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Full-fat black soldier fly larvae (*Hermetia illucens*) meal and paste in extruded diets for Atlantic salmon (*Salmo salar*): Effect on physical pellet quality, nutrient digestibility, nutrient utilization and growth performances

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ABSTRACT

The present study investigated the effect of graded levels of black soldier fly larvae (BSFL) (*Hermetia illucens*) meal and paste on physical pellet quality, digestibility and utilization of nutrients and growth performances in extruded diets for Atlantic salmon (*Salmo salar*). A total of 1260 Atlantic salmon with 34 g of mean initial weight were randomly distributed into 21 fiberglass tanks and fed with one of seven isonitrogenous, isolipidic and isoenergetic diets for seven weeks. The experimental diets consisted of a control diet based on fishmeal, soy protein concentrate, corn gluten, faba bean and fish oil (Control-1); three diets with increased levels of full-fat BSFL meal, substituting 6.25% (6.25IM), 12.5% (12.5IM) and 25% (25IM) of the protein content of Control-1; two diets with increased levels of full-fat BSFL paste, substituting 3.7% (3.7IP) and 6.7% (6.7IP) of protein from Control-1 and an extra control diet with 0.88% of formic acid (Control-2). Pellet durability and hardness were overall high for all diets. However, the expansion, sinking velocity and water stability of feed pellets were lower with increased inclusion of BSFL meal and paste. Dietary inclusion of BSFL meal or paste did not affect the feed intake of fish. Further, replacing the protein content of the control diet with up to 12.5% and 6.7% of BSFL meal and paste, respectively, did not compromise fish growth rate or feed conversion ratio, although polynomial contrast analysis showed that increasing BSFL meal level in the diet linearly ($p < .05$) decreased these parameters. However, apparent digestibility coefficient (ADC) of protein and lipid, protein efficiency ratio and lipid retention were reduced linearly ($p < .05$) with increasing inclusion level of BSFL meal. Further, increasing dietary levels of BSFL paste linearly ($p < .05$) reduced ADC of protein, protein efficiency ratio and phosphorous retention. Despite the decreased ADC of protein, protein retention was not compromised by the inclusion of BSFL meal or paste. Replacement of 25% of dietary protein with BSFL meal decreased ($p < .05$) growth rate, accompanied by lower ($p < .05$) ADC and utilization of lipids and protein efficiency ratio. The present study showed that BSFL meal and paste could replace up to 12.5% and 6.7% of dietary protein, respectively, without compromising growth performance in Atlantic salmon.

1. Introduction

In recent years, insects have received growing attention as a sustainable ingredient for aquafeed production (Henry et al., 2015; Makkar et al., 2014; Nesic and Zagon, 2019) although the production of insects in sufficient volumes to compete with fishmeal and plant protein sources is yet to be achieved (Sogari et al., 2019). The production of insects has environmental benefits such as lower greenhouse gas and ammonia emissions (Oonincx et al., 2010), high land use efficiency (Alexander et al., 2017) and efficient nutrient conversion (Oonincx et al., 2010; van Huis, 2013). The feed conversion ratio (FCR) of insects fed food by-products ranged from 1.4 to 19.1 and nitrogen (N) conversion efficiency ranged from 22 to 87% depending on the insect species and growth media (Oonincx et al., 2015). The use of processed

Abbreviations: FCR, feed conversion ratio; N, nitrogen; BSFL, black soldier fly larvae; AA, amino acids; Ca, calcium; DM, dry matter; SPC, soy protein concentrate; ADC, apparent digestibility coefficient; WHC, water holding capacity; FA, fatty acids; Y, yttrium; Mg, magnesium; K, potassium; Na, sodium; P, phosphorous; SME, specific mechanical energy; SGR, specific growth rate; FBW, final body weight; PER, protein efficiency ratio; LER, lipid efficiency ratio; SFA, saturated fatty acids

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insects in feed for aquaculture animals was recently allowed by the European Commission (Regulation 2017/893/EC, 2017), which promotes upscaling of this novel feed ingredient.

One of the most promising insect species for feed purposes is black soldier fly larvae (BSFL) (*Hermetia illucens*) (van Huis, 2013). BSFL efficiently convert low-grade organic matter to high-quality nutrients (Diener et al., 2009). As shown by Oonincx et al. (2015), the N conversion efficiency can reach up to 43–55% in black soldier fly fed diets composed of food by-products. BSFL contain a moderate level of protein (31–59%) and has an amino acid (AA) profile closer to fishmeal and superior to soybean meal (Barroso et al., 2014; Makkar et al., 2014; Nogales-Mérida et al., 2019). In addition, BSFL is a good source of lipid (11–49%) (Makkar et al., 2014; Nogales-Mérida et al., 2019) and minerals, particularly calcium (Ca) (Finke, 2013; Makkar et al., 2014).

BSFL have successfully been used as a protein and lipid source in diets for Atlantic salmon (*Salmo salar*) reared in freshwater and seawater. Dietary inclusion of dried, defatted and chitin-reduced BSFL meal (60%) combined with BSFL oil (4.8%) (Belghit et al., 2018) and dried BSFL meal (10–20%) (Fisher et al., 2020) resulted in similar growth performance when compared with fishmeal and other protein sources in Atlantic salmon pre-smolts. Similar findings have also been shown in Atlantic salmon post-smolts when feeding partially defatted, dried BSFL meal (5–15%) (Belghit et al., 2019b; Lock et al., 2016). Hence, previous research demonstrated that dried or partially defatted BSFL meal, has potential as an alternative protein source in salmon feeds.

When considering commercial production, the processing of insect meal, particularly defatting is an additional cost. Thus, the use of whole BSFL meal is more cost-efficient. The use of full-fat insect meals can be a challenge to the feed industry due to its high lipid content that can interfere with the extrusion process (Lin et al., 1997), hence reducing the pellet quality (Sørensen et al., 2009). Thus, processing of BSFL biomass into partially defatted protein-rich meal has become a common practice, which allow high inclusion levels of insect meal in fish diets without reducing the technical quality of extruded diets (Dumas et al., 2018). Several studies have used full-fat dried BSFL meal in pelleted diets for rainbow trout (Sealey et al., 2011) and yellow catfish (Xiao et al., 2018), and extruded diets for rainbow trout (Józefiak et al., 2019b) and Siberian sturgeon (Józefiak et al., 2019a). However, none of them reported information on feed processing conditions or the impact of full-fat BSFL meal on extruder parameters and physical pellet quality.

High-temperature processing can reduce the nutritional quality of protein feed resources (Ljøkjel et al., 2000; Opstvedt et al., 1984; Opstvedt et al., 2003). Thus, processing BSFL at low temperatures to produce a paste, maintaining the nutritional value and reducing the production cost, could be beneficial for the aquaculture industry. As reported by Xu et al. (2020) feeding diets containing undried BSFL pulp (4.4–17.5%) to mirror carp did not affect growth rate and FCR. To our knowledge, there is no literature available regarding the use of undried full-fat BSFL ingredients in salmon diets.

Therefore, the present study used two types of BSFL; full-fat dried BSFL meal and BSFL paste (ground frozen BSFL preserved with formic acid) and investigated their effect on nutrient digestibility, nutrient utilization and growth performances when added in graded levels in extruded diets for Atlantic salmon pre-smolts. In addition, the effect of increasing levels of BSFL meal or paste on extruder parameters and physical pellet quality was also investigated.

2. Materials and methods

2.1. Experimental diets

BSFL meal and BSFL paste were produced at HiProMine S.A., Poznań, Poland. The BSFL feed was normalized in terms of dry matter (DM) content by the addition of wheat middlings (17%) to fresh vegetables and fruit mix, consisting of apples (15%), carrots (50%), potatoes (15%), and cabbage (20%) and established at the level of 22% DM. Fresh vegetable and fruit pre-consumer waste was ground (2000 rpm/1 min, (HPM milling system, 55 kw, Poland) to pass 2 mm screen and offered *ad libitum* to BSFL. Substrates were not contaminated by any animal products in accordance with EC regulation (no 1069/09).

At the prepupal stage (10th day of rearing), larvae were harvested, sieved through 3 mm screen and washed with water on drum separator at 90 °C for 10 min (HPM cleaning system, Poland). A batch of BSFL was divided into two parts and frozen at −50 °C to produce BSFL paste or dried for meal. The BSFL were dried first at 130 °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., Poznań, Poland) to produce BSFL meal. In the case of BSFL paste, BSFL were ground to pass 4 mm screen on continue flow homogenizer (HPM milling system, 25 kw, Poland) and preserved with formic acid (2.5%). The analyzed chemical composition of BSFL meal and paste is shown in Table 1.

