



BLACK SOLDIER FLY FULL-FAT MEAL IN ATLANTIC SALMON NUTRITION – PART A: EFFECTS ON GROWTH PERFORMANCE, FEED UTILIZATION, SELECTED NUTRIPHYSIOLOGICAL TRAITS AND PRODUCTION SUSTAINABILITY IN FRIES*

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Abstract

Black soldier fly larva meal (BSFM) application as an innovative feed material has been developed in aquaculture nutrition. However, recent scientific literature is still lacking in the case of insect meal effects on different stages of salmonid fish, and the vast majority of research is focused on farmed Atlantic salmon. Therefore, the holistic approach was undertaken in the cycle of two studies carried out: on fries and pre-smolts. This study aimed to evaluate the effect of fish meal (FM) replacement by full-fat BSFM in Atlantic salmon fry diets on the growth performance, feed utilization, crude protein and crude fat digestibility, somatic indices, histomorphology of intestines, and environmental sustainability. Additionally, pellet quality was assessed. Two experiments were performed on 360 Atlantic salmon fries – each of which lasted 60 days. For the first experiment, 240 individuals were distributed to four experimental groups: six replicates (tanks) per group and 10 fish per tank. For the second experiment, 120 individuals were distributed to four experimental groups: three replicates (tanks) per group and 10 fish per tank were used. The following groups were applied: CON – without the addition of full-fat BSFM and with 39% FM; BSFM5 – with 5% addition of full-fat BSFM and 36.1% FM; BSFM10 – with 10% full-fat BSFM and 33.3% FM; BSFM15 – with 15% addition of full-fat BSFM and 30.3% FM. The present study showed satisfactory results of BSFM inclusion at the levels of 5% and 10% as a replacement for fish meal in feeds for Atlantic salmon fries. No negative effects on growth performance, feed utilization, protein and fat digestibility, or intestinal structure were recorded. Simultaneously, insect meal inclusion in Atlantic salmon diets significantly improved the environmental sustainability of the feed. In the case of BSFM15, decreases in body weight gain, protein lipid efficiency ratio, and lipid efficiency ratio, as well as increases in feed conversion ratio, were observed. Therefore, the use of shares higher than 10% of BSFM in Atlantic salmon fries may not be recommended.

Key words: black soldier fly, Atlantic salmon nutrition, innovative feed materials, sustainability, insect meals

A wide spectrum of insect meals as a replacement for fish meal (FM) have been studied in various salmonids: rainbow trout (*Oncorhynchus mykiss*) (Cardinaletti et al., 2019; Jóźefiak et al., 2019; Bruni et al., 2020), sea trout (*Salmo trutta* m. *trutta*) (Hoffmann et al., 2020, 2021 b; Mikołajczak et al., 2020), and brown trout (Mikołajczak et al., 2022). In addition to these new innovative products, those produced from insects have been started to R&D for trout feed (Acar et al., 2021). However, in Atlantic salmon (*Salmo salar*) nutrition, the main focus is on black soldier fly larvae meal (BSFM; *Hermetia illucens*) due to the large aquaculture scale and the highest

meal availability (Belghit et al., 2019 a; Biancarosa et al., 2019; Stenberg et al., 2019; Li et al., 2020; Weththasinghe et al., 2021 a). The BSFM application as an innovative feed material has been developed in aquaculture nutrition due to its sustainability in terms of land use, water consumption, CO₂ emissions, short larval rearing period, and especially its ability to convert food waste to high-quality feed (Sprangers et al., 2017; Meneguz et al., 2018; Zarantonello et al., 2021). Additionally, in recent scientific literature, insect meals are considered a source of antimicrobial peptides (AMPs) (Xia et al., 2021), chitin (Gaudioso et al., 2021), and lauric acid (Belghit et

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al., 2019 b), which seem to be chemical compounds that positively affect the microbiome of the gastrointestinal tract and immune system.

The vast majority of research and review papers point to environmental sustainability as the main argument for the use of insects in aquaculture nutrition (Arru et al., 2019; Goyal et al., 2021). However, only a few research works focused on growth performance and feed utilization simultaneously apply calculations that would confirm the environmental balance of insects (Stejskal et al., 2020; Rawski et al., 2021). One of the simplest methods to assess the sustainability of novel protein sources is the application of the fish-in-fish-out ratio, which is the combination of an improved feed conversion ratio (FCR) and a reduction in FM and fish oil ratios (Naylor et al., 2021). Based on the data already obtained during growth trials, it is possible to easily assess the environmental sustainability of the innovative feed materials used, which in the future will allow for more effective meta-analyses in this field, as well in species other than salmonids. Additionally, there is a lack of data about full-fat BSFM usage among stages of Atlantic salmon production. Due to their natural life cycle, the species reproduces in two environments – fresh and salt waters (Hosfeld et al., 2009). In nature they undergo long-distance migrations, which is an important step in their maturity, and the change in the environment from fresh to seawater is connected with several physiological responses, a process called smoltification (Juntti and Fernald, 2016).

Considering significant changes taking place during this process in salmon physiology, it can be assumed that it is important to implement similar experimental diets in different life stages of Atlantic salmon as a continuation in a cycle of two treatments. Because the results obtained in the case of young and older individuals cannot be compared with each other in any way, it is important to create a niche in which nutritional research will be extended for the further period of salmon rearing. For instance, Lazzarotto et al. (2015) showed that the effect of applied diets in salmonids can be related to fish age. Based on this research, the early inclusion of plant-based materials may significantly influence reproduction performance and survival rates, while older fish are less susceptible to this effect. A comprehensive approach to fish nutrition, especially in terms of life stage, will help to explain alimentary factor modes of action and effects on the growth performance, feed utilization, and health of fish, especially in the case of studies on innovative feed materials. Additionally, a key factor, which is production sustainability, is omitted in most scientific papers. Therefore, the holistic approach was undertaken in the cycle of two studies carried out: on fries and pre-smolts.

