019), respectively. The replacement of dietary protein with de-chitinised insect meal supported higher growth rate of fish, but decreased compared to full-fat insect meal, indicating BSFL chitin might have a positive effect on growth rate of salmon. The present results also showed that dietary inclusion of 7.2% insect exoskeleton improved FCR in fish than full-fat meal, which further confirmed that the exoskeleton fraction of BSFL might not have negative impact in salmon. On the other hand, it should be noted that the improved growth rate in fish fed de-chitinised meal diet can also be due to higher protein: lipid ratio in this diet.

Dietary inclusion of defatted insect meal, de-chitinised insect meal and insect exoskeleton reduced protein digestibility. This might partially be attributed to the presence of chitin in these diets; 1.4% in defatted meal and exoskeleton diets and 0.5% in de-chitinised meal diet. The exoskeleton of BSFL contains non-protein N from chitin and amino acids bound to a matrix (Finke, 2007), and thus probably have a low digestibility. The chitin digestibility capacity of salmon is low (13-40%) (Olsen *et al.*, 2006), leading to increased faecal N excretion. The protein content was calculated as total N×6.25, which overestimate the protein content in the faeces and consequently underestimate the ADC of protein. In addition, chitin might reduce the availability of insect protein for protease enzymes (Henry *et al.*, 2015) or activity of protease enzymes (Belghit *et al.*, 2018; Muzzarelli, 1980). On the other hand, full-fat insect meal diet also contained similar level of chitin as defatted meal and exoskeleton diets, but the full-fat insect meal diet did not reduce the ADC of protein in the present study. The chitin may lead to a reduced protein digestibility in fish, but in accordance with the present study and other studies, limited effect on protein digestibility in salmon (Fisher *et al.*, 2020) and rainbow trout (Melenchón *et al.*, 2021) fed BSFL meal has been reported.

Despite the reduced ADC of protein, the inclusion of meals or fractions of insects did not compromise protein retention. In line with the present results, BSFL meal in diets for salmon (Weththasinghe *et al.*, 2021a) and rainbow trout (Melenchón *et al.*, 2021) did not affect protein retention. In the present study, dietary inclusion of defatted insect meal even increased digested protein retention in salmon compared to both the control diet and the full-fat meal diet. In defatted insect meal fed fish, this was also accompanied by lower FCR compared to the fish fed full-fat meal. Thus, the replacement of 15% of dietary protein with defatted insect meal gave better feed utilisation than the full-fat insect meal. This emphasised the importance of applying a defatting process as a strategy to improve the nutritional value of BSFL.

Neither the insect meals nor the fractions adversely affected ADC of lipid in the present study. In several treatments, the lipid retention values were above 100%. Similarly, Weththasinghe *et al.* (2021a) also reported lipid retention values above 100% in salmon, whereas Dumas *et al.* (2018) reported efficiency of lipid deposition values above 100% in rainbow trout, and indicated lipid synthesis outweighed lipid catabolism. The insect diets gave similar lipid retentions as the control diet, except for the de-chitinised meal diet which gave a higher lipid retention as well as LER in the present study. The lauric acid is shown a larger extent to be oxidised and a lesser extent to be deposited, resulting in reduced tissue lipid deposition (Belghit *et al.*, 2019b; Renna *et al.*, 2017) and subsequently reduced lipid retention. However, in the present study, de-chitinised meal diet with the highest proportion of lauric acid, had the highest lipid retention, indicating lipid deposited rather than oxidation. This is most likely due to the lower lipid content in this diet. On the other hand, the lower lipid retention in fish fed full-fat meal diet compared to other insect diets, might indicate a higher utilisation of energy from lipid, thus increasing the amount of dietary protein used for tissue synthesis due to a protein sparing effect (Francis and Turchini, 2017; Karalazos *et al.*, 2011) and subsequently improved fish growth.

Corresponding to the higher retention of digested protein, defatted insect meal reduced the dissolved N discharges in salmon. Furthermore, the diets containing full-fat insect meal and de-chitinised insect meal reduced faecal P excretion indicating improved P digestibility, whereas the fish fed the diet containing defatted insect meal showed higher P retention. This indicates that the P in BSFL might be more bioavailable than the P in fishmeal and plant protein sources. Thus, the present results suggest that feeding fish with diets containing full-fat, defatted or de-chitinised insect meals reduced environmental impact of salmon production by reducing either dissolved N or faecal P excretion. On the other hand, feeding diets with defatted meal, de-chitinised meal and exoskeleton also increased environmental impact by increasing faecal N excretion.

5. Conclusions

In commercial production, less processed full-fat insect meal is more resource-efficient because processing of BSFL to remove lipid or exoskeleton fractions is an additional cost. It is, thus, important to determine if further processing of BSFL is necessary. In the present study, the full-fat insect meal improved feed intake and growth rate in salmon when replacing 15% of dietary protein from fishmeal and plant protein sources, although defatted meal gave better feed utilisation than full-fat meal. The present results suggest that BSFL might be optimal to use in less processed full-fat form in diets for salmon compared with processing to

remove the lipid and exoskeleton fractions. However, the inclusion of lipid-rich full-fat and de-chitinised insect meals in the diet numerically reduced hardness, expansion, and water stability of the pellets. Future studies are needed to optimise the use of BSFL in extruded fish diets.

Supplementary material

Supplementary material can be found online at https://doi.org/10.3920/JIFF2021.0094

Table S1. Fatty acid composition (% of total fatty acids) of black soldier fly larvae oil.

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Conflict of interest

The authors declare no competing conflicts of interest.

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