Seven isonitrogenous, isolipidic and isoenergetic diets were formulated. The diets were formulated to meet or exceed NRC (2011) requirements for all indispensable AA and other nutrients for Atlantic salmon. The experimental diets consisted of a control diet based on fishmeal, soy protein concentrate (SPC), corn gluten, faba bean and fish oil (Control-1); three diets with increasing levels of full-fat BSFL meal, substituting 6.25% (6.25IM), 12.5% (12.5IM) and 25% (25IM) of the protein content of Control-1. In addition, two diets with increasing levels of full-fat BSFL paste, substituting 3.7% (3.7IP) and 6.7% (6.7IP) of the protein content Control-1 and an extra control with 0.88% of formic acid (Control-2) were evaluated. The diet Control-2 was included as a control for BSFL paste diets, since the BSFL paste was preserved with formic acid. Yttrium oxide was included in all the diets as an internal

Table 1
Analyzed chemical composition (%, dry matter) of black soldier fly larvae (BSFL) meal and paste.

Nutrient	BSFL meal	BSFL paste
Dry matter (%)	90.5	23
Crude protein	42	40.5[a]
Crude lipid	32	34.2[b]
Ash	9.25	
Formic acid	0	2.47
Total phosphorous	0.86	
Calcium	1.92	
Magnesium	0.27	
Chitin	8	
Amino acids[c]		
Essential amino acids		
Methionine	0.63	0.57
Threonine	1.34	1.26
Valine	1.9	1.62
Isoleucine	1.48	1.37
Leucine	2.9	2.17
Phenylalanine	1.48	1.43
Histidine	0.88	1.11
Lysine	2.12	2.28
Arginine	1.65	1.42
Tryptophan	0.77	0.12
Non-essential amino acids		
Cysteine	0.25	0.26
Aspartic acid	3.18	2.9
Serine	1.23	1.29
Glutamic acid	4.25	4.12
Proline	1.92	1.97
Glycine	1.53	1.53
Alanine	2.45	2.04
Tyrosine	1.84	2.77
Total amino acids	31.8	30.2

[a] Corresponds to 9.32% in BSFL paste.
[b] Corresponds to 7.87% in BSFL paste.
[c] Water corrected values.

Table 2
Ingredient and analyzed chemical composition of experimental diets with increased inclusion level of black soldier fly larvae (BSFL) meal and paste.[1]

	Control-1	6.25IM	12.5IM	25IM	Control-2	3.7IP	6.7IP
Ingredients (%)							
Fish meal[a]	25	23.24	21.48	17.69	25	20.27	16.62
SPC[b]	35.5	33.45	30.92	25.58	35.5	29.18	23.92
Corn gluten[c]	4	3.72	3.44	2.59	4	3.24	2.66
Faba bean[d]	1.85	1.72	1.59	1.03	1.85	1.5	1.23
BSFL meal[e]	0	8.07	16.13	32.27	0	0	0
BSFL paste[f]	0	0	0	0	0	19.8	35.12
Wheat flour[g]	13.64	13.64	13.64	13.64	13.64	11.91	10.56
Wheat bran[h]	4	2.47	1.28	0	3.12	2.16	0.98
Fish oil[i]	15	12.68	10.51	6.19	15	11.06	8.13
Formic acid[j]	0	0	0	0	0.88	0	0
Yttrium oxide[k]	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Vit/min premix[l]	0.65	0.65	0.65	0.65	0.65	0.57	0.5
Methionine[m]	0.2	0.2	0.2	0.2	0.2	0.17	0.15
Choline chloride[n]	0.15	0.15	0.15	0.15	0.15	0.13	0.12
Chemical composition (%, as is)							
Dry matter	92.9	92.5	91.5	90.8	91.1	91.9	91.9
Crude protein	46.8	47.4	46.4	45.7	45.8	47.2	47.9
Crude lipid	14.6	15.5	17.2	15.9	16.2	13	13.3
Starch	12.3	12.4	11.5	11.5	11.6	12	12.8
Ash	5.52	5.88	6.17	6.83	5.3	5.67	6.08
Formic acid	0	0	0	0	0.72	0.58	1.1
Gross energy (MJ kg^{-1})	21.9	21.7	21.7	21.5	21.6	21.4	21.1
Macro mineral composition (%, as is)							
Total phosphorous	0.91	0.89	0.87	0.88	0.91	0.9	0.89
Calcium	0.93	1.02	1.1	1.21	1.01	1.06	1.05
Magnesium	0.1	0.13	0.12	0.15	0.11	0.11	0.12
Potassium	0.52	0.62	0.66	0.66	0.46	0.58	0.61
Sodium	0.46	0.45	0.48	0.39	0.46	0.44	0.4
Amino acid composition[o] (%, as is)							
Essential amino acids							
Methionine	0.96	0.97	0.94	0.91	0.92	0.96	0.83
Threonine	1.54	1.56	1.53	1.5	1.52	1.55	1.52
Valine	1.58	1.61	1.61	1.62	1.57	1.63	1.67
Isoleucine	1.69	1.72	1.69	1.66	1.67	1.75	1.78
Leucine	3.19	3.14	3.07	2.92	3.12	3.19	3.2
Phenylalanine	1.9	1.89	1.85	1.81	1.91	1.93	1.93
Histidine	1.02	1.05	1.04	1.03	1	1.06	1.08
Lysine	2.48	2.46	2.43	2.36	2.4	2.5	2.58
Arginine	2.54	2.51	2.43	2.22	2.64	2.6	2.54
Non-essential amino acids							
Cysteine	0.44	0.44	0.43	0.39	0.43	0.45	0.46
Aspartic acid	3.98	3.97	3.84	3.71	3.85	3.99	4.12
Serine	1.77	1.78	1.72	1.66	1.76	1.79	1.82
Glutamic acid	7.45	7.34	7.11	6.6	7.2	7.42	7.49
Proline	2.15	2.08	1.96	2.06	2.07	2.16	2.19
Glycine	1.74	1.75	1.73	1.72	1.74	1.79	1.79
Alanine	1.89	1.94	1.97	2.04	1.86	1.94	1.99
Tyrosine	1.26	1.36	1.45	1.79	1.29	1.38	1.44
Total amino acids	37.58	37.57	36.8	36	36.95	38.09	38.43

[1] Control-1: Control diet. 6.25IM, 12.5IM and 25IM: BSFL meal substituted 6.25%, 12.5% and 25% of protein content of Control-1. Control-2: Control diet with 0.88% of formic acid. 3.7IP and 6.7IP: BSFL paste substituted 3.7% and 6.7% of protein content of Control-1.
[a] LT fishmeal, Norsildmel AS, Bergen, Norway
[b] Soy protein concentrate AX3®, Triple A AS, Hornsyld, Denmark.
[c] Corn gluten meal, Baolingbao Biology, Shangdong Yucheng, China.
[d] Faba beans, Norgesfôr, Oslo, Norway.
[e] Black soldier fly larvae meal, HiProMine S.A., Poznań, Poland.
[f] Black soldier fly larvae paste, HiProMine S.A., Poznań, Poland.
[g] Wheat flour 78%, batch number: 5093060546, Norgesmøllene, Bergen, Norway.
[h] Wheat bran, Norgesmøllene, Bergen, Norway.
[i] Fish oil, Norsildmel AS, Bergen, Norway.
[j] Formic acid, Ensil Maursyre 85%, Felleskjøpet, Norway.
[k] Yttrium oxide (Y_2O_3), Metal Rare Earth Limited, Shenzhen, China.
[l] Vit/min premix, Farmix, Trouw Nutrition, LA Putten, The Netherlands. Per kg of feed; retinol 2500.0 IU, cholecalciferol 32,400.0 IU, α-tocopherol SD 0.2 IU, menadione 40.000 mg, thiamine 15.0 mg, riboflavin 25.0 mg, d-Ca-pantothenate 40.002 mg, niacin 150.003 mg, biotin 3000.0 mg, cyanocobalamin 20.0 mg, folic acid 5.0 mg, pyridoxine 15.0 mg, ascorbate polyphosphate 0.098 g, Cu: Cu sulphate 5H2O 11.998 mg, Zn: Zn sulphate 89.992 mg, Mn: Mn(II) sulphate 34.993 mg, I: K-iodine 1.999 mg, Se: Na-selenite 0.200 mg, Cd Max. 0.0003 mg, Pd max. 0.028 mg, Ca 0.915 g, K 1.380 g, Na 0.001 g, Cl 1.252 g.
[m] L-methionine, Bestamino™ Cj Cheiljedang, Seoul, Korea.
[n] Choline chloride 70%, $C_5H_{14}ClNO$, 139.6 g/mol, Vilomix, Hønefoss, Norway.
[o] Water corrected values.

marker for the determination of apparent digestibility coefficient (ADC) of nutrients. Crystalline methionine was added to all the diets to ensure that the diets met or exceeded the methionine requirement of Atlantic salmon pre-smolts (0.7%, DM basis (NRC, 2011)). The ingredient and analyzed chemical composition of the experimental diets are shown in Table 2.