The current study aimed to present the results of study performed on Atlantic salmon fries at the laboratory scale. The scientific goals of the study were to assess the possibility of dietary application of BSFM at the levels of 5, 10, and 15% as the substitution of FM, and their effects on growth performance, feed utilization, and

selected nutrphysiological traits of fish, as well as to calculate the environmental potential of this innovative feed material. To meet this question, the research hypothesis was that the usage of BSFM in diets for Atlantic salmon will not affect growth parameters, feed utilization, and designated gastrointestinal tract functions and structures, and at the same time, it will increase the environmental sustainability of fish feed.

Material and methods

Ethical statement

The experimental protocols and methods utilized during this study complied with the recommendations of Directive 2010/63/EU of the European Parliament and the Council of 22 September 2010 on the protection of animals used for scientific purposes, the Polish law of 15 January 2015 on the protection of animals used for scientific purposes (Journal of Laws 2015, item 266), and the good practices and recommendations of the National Ethics Committee for Animal Experiments and the Local Ethics Committee for Animal Experiments of Poznań University of Life Sciences. The place of experimentation was certified for animal experiments by the National Ethics Committee for Animal Experiments (based on authorization by the Ministry of Science and Higher Education – approved unit no. 0091) – the Laboratory of Inland Fisheries and Aquaculture (the Faculty of Veterinary Medicine and Animal Science of Poznań University of Life Sciences).

Insect full-fat meal preparation

Insect biomass was purchased from HiProMine S.A., Robakowo, Poland. BSF larvae were fed a mix of plant byproducts normalized in terms of dry matter (DM) content by the addition of wheat middlings (Weththasinghe et al., 2021 a). The biomass of insects was frozen at -20°C and then dried first at 130°C for 1 hour and then at 80°C for 23 hours until a constant weight was reached using a chamber air dryer (HiProMine S.A., Robakowo, Poland). The prepared insects were homogenized using a beater mill (diameter less than 0.1 mm) to obtain a full-fat meal. The chemical analyses of meals were performed in the Laboratory of the Department of Animal Nutrition (Poznań University of Life Sciences). Ingredients were analysed according to the procedures described in detail below.

Amino acid profile analyses were performed to assess the amount of protein and amino acids as well as for conversion factor establishment for BSFM. The BSFM protein content was estimated based on the amino acid content. In further calculations for the diet, the conversion factor (Kp) for BSFM was calculated according to the following formula:

$$Kp = (\text{total amino acid content} / \text{total protein content based on } N \times 6.25 \text{ analysis}) \times 6.25$$

The formula was based on the ratio of amino acids to total protein content with $N \times 6.25$ conversion, as previously established by Janssen et al. (2017). Due to the presence of chitin in insect biomass, which could lead to overestimation of protein, a calculation based on the analysis of BSFM used for the present experiment was performed, i.e.:

$$BSFM\ Kp = (412.1\ g/503.1\ g) \times 6.25 = 5.12$$

where:

412.1 g was the sum of amino acids in 1000 g of BSFM DM;

503.1 g was the total protein content in 1000 g of BSFM calculated on an $N \times 6.25$ basis;

6.25 was the traditionally used *Kp*;

5.12 was the *Kp* calculated for BSFL.

The nutritional composition and amino acid profile of the meals corrected by calculated KP are presented in Table 1.

Diet formulation and preparation

The isonitrogenous, isoenergetic, and isolipidic diets were manufactured: a control diet (CON) and three experimental diets. The arrangement of diets was as follows: CON – without the addition of full-fat BSFM and with 39% FM; BSFM5 – with 5% addition of full-fat BSFM and 36.1% FM; BSFM10 – with 10% full-fat BSFM and 33.3% FM; BSFM15 – with 15% addition of full-fat BSFM and 30.3% FM. All experimental diets were manufactured at the Experimental Station of Feed Production Technology and Aquaculture in Muchocin, Poland using a single-screw extruder (Metalchem S-60, Gliwice, Poland). The extrusion conditions were 90°C in the cylinder and 110°C in the head, 52 rpm screw speed, and a matrix size of 2 mm (Rawski et al., 2020). After drying and screening of dusty fractions, pellets were sprayed at 40°C with fish oil in the amount specified in the recipe. The diets were packed in plastic bags and stored at –18°C. The ingredient composition of the diets used in the fry experiments is presented in Table 2.

Biological material analyses

Experimental diets and faeces (sampled during digestibility trial – description below) were analysed according to the AOAC (AOAC, 2005) methodology for crude protein (976.05), ether extract (920.39), crude ash (920.153), and crude fibre (985.29). The dry matter content was analysed according to the ISO 6496 method. TiO_2 analyses were performed according to the recommendations of Myers et al. (2004). The gross energy content was analysed according to the ISO 9831 method using an adiabatic bomb calorimeter (KL 12Mn, Precyzja-Bit PPHU, Poland) standardized with benzoic acid. To determine the number of macro elements (C and P), the methodology described by Ptak et al. (2013) was applied. The amino acid profile was determined using an AAA-400 automatic amino acid analyser (Ingos Ltd., Prague,

Czech Republic) and ninhydrin for postcolumn derivatization. Before carrying out profile analyses, hydrolysis in 6 N HCl for 24 h at 110°C was applied according to the AOAC procedure (994.12) (AOAC, 2005). The chemical composition and amino acid profile of the experimental diets are presented in Table 3.