2.2. Production of experimental diets

The experimental diets were produced at the Norwegian University of Life Sciences (NMBU) Centre for Feed Technology (Fôrtek), Ås, Norway. The BSFL paste was ground frozen using a meat grinder (Tripas-Wexiö, RK-82, Sweden). After thawing overnight at room temperature, water was added to the paste (1 kg of water per 6 kg of paste) and ground using a pump grinder (Pedrollo TR 1.1, San Bonifacio (VR), Italy). The macro ingredients including SPC, fishmeal, corn gluten meal, wheat bran, faba beans, wheat flour, and BSFL meal were weighed and mixed with an ISDECA mixer (60-l paddle-mixer, prototype, Fôrtek, Forberg, Norway) for 2.5 min. The material mix was then ground in a small Hammer mill (Bill bliss, horizontal, 18.5 kW, USA) with a 1 mm sieve. The ground macro ingredients mixture was mixed with the micro-ingredients. BSFL paste was also added using the ISDECA mixer when producing the 3.7IP and 6.7IP diets. Formic acid was added to the Control-2 diet using the ISDECA mixer equipped with spray nozzles (nozzle type: 11004, Spraying Systems Co., Norway).

The diets were extruded in a five-section Bühler twin-screw extruder (BCTG 62/20 D, Uzwil, Switzerland) with reduced capacity, bypassing the conditioner, fitted with four 2.5 mm die holes. A small K-tron feeder was used to feed the material directly into the first extruder section. A screw configuration suitable for reduced extruder capacity was used for all the diets (Fig. 1). The screw speed was increased when the BSFL meal or paste content was increased in the diet. The pellets were dried at 60 °C using fan heaters (15KW, Inelco heaters, Dania-heater 15 kW, Fjerritslev, Denmark) for 1 h and cooled at room temperature. The dried uncoated pellets were sieved (1.6 mm screen) and the percentage of dust/broken pieces was calculated. Dried extruded pellets were vacuum coated with fish oil in Gentle Vacuum Coater (GVC) – 80 prototype (Fôrtek, Amandus-Kahl).

2.3. Fish experiment and rearing facilities

The fish experiment was conducted at the Center for Fish Research, NMBU, Ås, Norway. The experimental procedures were performed in accordance with the national guidelines for the care and use of animals (The Norwegian Animal Welfare Act and the Norwegian Regulation and Animal Experimentation). A total of 1260 Atlantic salmon (Aqua Gen Atlantic QLT-innOva SHIELD) with 34 g of mean initial weight were distributed into 21 fiberglass tanks (300 l capacity) with 60 fish per tank. The fish were kept under continuous light in recirculated freshwater with a water supply of 8.5 l min^{-1}. The average water temperature was 14.8 °C during the experimental period. Dissolved oxygen levels were kept above 7.0 mg l^{-1} in the outlet water. Triplicate tanks of salmon were fed one of seven experimental diets over a period of seven weeks. Fish were fed *ad libitum* (i.e. 10% excess) with electrically driven belt feeders twice a day for 2.5 h. Daily feed intake in each tank was quantified according to Helland et al. (1996), by collection of uneaten feed using wedge wire screens as explained by Shomorin et al. (2019). Fish weight was measured at the start and end of seven-week experimental period. Fifteen fish at the start of experiment and five fish from each tank at the end of the seven-week experimental period were randomly sampled and euthanized by a sharp blow to the head and stored at −20 °C. All sampled fish at the start of experiment and all sampled fish per tank at the end of the seven-week experimental period were pooled, homogenized and freeze dried prior to analysis of the chemical composition. After the seven-week experimental period, fish were fed the experimental diets for another two weeks for fecal collection. Fish were carefully stripped three times with seven days interval (i.e. 0, 7 and 14 days after whole body sampling) for fecal collection from the posterior intestine according to Austreng (1978). The feces were immediately weighed and stored at −20 °C prior to freeze drying. Prior to weighing, sampling and stripping, fish were anesthetized with tricaine methanesulfonate (MS-222) (80 mg l^{-1}) in small aerated tanks.

2.4. Physical pellet quality analysis

Physical quality parameters of oil-coated pellets were measured. Water stability of pellets during 30, 60 and 120 min were measured according to Baeverfjord et al. (2006). The durability of the pellets was estimated in triplicates using a Doris pellet tester (AKVAsmart, Bryne, Norway) according to Hansen et al. (2010). Hardness was measured using 15 randomly picked pellets from each diet with a Texture analyzer equipped with a 5 kg load cell (Tinius Olsen, H5KT, Salfords, England) according to Øverland et al. (2009). The width of 30 randomly selected pellets per diet was recorded using the Texture analyzer (Tinius Olsen, H5KT, Salfords, England) to determine expansion. The expansion (%) was calculated as ((Pellet width-die diameter) × die diameter^{-1}) × 100. The sinking velocity was determined by measuring the mean value of the time required for 10 randomly picked pellets to sink 1 m in 17 °C tap water. In addition, the water holding capacity (WHC) was measured according to Nguyen et al. (2015). The degree of starch gelatinization of feed was analyzed by Nofima AS, Ås, Norway, according to Kraugerud and Svihus (2011).

2.5. Chemical analysis

The feed and freeze-dried feces and fish were ground. DM content was measured by oven drying at 104 °C until a constant weight was reached. Ash content was determined by combustion at 550 °C. The N content of feed, feces and fish was analyzed by CHNS Elemental Analyzer (Vario El Cube elemental analyzer system GmbH, Hanau, Germany) and crude protein content was determined as N × 6.25. The crude protein content of BSFL meal and paste was estimated by Kjeldahl N × 6.25 according to Commission Regulation (EC) No 152/ 2009. Samples were extracted with petroleum ether and acetone (70/30) and crude lipid content was determined using an Accelerated Solvent Extractor (ASE200; Dionex Corp., Sunnyvale, CA, USA). The starch content was determined as described by McCleary et al. (1994) with some modifications. Briefly, the samples were treated with heat-stable α-amylase and amyl glucosidase-enzymes to degrade starch into glucose and glucose content was measured by a spectrometer (RX4041 Randox Daytona +, England). Gross energy was measured with PARR 1281 Adiabatic Bomb calorimeter (Parr Instruments, Moline, IL, USA) according to ISO 9831. AA except tryptophan contents were analyzed

																	Inlet		
Outlet	40	40	60	60	60	80	80	20	20	120	100	80	60	40	60	100	80	80	80
	R	R	R	R	R	R	R	L	L	R	R	R	R	R	R	R	R	R	R
										P					P		UC	UC	

Fig. 1. The screw configuration used during the extrusion of experimental diets. 20; 40; 60; 80; 100; 120: Length in cm of each screw element. R: Right. L: Left (Flow direction of each screw element). P: Polygon. UC: Undercut conveying screw element (larger channel depth than the other conveying screw elements). The arrows indicate 5 mm spacer ring and 90^0 offset between the screw elements.

according to Commission Regulation (EC) No 152/2009 on a Biochrom 30 AA Analyzer (Biochrom Ltd., Cambridge, UK). Tryptophan content was analyzed using a Dionex Ultimate 3000 HPLC system (Dionex Softron GmbH, Germering, Germany) equipped with a Shimadzu RF-535 fluorescence detector (Shimadzu Corporation, Kyoto, Japan) according to Commission Regulation (EC) No 152/2009. The fatty acid (FA) content was determined using Trace GC Ultra gas chromatograph (Thermo Fisher Scientific, US) according to O'fallon et al. (2007) by synthesizing the FA to FA methyl esters (FAME). Yttrium (Y), Ca, magnesium (Mg), potassium (K) and sodium (Na) contents were measured using a microwave plasma atomic emission spectrometer (MP-AES 4200, Agilent Technologies, USA) after acid decomposition in a microwave digestion system (Start D, Milestone Srl, Italy). Total phosphorous (P) content was analyzed using a commercial spectrophotometric kit (PH8328, Randox laboratories, County Antrim, UK) after combustion and acid digestion according to Commission Regulation (EC) No 152/2009. The chitin content of BSFL meal was measured according to Finke (2007). The formic acid content in BSFL paste, Control-2 diet and diets containing BSFL paste was determined using HPLC-UV at Eurofins Agro Testing Norway AS.

2.6. Calculations

Specific mechanical energy (SME) (Wh kg^{-1}) was calculated as $(2 \times \pi \times 60^{-1}) \times (S_{rpm} \times T_{knm} \times T_{t/h}{}^{-1})$, where S_{rpm} is screw speed, T_{knm} is Torque and $T_{t/h}{}^{-1}$ is throughput. Specific growth rate (SGR) (% body weight day^{-1}) was calculated as [(ln (Final body weight (FBW) (g $fish^{-1}$)) – ln (Initial body weight (g $fish^{-1}$)))/ Experimental period (days)] × 100%. Feed intake (g DM $fish^{-1}$) was calculated as Total feed intake (g DM $tank^{-1}$)/Number of fish per tank. FCR (g g^{-1}) was calculated as Feed intake (g DM $fish^{-1}$)/ (FBW (g $fish^{-1}$) - Initial body weight (g $fish^{-1}$)). ADC of nutrients (%) was calculated as (1- [(Y concentration in diet/Y concentration in feces) × (Nutrient concentration in feces/Nutrient concentration in diet)]) × 100. Fecal excretion of minerals and N (%) was calculated as (100 – ADC of minerals or N). The dissolved N fraction (g kg^{-1} fish body weight gain) was calculated as (([Feed intake (g $fish^{-1}$) × N content in feed (%)/100] × ADC of N/100) – ([(FBW (g) × Final N content in fish (g g^{-1})) – (Initial body weight (g) × Initial N content in fish (g g^{-1}))])/ ([FBW (g $fish^{-1}$) - Initial body weight (g $fish^{-1}$)]/1000). Protein and lipid efficiency ratios (g g^{-1}) were calculated as (FBW (g $fish^{-1}$) - Initial body weight (g $fish^{-1}$))/ [Feed intake (g $fish^{-1}$) × Protein or lipid content in feed (%)/100]. Apparent nutrient retention (% intake) was calculated as [(FBW (g) × Final nutrient content in fish (g g^{-1})) – (Initial body weight (g) × Initial nutrient content in fish (g g^{-1}))]/ [Feed intake (g $fish^{-1}$) × Nutrient content in feed (%)/100] × 100.

2.7. Statistical analysis

The data were analyzed using one-way ANOVA, followed by a Tukey's test for comparison of means. Differences at $p < .05$ were considered as significant. Linear and quadratic polynomial contrasts were used to evaluate the relationship between different parameters and dietary BSFL meal or BSFL paste levels as indicated in tables. The chosen level of significance was $p < .05$ and threshold level of tendency was $p < .1$. All the statistical analyses were performed using IBM SPSS Statistics 26 software.

3. Results

3.1. Feed production and pellet quality

The extruder parameters used during the production of experimental diets are shown in Table 3. The full-fat BSFL meal increased the lipid content and the BSFL paste increased the moisture content of the feed mash prior to extrusion. To compensate this, the screw speed of the extruder was increased to obtain pellet with desirable expansion and physical quality. Despite the increased screw speed, decreased die pressure and torque were observed, which resulted in a decreased SME. In addition to increased screw speed, the water added to the BSFL paste diets in the extruder was reduced.

Inclusion of BSFL meal and paste in the diets numerically increased the amount of fines before coating. Pellet durability and hardness were overall high for all diets, but with a numerical reduction in durability for the diet with 12.5% BSFL meal replacement (Table 4). The expansion, sinking velocity and water stability of pellets after 30, 60 and 120 min were numerically lower with increased inclusion of both BSFL meal and paste (Table 4 and Fig. 2). Starch gelatinization varied among the experimental diets, ranging from 54.4 to 95.2%, but with high analytical variation within the treatments.

3.2. Fish performance

Only one fish died throughout the experimental period. Fish fed the BSFL meal diets had similar feed intake compared to Control-1. According to the linear polynomial contrasts, FBW and SGR reduced ($p < .05$) with increasing dietary BSFL meal level. According to ANOVA, there were no differences in FBW and SGR between the fish fed 6.25IM and 12.5IM diets and the fish fed Control-1 diet, while FBW and SGR were lower ($p < .05$) in fish fed 25IM diet than fish fed Control-1 diet. FCR of fish fed the BSFL meal diets were not different from the fish fed Control-1, though it was lower ($p < .05$) in 12.5IM than 25IM diet. In addition, a linear relationship ($p < .05$) was observed between FCR and BSFL meal level in the diet (Table 5).

In contrast, 3.7IP and 6.7IP diets did not affect the growth performances of fish compared to Control-1 or Control-2. A positive linear tendency ($p = .08$) was, however, observed between dietary BSFL paste level and FCR of fish. Growth performances did not differ between the two controls (Table 5).

3.3. Digestibility of nutrients, fecal excretion of minerals and dissolved fraction of N

Although ADC of protein and energy in BSFL meal diets did not differ from Control-1, ADC of protein decreased linearly ($p < .05$) with increasing dietary BSFL meal level. ADC of lipid was lower ($p < .01$) for the 12.5IM and 25IM diets compared to the Control-1, whereas ADC of starch was highest ($p < .01$) for the 25IM diet. Furthermore, a negative liner relationship ($p < .001$) was found between dietary BSFL meal level and ADC of lipid, and a positive linear relationship ($p < .01$) was found in ADC of starch (Table 6).

ADC of AA, except tyrosine in the BSFL meal diets did not differ from the Control-1. Tyrosine digestibility was lower ($p < .01$) in 12.5IM and 25IM diets when compared with the Control-1. It was also observed linear reductions ($p < .05$) of the ADC of phenylalanine, histidine, lysine and tyrosine with increasing level of BSFL meal in the diet. The same trend was observed for ADC of arginine ($p = .05$) and cysteine ($p = .07$). However, the ADC of total AA was unaltered by the dietary inclusion of BSFL meal (Table 7).

The ADCs of nutrients in salmon fed the BSFL paste diets did not differ from fish fed the Control-1 or the Control-2 diets. However, negative linear effects ($p < .05$) were observed between dietary BSFL paste level and ADCs of protein and energy (Table 6).

Fecal excretion of P and Mg were not affected by dietary inclusion of BSFL meal. Fecal Ca and K excretions were lower ($p < .05$) in 25IM diet and 12.5IM diet respectively, compared with Control-1. Further, linear relationships ($p < .05$) were observed between fecal Ca and K excretions and dietary BSFL meal level (Table 6).

Fecal P excretion increased linearly ($p < .001$) with increasing level of BSFL paste in the diet and BSFL paste diets showed higher ($p < .01$) P excretion than Control-2. Linear ($p < .05$) and quadratic ($p < .05$) relationships were observed between fecal Ca and Mg

Table 3
Extruder parameters during the production of experimental diets with increased inclusion level of black soldier fly larvae (BSFL) meal and paste.[1]

Extruder parameter	Control-1	6.25IM	12.5IM	25IM	Control-2	3.7IP	6.7IP
Throughput (kg h^{-1})	54.3	54.3	54.3	54.7	54.3	46.8	43.0
Barrel 1 (°C)	38.9	31.4	29.9	34.3	40.3	35.0	34.2
Barrel 2[a] (°C)	61.2	47.1	47.9	68.9	63.6	62.3	56.5
Barrel 3[a] (°C)	108	96.6	92.5	96.1	97.2	112	109
Barrel 4[a] (°C)	117	118	115	104	112	114	112
Barrel 5[b] (°C)	75.1	64.2	58.7	53.8	79.9	84.7	85.2
Die temperature (°C)	96.5	92.5	90.5	78.5	92.0	92.5	94.0
Die pressure (Bar)	29.5	20.2	16.2	1.35	22.8	6.80	3.95
Screw speed (rpm)	270	290	345	475	275	400	400
Torque (Nm)	339	270	226	103	277	180	156
Drive power (kW)	9.30	8.25	8.10	5.10	7.75	7.50	6.50
SME[c] (Wh kg^{-1})	177	151	150	93.6	147	161	152
Water addition[d] (kg h^{-1})	14.0	14.0	14.0	15.5	14.0	6.50	0
Lipid[e] (%)	2.67	5.04	7.64	12.6	2.67	4.32	6.14

[1] Control-1: Control diet. 6.25IM, 12.5IM and 25IM: BSFL meal substituted 6.25%, 12.5% and 25% of protein content of Control-1. Control-2: Control with 0.88% of formic acid. 3.7IP and 6.7IP: BSFL paste substituted 3.7% and 6.7% of protein content of Control-1.
[a] Heating around these sections of the extruder barrel.
[b] Cooling around this section of the extruder barrel.
[c] Specific mechanical energy.
[d] Water added into the extruder.
[e] Percentage of lipid in the feed mash prior to extrusion, on dry matter basis.