Table 1. Chemical composition and amino acid profile of insect larval meal and fish meal used in experimental diets

Nutrients	BSFM	FM
% of DM		
crude protein	41.21	61.80
crude fat	16.19	16.50
nitrogen-free extract ¹	9.54	4.20
ash	11.91	17.50
crude fibre	12.06	0.71
Ca	1.32	3.92
P	2.35	2.16
Amino acid profile (% of crude protein)		
Indispensable amino acids (IAA)		
arginine	5.47	6.07
histidine	3.25	2.09
isoleucine	4.73	4.24
leucine	7.83	7.48
lysine	6.83	6.63
methionine	2.13	2.53
phenylalanine	4.76	3.07
threonine	4.43	4.10
valine	6.80	5.79
Dispensable amino acids (DAA)		
alanine	8.22	6.87
aspartic acid	7.30	9.40
cysteine	0.77	9.59
glycine	6.15	6.41
glutamic acid	13.07	14.50
proline	6.68	4.28
serine	4.88	4.17
tyrosine	6.71	3.00

BSFM = black soldier fly larvae meal; FM = fish meal (brown fish meal, 62% total protein, 16% fat, Agro-Fish, Kartoszyno, Poland).

¹Nitrogen-free extract = dry matter % – (crude protein % + crude fat % + crude fibre % + ash %).

Feed physical properties

All feed physical property assessments were performed according to Rawski et al. (2020) and conducted in 10 replicates for each parameter. The analysis conditions were a temperature of 21°C and 60% air-controlled humidity (Toyotomi, YD-C312, Nagoya, Japan).

1. Pellet bulk density (PD) analysis was conducted using a modified method described by Irungu et al. (2018). A total of ten randomly sampled replications of 1000 mL of feed were weighed (W), and the density was expressed as g/dm³.

$$PD\ (g/dm^3) = \text{sample weight}/dm^3$$

Table 2. Ingredient composition of the experimental diets in Atlantic salmon fry

Ingredients (g/kg)	CON	BSFM5	BSFM10	BSFM15
FM ¹	390	361	333	303
BSFM ²	0	50	100	150
Krill meal ³	50	50	50	50
Blood cells ⁴	100	100	100	100
Soy protein isolate ⁵	100	100	100	100
Wheat gluten ⁶	50	50	50	50
Wheat meal	90	73	54	38
Maltodextrin	50	50	50	50
Fish oil ⁷	125	121	118	114
Lecithin ⁸	10	10	10	10
Premix ⁹	15	15	15	15
Vitamin mix ¹⁰	1	1	1	1
Choline chloride	2	2	2	2
Chalk	14	14	14	14
TiO ₂	3	3	3	3
Total (g)	1000	1000	1000	1000

FM = fish meal; BSFM = black soldier fly larvae meal; CON = control group, without the addition of full-fat BSFM and with 39% FM; BSFM5 = 5% addition of full-fat BSFM and 36.1% FM; BSFM10 = 10% addition of full-fat BSFM and 33.3% FM; BSFM15 = 15% addition of full-fat BSFM and 30.3% FM.

¹Brown fish meal, 62% total protein, 16% fat, Agro-Fish, Kartoszyno, Poland.

²HiProMine S.A., Poland.

³Krill Meal (Antarctic), 60% total protein, 23% fat, less than 13% of ash, CC Moore, England.

⁴AP 301 P, 92% total protein, APC (GB) Ltd, Ings Road, Doncaster, UK.

⁵SUPRO 590, 87.5% total protein, 3.2% fat, Solae Belgium N.V., Belgium.

⁶GluVital, Cargill, Poland.

⁷Agro-Fish, Kartoszyno, Poland.

⁸BergaPure, deoiled lecithin, 97% pure lecithin, Berg + Schmidt GmbH & Co. KG, Hamburg, Germany.

⁹Premix (g kg⁻¹): vitamin A, 1,000,000 IU; vitamin D₃, 200,000 IU; vitamin E, 1.5 g; vitamin K, 0.2 g; vitamin B₁, 0.05 g; vitamin B₂, 0.4 g; vitamin B₁₂, 0.001 g; nicotinic acid, 2.5 g; inositol, 35 g; D-calcium pantothenate, 1.0 g; myoinositol, 500 IU; choline chloride, 7.5 g; folic acid, 0.1 g; methionine, 150.0 g; lysine, 150.0 g; Fe, 2.5 g; Mn, 6.5 g; Cu, 0.8 g; Co, 0.04 g; Zn, 4.0 g; I, 0.008 g; carrier > 1000.0 g.

¹⁰Vitamin mix (g kg⁻¹): vitamin A, 50,000 IU; vitamin D₃, 5000 IU; vitamin E, 30.0 mg; vitamin C, 100.0 mg.

Table 3. Chemical composition and amino acid profile of the experimental diets used in Atlantic salmon fry

Nutrients	CON	BSFM5	BSFM10	BSFM15
% of DM				
crude protein	47.99	48.00	48.05	48.01
crude fat	20.05	20.00	20.06	20.00
nitrogen-free extract ¹	15.63	15.34	14.92	14.70
ash	10.82	10.90	11.00	11.07
crude fibre	0.88	1.42	1.94	2.48
Ca	1.50	1.50	1.50	1.49
P	1.10	1.12	1.16	1.19
gross energy (MJ/kg)	21.84	21.77	21.74	21.66
energy/protein ratio	45.51	45.36	45.23	45.13
Amino acid profile (% of crude protein)				
arginine	5.49	5.36	5.23	5.09
histidine	2.74	2.71	2.68	2.65
lysine	6.18	6.07	5.96	5.85
tryptophan	0.87	0.84	0.81	0.78
phenylalanine + tyrosine	6.22	6.15	6.08	6.01
methionine + cysteine	2.38	2.33	2.27	2.22
threonine	3.64	3.57	3.49	3.41
leucine	7.65	7.50	7.35	7.20
isoleucine	7.65	4.14	4.06	3.98
valine	5.76	5.67	5.57	5.47

FM = fish meal; BSFM = black soldier fly larvae meal; CON = control group, without the addition of full-fat BSFM and with 39% FM; BSFM5 = 5% addition of full-fat BSFM and 36.1% FM; BSFM10 = 10% addition of full-fat BSFM and 33.3% FM; BSFM15 = 15% addition of full-fat BSFM and 30.3% FM.