Table 4
Physical pellet quality of experimental diets with increased inclusion level of black soldier fly larvae (BSFL) meal and paste.[1]

Pellet quality parameter	Control-1	6.25IM	12.5IM	25IM	Control-2	3.7IP	6.7IP	SEM[2]
Fines before coating (%)	0.25	1.31	4.08	1.93	0.25	0.62	0.73	
Durability (%)	99.1	98.9	96.3	98.8	99.6	99.0	98.6	0.28
Hardness (N)	35.5	37.0	35.5	31.3	46.4	32.7	34.4	0.66
Expansion (%)	13.8	6.9	3.8	−9.0	12.2	3.6	−2.9	0.66
Sinking velocity (m S^{-1})	0.09	0.10	0.08	0.06	0.09	0.07	0.07	0.00
Water holding capacity (g wet/g dry)	2.59	2.48	2.53	2.36	2.58	2.10	1.84	0.06
Gelatinization (%)	72.7	95.2	75.8	84.2	83.0	54.4	67.9	3.75

[1] Control-1: Control diet. 6.25IM, 12.5IM and 25IM: BSFL meal substituted 6.25%, 12.5% and 25% of protein content of Control-1. Control-2: Control diet with 0.88% of formic acid. 3.7IP and 6.7IP: BSFL paste substituted 3.7% and 6.7% of protein content of Control-1.
[2] Standard error mean.

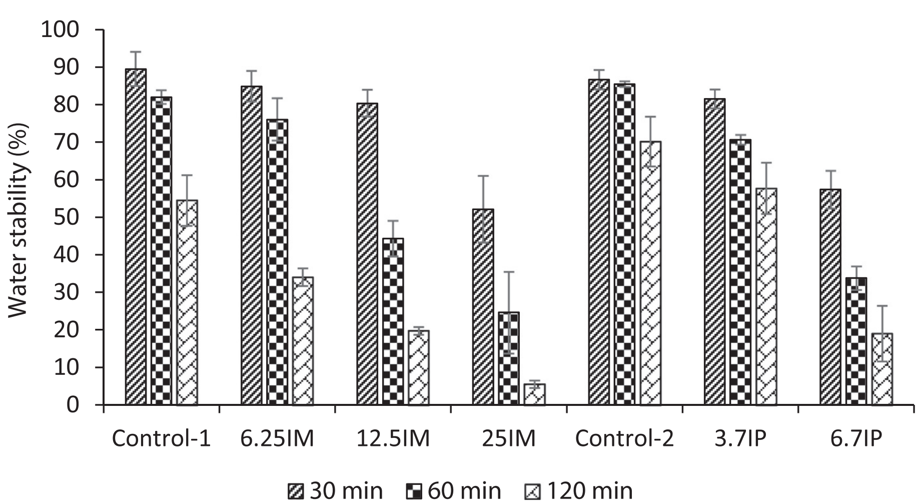

Fig. 2. Water stability (dry matter retention %) of pellets with increased inclusion of black soldier fly larvae (BSFL) meal and paste after 30, 60 and 120 min. Error bars indicate standard deviation. Control-1: Control diet. 6.25IM, 12.5IM and 25IM: BSFL meal substituted 6.25%, 12.5% and 25% of protein content of Control-1. Control-2: Control diet with 0.88% of formic acid. 3.7IP and 6.7IP: BSFL paste substituted 3.7% and 6.7% of protein content of Control-1.

excretions and dietary BSFL paste level. 6.7IP diet had higher ($p < .01$) Ca excretion compared to Control-2 (Table 6).

The dietary inclusion of BSFL meal or paste did not affect the dissolved N discharges, whereas fecal N excretion increased linearly ($p < .05$) when increasing the level of BSFL meal and paste in the diet (Table 6).

3.4. Nutrient utilization

The protein efficiency ratio (PER) linearly decreased ($p < .05$) with increasing BSFL meal level and 25IM diet showed a lower ($p < .05$) PER than Control-1, but dietary inclusion of BSFL meal did not affect the apparent protein retention in fish. Lipid efficiency ratio (LER) was lower ($p < .01$) in all BSFL meal diets compared to Control-1. Further, linear ($p < .001$) and quadratic ($p < .001$) relationships were

Table 5
Performance of fish fed experimental diets with increased inclusion level of black soldier fly larvae (BSFL) meal and paste.[1]

									Comparison 1 – BSFL meal diets[2]			Comparison 2 – BSFL paste diets[2]		
Performance indicator	Control-1	6.25IM	12.5IM	25IM	Control-2	3.7IP	6.7IP	SEM[3]	p_{ANOVA}[4]	p_{linear}[4]	p_{quad}[4]	p_{ANOVA}[5]	p_{linear}[5]	p_{quad}[5]
Initial body weight (g)	34.4	34.3	34.3	34.3	34.3	34.4	34.3	0.03	0.79	0.36	0.89	0.75	0.60	0.59
Final body weight (g)	94.4^{a}	92.4^{ab}	93.9^{a}	89.1^{b}	85.2	94.8	89.3	1.13	0.03	0.01	0.3	0.23	0.43	0.18
Specific growth rate (%)	2.25^{a}	2.20^{ab}	2.24^{ab}	2.12^{b}	2.02	2.25	2.12	0.03	0.04	0.014	0.33	0.28	0.47	0.22
Feed intake (g)	46.4	45.4	45.5	44.3	37.5	46.1	44.1	0.87	0.65	0.25	0.99	0.069	0.10	0.16
Feed conversion ratio	0.77^{ab}	0.78^{ab}	0.76^{b}	0.81^{a}	0.74	0.76	0.81	0.01	0.028	0.023	0.06	0.18	0.08	0.64

p_{ANOVA}: p value for one-way ANOVA. Values in the same row of Control-1, 6.25IM, 12.5IM and 25IM diets (Comparison 1) with different superscripts (a-b) are statistically different ($p < .05$) according to Tukey's multiple comparison test. p_{linear} and p_{quad} are the p values of linear and quadratic components of the polynomial contrast analysis between each performance indicator and BSFL meal/paste protein level in the diet: Control-1 was excluded in the polynomial contrast analysis of BSFL paste diets (Comparison 2).

[1] Control-1: Control diet. 6.25IM, 12.5IM and 25IM: BSFL meal substituted 6.25%, 12.5% and 25% of protein content of Control-1. Control-2: Control diet with 0.88% of formic acid. 3.7IP and 6.7IP: BSFL paste substituted 3.7% and 6.7% of protein content of Control-1.

[2] Two group comparisons were conducted: Comparison 1, between Control-1, 6.25IM, 12.5IM and 25IM diets; Comparison 2, between Control-1, Control-2, 3.7IP, and 6.7IP.

[3] Standard error mean.

[4] p values for Comparison 1.

[5] p values for Comparison 2.

observed between the LER and dietary BSFL meal level. Apparent lipid retention and apparent energy retention decreased linearly ($p < .01$) with increasing dietary BSFL meal level, where 25IM diet showed the lowest ($p < .05$) retentions (Table 8).

PER decreased linearly ($p < .05$) with increasing level of BSFL paste in the diet, and PER was lower ($p < .05$) in the 6.7IP diet than in the Control-2 diet. However, apparent protein retention was not affected by dietary inclusion of BSFL paste. The 3.7IP diet showed higher LER ($p < .05$) compared to the two controls and higher apparent lipid retention ($p < .01$) compared to Control-2. Linear ($p < .05$) and quadratic ($p < .01$) relationships were also observed between LER and apparent lipid retention and the level of BSFL paste in the diet. Further, apparent P retention decreased linearly ($p < .05$) when increasing dietary BSFL paste level (Table 8).

4. Discussion

4.1. Feed production and physical pellet quality

Adding the BSFL meal and paste to the diets led to an increased lipid and moisture content in the mash prior to extrusion, respectively. Lipids act as lubricants, therefore a high lipid level in extrusion increases the lubrication and reduces the friction in the extruder (De Pilli et al., 2015; Ilo et al., 2000; Lin et al., 1997), resulting in a decreased dough

Table 6
Apparent digestibility coefficient (ADC) of nutrients (%), fecal excretion of minerals and nitrogen (%) and dissolved fraction of nitrogen (g kg^{-1} fish body weight gain) of fish fed experimental diets with increased inclusion level of black soldier fly larvae (BSFL) meal and paste.[1]

									Comparison 1 – BSFL meal diets[2]			Comparison 2 – BSFL paste diets[2]		
	Control-1	6.25IM	12.5IM	25IM	Control-2	3.7IP	6.7IP	SEM[3]	p_{ANOVA}[4]	p_{linear}[4]	p_{quad}[4]	p_{ANOVA}[5]	p_{linear}[5]	p_{quad}[5]
Apparent digestibility coefficients														
Crude protein	87.2	86.9	84.7	83.3	89.6	88.9	86.7	0.55	0.12	0.027	0.92	0.33	0.03	0.36
Crude lipid	95.2^{a}	94.6^{a}	91.3^{b}	88.0^{c}	96.0	95.2	94.5	0.62	< 0.001	< 0.001	0.76	0.54	0.12	0.91
Starch	64.3^{b}	65.1^{b}	66.5^{b}	70.4^{a}	63.2	66.3	62.7	0.60	0.004	0.001	0.39	0.07	0.81	0.018
Energy	77.1	77.1	75.8	75.4	80.0	79.6	77.1	0.51	0.717	0.31	0.93	0.30	0.045	0.27
Fecal excretion of minerals and nitrogen														
Phosphorous	49.1^{X}	48.0	51.2	44.6	39.8^{Y}	44.6^{XY}	50.6^{X}	1.28	0.68	0.46	0.49	0.004	< 0.001	0.4
Calcium	$105^{a,\ X}$	98.1^{ab}	95.9^{ab}	92.7^{b}	80.8^{Y}	81.1^{Y}	99.3^{X}	2.06	0.017	0.004	0.17	< 0.001	0.004	0.025
Magnesium	53.7	43.4	52.9	47.5	46.2	43.1	53.2	1.24	0.11	0.47	0.66	0.08	0.038	0.016
Potassium	13.7^{a}	9.77^{ab}	8.80^{b}	8.82^{ab}	10.9	9.03	9.95	0.47	0.036	0.02	0.052	0.09	0.31	0.14
Nitrogen	12.8	13.1	15.3	16.7	10.4	11.1	13.3	0.55	0.12	0.027	0.92	0.33	0.03	0.36
Dissolved fractions														
Nitrogen	22.2	22.7	19.9	22.2	20.0	23.2	24.2	0.54	0.26	0.78	0.22	0.34	0.14	0.70

p_{ANOVA}: p value for one-way ANOVA. Values in the same row that share same superscripts are not statistically different ($p > .05$) according to Tukey's multiple comparison test. The letters a-c denote significant differences among Control-1, 6.25IM, 12.5IM and 25IM diets (Comparison 1), whereas the letters X-Y denote significant differences among Control-1, Control-2, 3.7IP, and 6.7IP diets (Comparison 2). p_{linear} and p_{quad} are the p values of linear and quadratic components of the polynomial contrast analysis between each parameter and BSFL meal/paste protein level in the diet: Control-1 was excluded in the polynomial contrast analysis of BSFL paste diets (Comparison 2).

[1] Control-1: Control diet. 6.25IM, 12.5IM and 25IM: BSFL meal substituted 6.25%, 12.5% and 25% of protein content of Control-1. Control-2: Control diet with 0.88% of formic acid. 3.7IP and 6.7IP: BSFL paste substituted 3.7% and 6.7% of protein content of Control-1.

[2] Two group comparisons were conducted: Comparison 1, between Control-1, 6.25IM, 12.5IM and 25IM diets; Comparison 2, between Control-1, Control-2, 3.7IP, and 6.7IP.

[3] Standard error mean.

[4] p values for Comparison 1.

[5] p values for Comparison 2.

Table 7
Apparent digestibility coefficient (ADC) of amino acids (%) of fish fed experimental diets with increased inclusion level of black soldier fly larvae (BSFL) meal.[1]

Amino acid	Control-1	6.25IM	12.5IM	25IM	SEM[2]	p_{ANOVA}[3]	p_{linear}[4]
Essential amino acids							
Methionine	93.0	92.8	92.0	91.6	0.34	0.46	0.15
Threonine	85.7	86.1	84.6	84.6	0.68	0.86	0.53
Valine	89.2	89.1	87.9	87.5	0.51	0.60	0.22
Isoleucine	90.5	90.4	89.2	88.8	0.49	0.56	0.21
Leucine	91.4	91.3	90.4	89.7	0.42	0.50	0.16
Phenylalanine	91.5	90.7	89.6	88.7	0.46	0.14	0.029
Histidine	88.2	87.9	86.3	84.7	0.63	0.19	0.042
Lysine	91.3	90.6	89.4	88.4	0.51	0.17	0.036
Arginine	95.0	94.8	94.1	93.3	0.30	0.22	0.05
Non-essential amino acids							
Cysteine	75.8	77.3	73.1	70.4	1.25	0.23	0.07
Aspartic acid	81.5	82.5	81.6	82.8	0.74	0.92	0.65
Serine	87.9	88.3	87.0	86.9	0.59	0.84	0.49
Glutamic acid	91.9	92.2	91.1	90.4	0.45	0.56	0.21
Proline	89.1	89.1	87.0	87.4	0.54	0.39	0.2
Glycine	83.9	84.1	82.2	82.4	0.65	0.69	0.36
Alanine	90.0	89.9	89.0	88.8	0.44	0.75	0.34
Tyrosine	87.4[a]	83.1[ab]	78.8[bc]	75.6[c]	1.44	0.001	< 0.001
Total amino acids	89.2	89.2	87.8	87.2	0.53	0.49	0.16

[1] Control-1: Control diet. 6.25IM, 12.5IM and 25IM: BSFL meal substituted 6.25%, 12.5% and 25% of protein content of Control-1.
[2] Standard error mean.
[3] p value for one-way ANOVA. Values in the same row with different superscripts are statistically different ($p < .05$) according to Tukey's multiple comparison test.
[4] p values of linear component of the polynomial contrast analysis between each ADC and BSFL meal protein level in the diet. Only the linear component of the polynomial contrasts is shown because all quadratic contrasts were not significant.

temperature (Hansen et al., 2011; Lin et al., 1997). Similarly, increased water content in the extruder can also act as a lubricant and decrease friction, leading to reduced dough temperature (Huang et al., 1995; Lin et al., 1997). Lower dough temperature can reduce starch gelatinization (Garber et al., 1997; Lin et al., 1997; Morken et al., 2012), which results in reduced expansion (Garber et al., 1997) and physical pellet quality (Morken et al., 2012). Hence, to reduce the adverse effect of high lipid and moisture contents in the mash during extrusion, the screw speed was increased with increasing BSFL meal and paste levels in the diet.

In general, a higher screw speed creates higher SME and leads to a higher dough temperature during extrusion (Morken et al., 2012; Rolfe et al., 2001). A decreased dough temperature with increased screw speed can also occur due to a decreased filling rate of the extruder (Huang et al., 1995) or decreased residence time of the dough in the extruder, resulting in a less efficient heat transfer between the extruder barrel and the dough (Della Valle et al., 1987; Huang et al., 1995; Lin et al., 1997). A similar effect might have occurred in the present study, as indicated by the reduced SME, die pressure and torque, because the resistance to screw rotation was proportional to the filling rate (Akdogan, 1996). In addition, the low barrel and die temperature, torque and SME in the high BSFL diets indicates that increased screw speed was not optimal in compensating for the increased lipid level in the present study. Further, modification of the screw configuration during extrusion of the high BSFL containing diets could have given better results, as this has large effect on the extrusion parameters (Gogoi et al., 1996).