¹Nitrogen-free extract = dry matter % – (crude protein % + crude fat % + crude fibre % + ash %).

2. Sinking velocity (SV) was conducted using a modified method described by Das et al. (1993). A sedimentation column (Terra-Robson, Wierzbno, Poland) with a height of 105 cm was filled with 100 cm of water. A total of twenty randomly selected pellets per feed batch were inserted into the water individually, and the time taken to reach the bottom was measured with an accuracy of 0.1 s.

$$SV (s/100 \text{ cm}) = \text{sinking time (s)} / \text{water column height (cm)}$$

3. Water stability (WS) was conducted using modified methods proposed by Das et al. (1993) and Umar et al. (2013). For each feed, 100 g was placed in a flask, and 500 mL of distilled water was added and placed on a horizontal shaker. Then, the feed was filtered after 10 minutes through a 1-mm strainer on paper filters. After water filtration, the residues were dried at 50°C for 24 h and weighed to calculate the disintegrated feed proportion.

$$WS (\%) = ((\text{final sample weight} - \text{initial sample weight}) / \text{initial sample weight}) \times 100$$

4. Volume increase (VI) was conducted using the method described by Rawski et al. (2020). A volume of 25 mL of feed was placed in a volumetric flask, and the flask was filled with 100 mL of water. After 10 minutes, the volume of the feed was measured, and the expansion in water was calculated.

$$VI (\%) = ((\text{final volume} - \text{initial volume}) / \text{initial volume}) \times 100$$

5. The expansion ratio (ER) was calculated according to the formula described by Khater et al. (2014). The result is presented as the ratio of pellet diameter to extrusion matrix perforation diameter. The analysis was performed using an electronic calliper (Yato, YT-7201, Shanghai, China) with an accuracy of up to 0.01 mm.

$$ER (\%) = (\text{pellet diameter (mm)} / \text{matrix diameter (mm)}) \times 100$$

Animal handling and growth performance trial

Atlantic salmon fries were transported from the Experimental Station of Feed Production Technology and Aquaculture in Muchocin to the Laboratory of Inland Fisheries and Aquaculture. The study was carried out using an experimental Recirculated Aquaculture System (eRAS). During acclimation, fish were kept in a 500 L tank. One week before the right part of the experiment, 240 healthy fish were randomly selected from the adaptation tank and transferred into experimental tanks (24 tanks with a capacity of 50 L, 10 fish per tank, 6 tanks per group) and then fed commercial feed for salmonids (Aller Performa – 48% crude protein, 21% crude fat, 22.1 MJ gross energy/kg, Aller Aqua, Poland). On the 1st day of the experimental period, all fish in each tank were bulk weighed (average body weight was $13.4 \text{ g} \pm 1 \text{ g}$). The eRAS was supplied with tap water, and technological water was purified by a biological filter and aerated using an aeration system powered with

a membrane blower (HiBlow, Japan). The water flow was 2 L/min per each experimental tank. The water parameters were recorded daily. The temperature was $15^\circ\text{C} \pm 0.5^\circ\text{C}$, the dissolved oxygen was $7.5 \text{ mg} \pm 0.3 \text{ mg L}^{-1}$, and the photoperiod was maintained at 16:8 (light:dark) during the experiment.

The experiment lasted 60 days. To reduce stress caused by contact with personnel, salmon were fed continuously using automatic belt feeders (12 h discharge time, FIAP Fishtech GmbH, Germany) during both acclimation and experimental periods. Each trial was carried out in six replications (tanks) per diet. The feed ratio was based on a feeding chart designed for Atlantic salmon, taking into consideration the average body weight and the water temperature (National Research Council, 2011). The fish mortality and health condition were monitored daily. All animals were weighed at 10-day intervals.

To calculate the growth performance and feed utilization factors, the following formulas were used:

1. Final Body Weight (FBW):

$$FBW (g) = \text{fish biomass in the tank (g)} / \text{number of fish in the tank}$$

2. Body Weight Gain (BWG):

$$BWG (g) = \text{final body weight (g)} - \text{initial body weight (g)}$$

3. Percent of Body Weight Gain (PWG):

$$PWG (\%) = ((\text{final body weight (g)} - \text{initial body weight (g)}) / \text{initial body weight (g)}) \times 100$$

4. Feed Intake (FI):

$$FI (g) = \text{applied feed (g)} - \text{uneaten feed (g)}$$

5. Feed Conversion Ratio (FCR):

$$FCR = \text{feed intake (g)} / \text{body weight gain (g)}$$

6. Specific Growth Rate (SGR):

$$SGR (\%/day) = 100 \times (\ln \text{final body weight} - \ln \text{initial body weight}) / \text{number of experimental days}$$

7. Survival Rate (SR):

$$SR (\%) = (\text{final number of live fish} / \text{initial number of live fish}) \times 100$$

8. Protein Efficiency Ratio (PER):

$$PER = (\text{body weight gain (g)}) / (\text{feed intake (g)} \times \text{protein level in the diet (\%)})$$

9. Lipid Efficiency Ratio (LER):

$$LER = (\text{body weight gain (g)}) / (\text{feed intake (g)} \times \text{lipid level in the diet (\%)})$$

All growth performance results are presented as the means, where $n = 6$ tanks per group (10 fish/tank, 60 fish/group).