Starch gelatinization was reported to be reduced at high lipid (Hansen et al., 2010; Hansen et al., 2011; Lin et al., 1997) and increased screw speed (Lin et al., 1997) due to low dough temperature and hydrophobic properties of high lipid in the extruder. However, no clear relationship was found between starch gelatinization and increased inclusion of dietary BSFL meal in the present study. The decreased starch gelatinization observed with increased inclusion of BSFL paste was probably due to reduced dough temperature from the higher moisture content in feed as shown by Lin et al. (1997) and this is associated with reduction of WHC (Artz et al., 1990).

The reduced pellet expansion with increased dietary BSFL meal in the present study is in line with previous reports indicating that a decreased extrudate expansion was due to high lipid content in mash (Hansen et al., 2011; Ilo et al., 2000; Navale et al., 2015). The decreased pellet expansion in high BSFL meal diets might also be related to lower barrel temperature, in particular the fifth barrel temperature (Bandyopadhyay and Rout, 2001; Kothakota et al., 2013; Pathania et al., 2013). The decreased expansion of BSFL paste diets might be associated with the higher screw speed and moisture content of the feed mash (Bandyopadhyay and Rout, 2001). This increased level of lipid content in the feed mash followed with reduced level of SME and pellet expansion is also, most probably, explaining the reduced pellet water stability in the present study as shown by Hansen et al. (2011).

Although Sørensen et al. (2009) showed that even a small increase in lipid content might adversely affect the physical quality of extruded diets, in the present study, pellet durability and hardness were not notably reduced with the inclusion of BSFL meal or paste in the diet. Similarly, BSFL and cricket meal did not affect the pellet durability in the extruded fish feed (Irungu et al., 2018). The presence of formic acid might also contribute to this in BSFL paste diets. As reported by the others, dietary supplementation of sodium diformate (Morken et al., 2011) and potassium diformate (Morken et al., 2012) increased durability/hardness of salmonid feed pellets.

4.2. Fish performance and nutrient digestibility and utilization

Dietary inclusion of both BSFL meal and paste did not affect the palatability of Atlantic salmon diets, as indicated by the similar feed intake among diets. Similar results were shown in Atlantic salmon pre-smolts (Belghit et al., 2018) and post-smolts (Belghit et al., 2019b) and rainbow trout (Dumas et al., 2018; Renna et al., 2017) fed BSFL meal diets.

As observed for both BSFL meal and paste in the present study, low dietary inclusion of BSFL meal, did not affect growth performance in Atlantic salmon pre-smolts (10–20%) (Fisher et al., 2020) or post-smolts (5–15%) (Belghit et al., 2019b) and rainbow trout (20%) (Józefiak et al., 2019b) compared to control diets based on fishmeal and other protein sources. Xu et al. (2020) also observed that dietary

Table 8
Nutrient retention parameters in fish fed experimental diets with increased inclusion level of black soldier fly larvae (BSFL) meal and paste.[1]

Nutrient retention parameter	Control-1	6.25IM	12.5IM	25IM	Control-2	3.7IP	6.7IP	SEM[3]	Comparison 1 – BSFL meal diets[2]			Comparison 2 – BSFL paste diets[2]		
									p_{ANOVA}[4]	p_{linear}[4]	p_{quad}[4]	p_{ANOVA}[5]	p_{linear}[5]	p_{quad}[5]
Protein efficiency ratio	2.57^{a,XY}	2.50^{ab}	2.58^{a}	2.46^{b}	2.70^{X}	2.56^{XY}	2.39^{Y}	0.03	0.02	0.031	0.28	0.03	0.016	0.75
Lipid efficiency ratio	8.21$^{a, YZ}$	7.65^{b}	6.98^{c}	7.07^{c}	7.62^{Z}	9.28^{X}	8.57^{XY}	0.18	< 0.001	< 0.001	< 0.001	0.003	0.022	0.008
Apparent protein retention (%)	51.5	51.6	52.5	49.0	55.9	51.9	51.0	0.64	0.29	0.17	0.22	0.20	0.10	0.58
Apparent lipid retention (%)	114^{a,X}	103^{ab}	97.4^{ab}	93.3^{b}	103^{Y}	123^{X}	113^{XY}	2.45	0.03	0.007	0.16	0.003	0.031	0.005
Apparent energy retention (%)	56.4^{a}	56.1^{a}	56.1^{a}	51.5^{b}	57.7	55.8	55.3	0.58	0.02	0.006	0.13	0.65	0.31	0.78
Apparent phosphorous retention (%)	55.3	51.5	56.3	57.6	62.4	57.3	53.3	0.94	0.10	0.11	0.36	0.06	0.032	0.97

p_{ANOVA}: p value for one-way ANOVA. Values in the same row that share same superscripts are not statistically different (p > .05) according to Tukey's multiple comparison test. The letters a-c denote significant differences among Control-1, 6.25IM, 12.5IM and 25IM diets (Comparison 1), whereas the letters X-Z denote significant differences among Control-1, Control-2, 3.7IP, and 6.7IP diets (Comparison 2). p_{linear} and p_{quad} are the p values of linear and quadratic components of the polynomial contrast analysis between each nutrient retention parameter and BSFL meal/paste protein level in the diet: Control-1 was excluded in the polynomial contrast analysis of BSFL paste diets (Comparison 2).

[1] Control-1: Control diet. 6.25IM, 12.5IM and 25IM: BSFL meal substituted 6.25%, 12.5% and 25% of protein content of Control-1. Control-2: Control diet with 0.88% of formic acid. 3.7IP and 6.7IP: BSFL paste substituted 3.7% and 6.7% of protein content of Control-1.

[2] Two group comparisons were conducted: Comparison 1, between Control-1, 6.25IM, 12.5IM and 25IM diets; Comparison 2, between Control-1, Control-2, 3.7IP, and 6.7IP.

[3] Standard error mean.

[4] p values for comparison 1.

[5] p values for Comparison 2.

fishmeal replaced with BSFL pulp (4.4–17.5%), which contained crushed fresh larvae, did not influence the SGR or FCR of juvenile mirror carp. Yet, the highest BSFL meal inclusion level which replaced 25% of dietary protein showed lower FBW and SGR in the present study. Similarly, FBW and/or SGR were also reported to be reduced in other studies where high levels of BSFL meal were included in the diets of Atlantic salmon pre-smolts (30%) (Fisher et al., 2020) and post-smolts (25%) (Lock et al., 2016) and rainbow trout (26.4%) (Dumas et al., 2018). In contrast, dietary inclusion of high levels of BSFL meal caused no adverse effect on growth performance in Atlantic salmon (60%) (Belghit et al., 2018) and rainbow trout (20–40%) (Renna et al., 2017).

The reduction of SGR in the present and previous studies may be attributed to the presence of chitin in the BSFL. Chitin is a major component of insect cuticle (Chapman, 1998; Tharanathan and Kittur, 2003). In the present study, whole BSFL meal including the cuticle was used. The chitin content in the BSFL meal (i.e. 8% in DM basis) corresponded to a chitin level of 0.6,1.2 and 2.3% for the meal diets and 0.4 and 0.6% for the paste diets. Previous studies reported reduced SGR in juvenile turbot fed BSFL meal containing chitin (1.6–7.3%) (Kroeckel et al., 2012), Atlantic salmon fed chitin from prawn shells (1–5%) (Karlsen et al., 2017) or krill meal containing chitin (2%) (Hansen et al., 2010) and reduced weight gain in tilapia fed chitin (2–10%) (Shiau and Yu, 1999). Furthermore, feeding high levels of chitin-reduced BSFL meal had no adverse effect on growth performance of Atlantic salmon (Belghit et al., 2018).