Digestibility trial

This trial was performed to determine the apparent digestibility coefficients of crude protein and crude fat in the Atlantic salmon fry experiment. Salmonids were transported from the Experimental Station of Feed Production Technology and Aquaculture in Muchocin to the Laboratory of Inland Fisheries and Aquaculture laboratory. Metabolic tanks were used as a modification of the Allan et al. (1999) design according to Rawski et al.

(2020). Tanks were equipped with a conical bottom with a separation grille and a two-valve bottom separation and drainage system. The tanks were supplied with filtrated tap water in an open-flow system. The temperature was $15^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$, the concentration of oxygen was $7.5 \text{ mg} \pm 0.3 \text{ mg L}^{-1}$, and the photoperiod was maintained at 16:8 (light:dark) during the entire experiment. The digestibility test lasted 45 days, including 15 days of the adaptation period and 30 days of the period of faecal sampling. For the experiment, 120 fish with an average body weight of 13.4 grams ($\pm 1 \text{ g}$) were used and divided into 4 groups (3 tanks/group and 10 fish/tank). Animals were fed the same diets used in growth performance trials but with the addition of titanium dioxide (TiO_2) as a digestibility marker in the amount of 0.3% (Kierończyk et al., 2020). The feed was given in the amount of 1% of fish weight per day for 6 hours (from 7:00 to 13:00) using automatic belt feeders. After feeding, the rest of the uneaten feed was removed. The accumulated faeces were obtained by opening the bottom separation system and collecting faeces for filtration vessels. The faecal collection was conducted 3 times per day between 7:00, 17:00, and 23:00. Filtration was carried out through cellulose filters. The obtained faeces were frozen at -20°C for further analysis. The coefficients of apparent ileal digestibility of crude protein and crude fat were calculated according to the following formulas described by Kierończyk et al. (2018):

1. Coefficients of apparent total digestibility (%):

$$\text{Coefficients of apparent total digestibility} = 1 - ((\text{TiO}_2 \text{ (g kg}^{-1} \text{ in the diet) / TiO}_2 \text{ (g kg}^{-1} \text{ in the faeces)}) \times (\text{nutrient (g kg}^{-1} \text{ in the faeces) / nutrient (g kg}^{-1} \text{ in the diet)}))$$

Sample collection and somatic indices

On the last day of the experiment, all fish were weighed individually, and 5 animals from each tank were euthanized using an overdose of MS-222 (Leary et al., 2013) and decapitated for dissection and sampling for further analysis. Measurements of the carcass, viscera, and liver were recorded to calculate the condition factor (CF), viscerosomatic index (VSI), and hepatosomatic index (HSI) as described by Hoffmann et al. (2020), while measurements of total intestines, proximal intestines, distal intestines, gastrointestinal tract, and total fish length were assessed to calculate numerous intestinal indices according to Rawski et al. (2021):

1. Condition Factor (CF):

$$\text{CF} = (\text{body weight (g) / fish total length (cm}^3)) \times 100$$

2. Viscerosomatic Index (VSI):

$$\text{VSI (\%)} = (\text{viscera weight (g) / body weight (g)}) \times 100$$

3. Hepatosomatic Index (HSI):

$$\text{HSI (\%)} = (\text{liver weight (g) / body weight (g)}) \times 100$$

4. Relative Gastrointestinal Tract Length (GIT/FTL):

$$\text{GIT/FTL (\%)} = (\text{gastrointestinal tract length (mm) / fish total length (mm)}) \times 100$$

5. Relative Intestinal Length (IL/FTL):

$$\text{IL/FTL (\%)} = (\text{intestine length (mm) / fish total length (mm)}) \times 100$$

6. Relative Proximal Intestine Length (PL/FTL):

$$\text{PL/FTL (\%)} = (\text{proximal intestine length (mm) / fish total length (mm)}) \times 100$$

7. Relative Distal Intestine Length (DL/FTL):

$$\text{DL/FTL (\%)} = (\text{distal intestine length (mm) / fish total length (mm)}) \times 100$$

8. Intestine Share in Total Gastrointestinal Tract Length (IL/GIT):

$$\text{IL/GIT (\%)} = (\text{intestine length (mm) / gastrointestinal tract length (mm)}) \times 100$$

9. Proximal Intestine Share in Total Gastrointestinal Tract Length (PL/GIT)

$$\text{PL/GIT (\%)} = (\text{proximal intestine length (mm) / gastrointestinal tract length (mm)}) \times 100$$

10. Distal Intestine Share in Total Gastrointestinal Tract Length (DL/GIT)

$$\text{DL/GIT (\%)} = (\text{distal intestine length (mm) / gastrointestinal tract length (mm)}) \times 100$$

All somatic index results are presented as the means, where $n = 32$ per group (6 tanks/treatment $\times$ 8 fish/tank).

Gut histomorphology

At the end of the experiment, 24 fish were sampled from each treatment (6 fish/group). Samples of the intestine were collected, submerged in Bouin's solution (Merck), and stored at 4°C . Histological examination was performed using the methodology described by Bogucka et al. (2016). The samples were dehydrated, cleared, and embedded in paraffin blocks. Formed blocks were cut on a rotary microtome (Thermo Shandon, Chadwick Road, Astmoor, Runcorn, Cheshire, United Kingdom) into slices of 10- μm thickness. The slices were placed on microscope slides coated with ovo-albumin with the addition of glycerol. The prepared samples were analysed using an AnMN-800 F microscope (OPTA-TECH, Warsaw, Poland) equipped with an Opta-View camera for recording microscopic images. MultiScan v. 18.03 microscope imaging software (Computer Scanning Systems II Ltd., Warsaw, Poland) was used to measure villus height and width and muscular layer thickness. Ten villi per fish were randomly selected from a cross-section. The length was measured from the top of the villus to its base, while the width was measured at half of its length.