Chitin was reported to contain around 17.1 kJ/g of energy content, which could constitute a substantial percentage of total energy intake (Gutowska et al., 2004), but, Atlantic salmon have been reported to have a poor capacity to digest chitin (13–40%) (Olsen et al., 2006). This indicates that chitin function as a filler with low digestible energy content (Karlsen et al., 2017) which might limit growth rate at high inclusion levels. In addition, the reduction in growth rate could also be a result of the reduced ADC of nutrients. In accordance with the decreased ADC of protein with increasing dietary levels of BSFL meal and paste in the present study, dietary inclusion of high levels of BSFL meal adversely affected ADC of protein/AA in salmon pre-smolt (60%) (Belghit et al., 2018) and rainbow trout (40%) (Renna et al., 2017). On the contrary, some research showed that ADC of protein and most of the AA were not affected by dietary BSFL meal inclusion in Atlantic salmon post-smolts (5–25%) (Belghit et al., 2019b; Lock et al., 2016) and rainbow trout (20%) (Dumas et al., 2018). In agreement with present results for BSFL meal diets, Belghit et al. (2018) and Belghit et al. (2019a) reported reduced ADC of lipid and most fatty acids with the inclusion of BSFL meal and oil in diets for Atlantic salmon. The lower ADC of nutrients might also be attributed to chitin, because previous studies showed that feeding diets containing chitin reduced ADC of nutrients in Atlantic salmon (Hansen et al., 2010; Karlsen et al., 2017) and tilapia (Shiau and Yu, 1999). The chitin in insect cuticle exists in a matrix with proteins, lipids and other compounds (Chapman, 1998; Kramer et al., 1995), which may reduce the access of digestive enzymes, thus reducing ADCs of nutrients (Henry et al., 2015). In addition, chitin might further reduce ADC of protein due to its capacity to bind proteins (Piccolo et al., 2017) and immobilize (Muzzarelli, 1980) or reduce the activity of proteolytic enzymes such as the brush border enzyme, leucine aminopeptidase that break down peptides into AA (Belghit et al., 2018). It has also been suggested that feeding chitin leads to decreased bile acid levels in the pylorus, and thereby reduce ADC of lipid as bile acid is essential for activation of lipase and efficient lipid absorption (Hansen et al., 2010). In addition, the FA composition of BSFL meal is presented in the supplementary table (Table A.1) showing that the majority of the FA in BSFL meal were saturated fatty acids (SFA) (65% of total FA), which might increase the SFA content in BSFL diets. High SFA dietary concentrations may also partially explain the decrease in ADC of lipid in the present study, as the ADC of lipid decreases linearly with an increasing concentration of dietary SFA. This has previously been demonstrated in salmonids (Hua and Bureau, 2009).

Based on the present results, acid detergent fiber fraction in BSFL meal contained 12% of AA, which was bound to chitin, and probably not available for digestion. The observed reduction of ADCs of several AA in the present study might be because these AA were trapped in chitin that is concealed for enzymatic digestion. Furthermore, in the analysis of AA, it appeared that the peak for tyrosine in the HPLC chromatogram was overlapped with glucosamine, which is the building block of chitin (Ng et al., 2001). Therefore, the reduced ADC of tyrosine was most likely linked to an overestimation of the tyrosine content in the feces. However, the dietary inclusion of BSFL meal did not affect the ADC of total AA although the ADC of protein was reduced. Thus, the observed reduction of ADC of protein might partially be explained by the poorly digestible chitin.

Despite the decreased ADC of protein, apparent protein retention was not compromised by the inclusion of BSFL meal and paste, probably indicating an increased utilization of digested proteins in the fish fed the BSFL diets. The similar ADC of total AA and dissolved N level among the diets might partially explain this. In addition, this might also partially be due to the content of nucleic acid in BSFL, which may have an N-sparing effect in salmon. As shown by other protein sources such as bacterial meal, nucleic acids were suggested to have an N-sparing effect and increased N retention in salmon, although the nutrient digestibility was slightly lower (Øverland et al., 2010). In line with the present results, dietary replacement of fishmeal with BSFL meal did not affect protein retention in Atlantic salmon post-smolts (Belghit et al., 2019b; Lock et al., 2016), gilthead seabream (Karapanagiotidis et al., 2014) and yellow catfish (Xiao et al., 2018). However, dietary inclusion of BSFL meal negatively affected PER and replacement of 25% of dietary protein with BSFL meal reduced PER in the present study. In contrast, replacement of fishmeal and/or plant protein with BSFL meal did not affect the PER in Atlantic salmon pre-smolts (Belghit et al., 2018) and rainbow trout (Józefiak et al., 2019b; Renna et al., 2017). Further, Fisher et al. (2020) reported even an increased PER at 30% inclusion level in Atlantic salmon pre-smolts.

The apparent lipid retention values above 100% observed in several treatments of the present study indicated lipid synthesis outweighed lipid catabolism. Both LER and apparent lipid retention were negatively affected by the dietary inclusion of BSFL meal and the effect was worse with increasing level of BSFL meal in the diet. In agreement with this, lipid retention decreased at dietary inclusion of 33% BSFL meal and higher in juvenile turbot (Kroeckel et al., 2012). In addition, two studies showed that dietary BSFL meal negatively affected the whole-body lipid composition in rainbow trout (St-Hilaire et al., 2007) and juvenile turbot (Kroeckel et al., 2012). The observed lower lipid utilization in salmon fed diets containing BSFL meal was accompanied by low ADC of lipid in these diets and can be attributed to the presence of chitin as discussed above. In addition, the most abundant SFA in BSFL was medium-chain lauric acid (40% of the total FA). Lauric acid is considered to be a good source of energy for salmonids as it seems to be oxidized to a larger extent and used less for lipid deposition, resulting in low tissue deposition (Belghit et al., 2019a; Renna et al., 2017) and subsequently reduce lipid retention and LER. The increased energy production by lauric acid might also explain the observed comparable protein retention of BSFL diets despite reduced ADC of protein due to a protein-sparing effect (Karalazos et al., 2011). Teo et al. (1989) also reported the potential protein-sparing effect of medium-chain triglycerides. In agreement with this, previous studies have also shown that dietary inclusion of medium-chain triglycerides improved N/protein retention in Atlantic salmon (Nordrum et al., 2000; Nordrum et al., 2003). In addition, protein synthesis is a highly energy requiring process (Nordrum et al., 2000) and the high energy contribution by lauric acid might, therefore, have a positive effect on protein retention. Nevertheless, the chitin and BSFL oil content in BSFL paste diets did not seem to be sufficient to cause a negative impact on ADC of lipid. However, in contrast to BSFL meal, it was observed that 3.7% replacement of dietary protein with BSFL paste improved both LER and apparent lipid retention. This might be due to improved utilization of digested nutrients when the BSFL were subjected to low temperature processing and preserved with formic acid or included in the diet at lower levels.

According to the results of present and previous studies (Finke, 2013; Fisher et al., 2020), BSFL meal is more abundant in micronutrients (P, Ca, Mg, K) and BSFL have a mineralized cuticle in which Ca is incorporated into the cuticle (Finke, 2013). In general P content in BSFL meal is lower than fishmeal (Liland et al., 2017), which was reflected by a slight reduction of P level in BSFL meal diets. But the P in insects is likely to be readily available, unlike plant-based phytate P (Finke, 2002). This might be the reason for unaltered fecal P excretion and P retention of BSFL meal diets in the present study. Similarly, whole fish P content was not altered by BSFL meal diets in Atlantic salmon pre-smolts (Belghit et al., 2018). The observed fecal Ca excretion values closer to or above 100% in the present study is most likely due to Ca uptake by fish from water. The decreased fecal Ca excretion when increasing BSFL meal level in the diet indicated that higher dietary inclusion of BSFL meal improved ADC of Ca. It has been reported that the supplementation of diets with formic acid affects the intestinal pH of rainbow trout and improves the ADC of P, Ca and Mg (Vielma and Lall, 1997). However, an opposite result was observed for BSFL paste containing formic acid, where the increased dietary level of BSFL paste increased fecal excretion of P indicated decreased ADC of P and accompanied by decreased P retention. Similarly, increased fecal Ca excretion was observed with increasing inclusion level of BSFL paste in the diet, indicating decreased ADC of Ca. The increased fecal N excretion of BSFL meal and paste diets and increased fecal P excretion of BSFL paste diets indicate an increased environment impact of low processed insect products as alternative protein sources, although the fecal P excretion of BSFL meal diets and dissolved N fraction of the BSFL meal and paste diets were similar to the control diet. Future work on further processing such as defatting and dechitinization can help alleviate potential adverse environmental effects of such insect ingredients.

5. Conclusions

The present study showed that BSFL meal and paste could replace up to 12.5% and 6.7% of dietary fishmeal and plant proteins, respectively, without compromising the growth performance or protein retention in Atlantic salmon. Nevertheless, protein and lipid digestibility, protein efficiency ratio and lipid retention decreased linearly with increasing dietary BSFL meal level, whereas increasing dietary BSFL paste level linearly decreased protein digestibility, protein efficiency ratio and phosphorous retention. At higher replacement level of 25% BSFL meal, the growth rate was reduced, accompanied by a reduction in digestibility and utilization of lipids and protein efficiency ratio.

Declaration of Competing Interest

None.

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Appendix A. Supplementary data

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