Environmental sustainability assessment

The environmental sustainability assessment was calculated based on raw data for growth performance and feed utilization parameters according to Rawski et al. (2020, 2021).

The relative usage of marine-derived feed materials – fish meal (FMU) and fish oil (FOU) – was calculated according to two formulas:

$$1. \text{FMU (g/kg of fish gain)} = \text{fish meal share in the diet (g kg}^{-1}) \times \text{feed conversion ratio (g/g)}$$

$$2. \text{FOU (g/kg of fish gain)} = \text{fish oil share in the diet (g kg}^{-1}) \times \text{feed conversion ratio (g/g)}$$

The fish-in-fish-out ratio (FIFO) was calculated as a practical measure of the quantity of live fish from cap-

ture fisheries required for each unit of farmed fish produced, as follows:

$$1. \text{FIFO} = ((\text{level of fish meal in the diet (g kg}^{-1}\text{)} + \text{level of fish oil in the diet (g kg}^{-1}\text{)}) / (\text{yields of fish meal from wild fish (g kg}^{-1}\text{)} + \text{yields of fish oil from wild fish (g kg}^{-1}\text{)})) \times (\text{feed intake (g)} / \text{body weight gain (g)})$$

where the yield of fish meal from wild fish was assumed to be 225 g kg⁻¹ of fresh fish weight and the yield of fish oil from wild fish was assumed to be 50 g kg⁻¹ of fresh fish weight (Stejskal et al., 2020).

Statistical analysis

R studio software was used to analyse the data for both experiments conducted in this study. To determine the normality of the data distribution and equality of variances, Kolmogorov–Smirnov and Levene's tests were used. One-way ANOVA was used, and if there were significant differences among treatments, further analysis was performed by correcting Duncan's post-hoc test. Data are presented as the mean ± standard error of the mean (SEM). The statistical significance level was declared at $P < 0.05$. The statistical analysis of variance was conducted according to the following general model:

$$Y_i = \mu + ai + \delta ij$$

where: Y_i is the observed dependent variable, μ is the overall mean, ai is the effect of the diet, and δij is the random error.

Results

Feed physical properties

In terms of the physical properties of feed, the increasing inclusion of BSFM meal in diets leads to decreasing results in PD and increasing results of VI ($P < 0.0001$). In groups BSFM10 and BSFM15, higher values in SV compared to CON and BSFM5 were observed ($P = 0.0005$). All groups with insect addition showed increased ER values compared to CON; however, there were no significant differences between groups BSFM10 and BSFM15 ($P < 0.0001$). Additionally, no significant differences were observed in terms of WS. All results of the feed physical property assessment are presented in Table 4.

Growth performance trial

In the case of the experiment conducted on Atlantic salmon fry, the BWG, PWG, SGR, PER, and LER showed decreases in the BSFM15 group compared to the CON ($P = 0.0064$; $P = 0.0064$, $P = 0.0161$; $P = 0.0111$; $P = 0.0111$, respectively), while the FCR in the mentioned group showed the highest result ($P = 0.0035$). However, there were no significant differences in FBW and FI results. No mortality during the entire experiment was recorded. The results of the growth performance trial on Atlantic salmon fry are presented in Table 5.

Table 4. Physical properties of experimental diets for Atlantic salmon fry

Parameter	CON	BSFM5	BSFM10	BSFM15	SEM	P value
PD (g/dm ³)	639 a	612 b	588 c	548 d	5.4185	<0.0001
SV (s/100 cm)	11.4 b	12.0 b	13.7 a	14.1 a	0.2869	0.0005
WS (%)	93.4	92.4	93.4	95.4	1.8123	0.3400
VI (%)	43.8 d	50.0 c	57.5 b	63.8 a	1.4979	<0.0001
ER (%)	119 c	126 b	127 ab	131 a	0.9906	<0.0001

FM = fish meal; BSFM = black soldier fly larvae meal; CON = control group, without the addition of full-fat BSFM and with 39% FM; BSFM5 = 5% addition of full-fat BSFM and 36.1% FM; BSFM10 = 10% addition of full-fat BSFM and 33.3% FM; BSFM15 = 15% addition of full-fat BSFM and 30.3% FM; PD – pellet density; SV – sinking velocity; WS – water stability; VI – volume increase; ER – expansion rate.

a-d – values within a row with different letters differ significantly at $P < 0.05$. Values are presented as the means ($n = 10/\text{treatment}$), pooled standard error of the mean, SEM.

Table 5. Growth performance and feed utilization parameters of Atlantic salmon fry fed experimental diets

Parameter	CON	BSFM5	BSFM10	BSFM15	SEM	P value
IBW (g)	13.7	13.2	13.0	13.6	0.2085	0.9410
FBW (g)	26.3	25.9	25.3	24.7	0.3144	0.3050
BWG (g)	12.6 a	12.7 a	12.4 a	11.0 b	2.0587	0.0064
PWG (%)	91.4 a	96.0 a	95.6 a	80.9 b	1.1587	0.0064
FI (g)	112	108	106	111	1.6325	0.5300
FCR	0.89 b	0.85 b	0.86 b	1.01 a	0.0192	0.0035
SGR	1.11 a	1.12 a	1.07 a	0.95 b	0.0223	0.0161
PER	2.43 a	2.55 a	2.56 a	2.15 b	0.0522	0.0111
LER	6.22 a	6.53 a	6.56 a	5.53 b	0.1340	0.0111

FM = fish meal; BSFM = black soldier fly larvae meal; CON = control group, without the addition of full-fat BSFM and with 39% FM; BSFM5 = 5% addition of full-fat BSFM and 36.1% FM; BSFM10 = 10% addition of full-fat BSFM and 33.3% FM; BSFM15 = 15% addition of full-fat BSFM and 30.3% FM; IBW – initial fish body weight; FBW – final fish body weight; BWG – fish body weight gain; PWG – percent of fish body weight gain; FI – feed intake; FCR – feed conversion ratio; SGR – specific growth rate; PER – protein efficiency ratio; LER – lipid efficiency ratio.

a-d – values within a row with different letters differ significantly at $P < 0.05$. Values are presented as the means ($n = 6/\text{treatment}$), pooled standard error of the mean, SEM.

Table 6. Somatic indices of Atlantic salmon fry fed experimental diets

Parameter	CON	BSFM5	BSFM10	BSFM15	SEM	P value
CF	1.21	1.22	1.20	1.18	0.0082	0.3090
VSI (%)	11.45	11.35	10.49	11.93	0.2024	0.0857
HSI (%)	1.99	2.17	1.93	1.89	0.0630	0.4140

FM = fish meal; BSFM = black soldier fly larvae meal; CON = control group, without the addition of full-fat BSFM and with 39% FM; BSFM5 = 5% addition of full-fat BSFM and 36.1% FM; BSFM10 = 10% addition of full-fat BSFM and 33.3% FM; BSFM15 = 15% addition of full-fat BSFM and 30.3% FM; CF – condition factor; VSI – viscerosomatic index; HIS – hepatosomatic index.

Values are presented as the means (n = 30/treatment), pooled standard error of the mean, SEM.

Table 7. Histomorphology of intestines of Atlantic salmon fry fed with experimental diets

Parameter	CON	BSFM5	BSFM10	BSFM15	SEM	P value
Villus height (μm)	346.42	393.55	467.70	393.55	19.4538	0.1720
Villus width (μm)	110.30	117.41	120.15	124.95	2.3168	0.1510
Villus area (mm ²)	122.72	147.90	178.81	156.92	9.2084	0.2010
Muscular layer thickness (μm)	44.73	47.04	45.06	40.78	1.9262	0.7350

FM = fish meal; BSFM = black soldier fly larvae meal; CON = control group, without the addition of full-fat BSFM and with 39% FM; BSFM5 = 5% addition of full-fat BSFM and 36.1% FM; BSFM10 = 10% addition of full-fat BSFM and 33.3% FM; BSFM15 = 15% addition of full-fat BSFM and 30.3% FM.

Values are presented as the means (n = 30/treatment), pooled standard error of the mean, SEM.

Table 8. Environmental sustainability parameters of Atlantic salmon fry fed experimental diets

Parameter	CON	BSFM5	BSFM10	BSFM15	SEM	P value
FCE (g/g)	1.12 a	1.17 a	1.18 a	0.99 b	0.0241	0.0111
FMU (g/kg)	349.0 a	308.2 b	285.9 b	307.0 b	6.5795	0.0017
FOU (g/kg)	111.8 ab	103.3 b	101.3 b	115.5 a	2.0281	0.0261
FIFO	1.68 a	1.50 b	1.41 b	1.54 ab	0.0301	0.0070

FM = fish meal; BSFM = black soldier fly larvae meal; CON = control group, without the addition of full-fat BSFM and with 39% FM; BSFM5 = 5% addition of full-fat BSFM and 36.1% FM; BSFM10 = 10% addition of full-fat BSFM and 33.3% FM; BSFM15 = 15% addition of full-fat BSFM and 30.3% FM; FCE – feed conversion efficiency; FMU – fish meal use; FOU – fish oil used, FIFO – the fish-in-fish-out ratio.

a-d – values within a row with different letters differ significantly at P<0.05. Values are presented as the means (n = 6/treatment), pooled standard error of the mean, SEM.

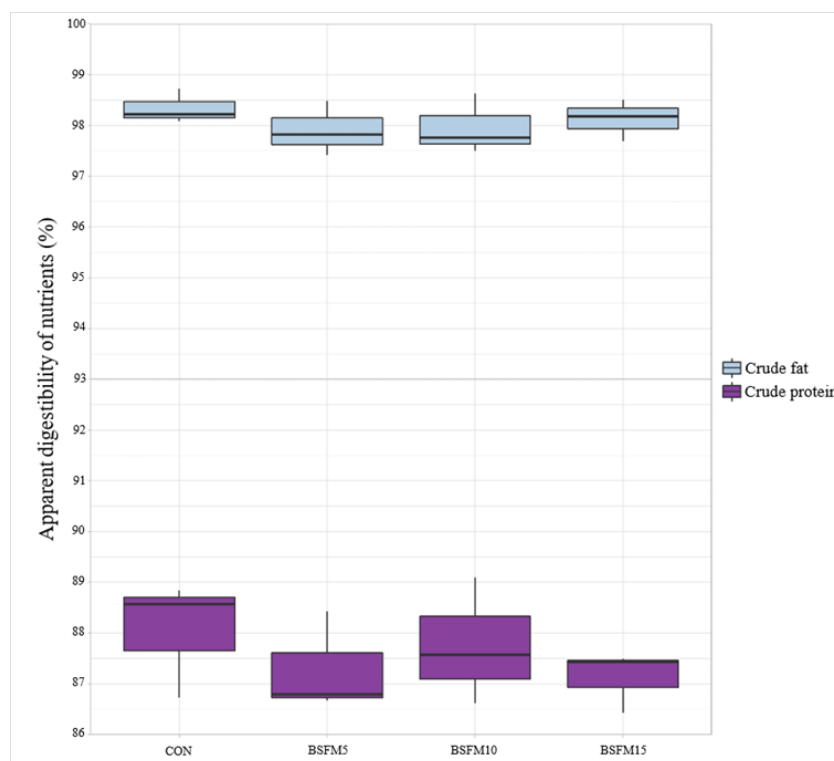


Figure 1. The effect of experimental diets on nutrients (crude fat – upper and crude protein – lower) digestibility of Atlantic salmon fry. FM = fish meal; BSFM = black soldier fly larvae meal; CON = control group, without the addition of full-fat BSFM and with 39% FM; BSFM5 = 5% addition of full-fat BSFM and 36.1% FM; BSFM10 = 10% addition of full-fat BSFM and 33.3% FM; BSFM15 = 15% addition of full-fat BSFM and 30.3% FM; No statistically significant differences were observed among the groups

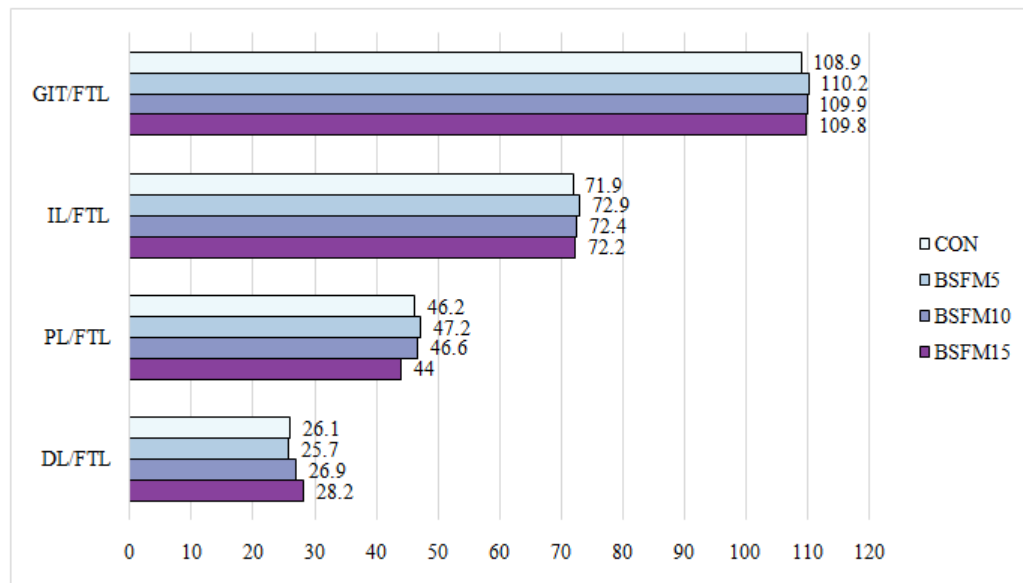


Figure 2. The effect of experimental diets on the percentage of length of individual parts of the gastrointestinal tract in the total body length of Atlantic salmon fry. FM = fish meal; BSFM = black soldier fly larvae meal; CON = control group, without the addition of full-fat BSFM and with 39% FM; BSFM5 = 5% addition of full-fat BSFM and 36.1% FM; BSFM10 = 10% addition of full-fat BSFM and 33.3% FM; BSFM15 = 15% addition of full-fat BSFM and 30.3% FM; GIT/FTL – relative gastrointestinal tract length; IL/FTL – relative intestinal length; PL/FTL – relative proximal intestine length; DL/FTL – relative distal intestine length; No statistically significant differences were observed among the groups ($P \geq 0.05$)

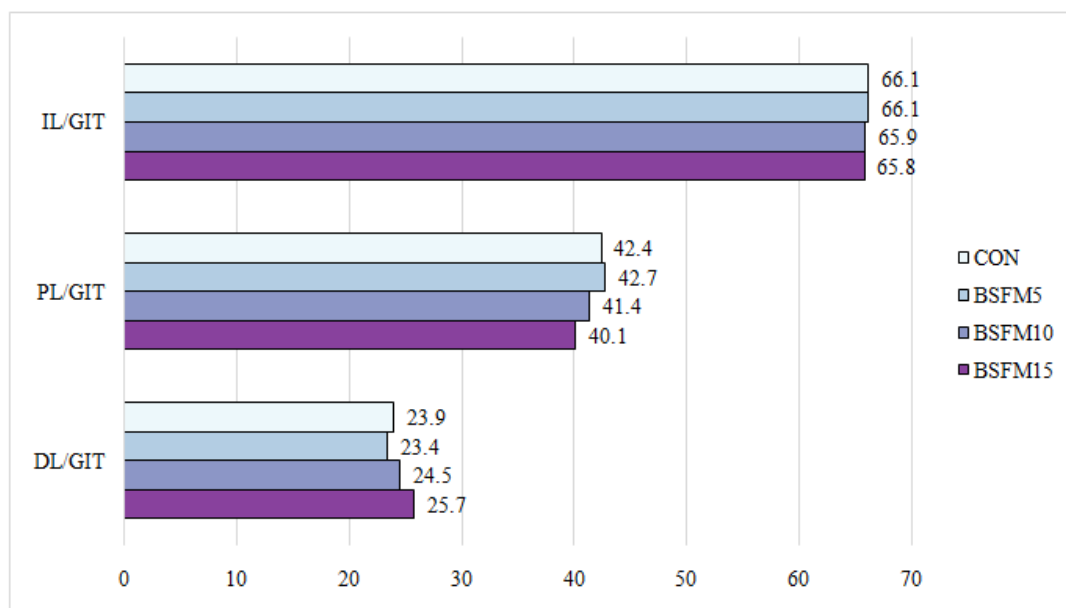


Figure 3. The effect of experimental diets on the percentage of length of individual parts of intestines in the total gastrointestinal tract length of Atlantic salmon fry. FM = fish meal; BSFM = black soldier fly larvae meal; CON = control group, without the addition of full-fat BSFM and with 39% FM; BSFM5 = 5% addition of full-fat BSFM and 36.1% FM; BSFM10 = 10% addition of full-fat BSFM and 33.3% FM; BSFM15 = 15% addition of full-fat BSFM and 30.3% FM; IL/GIT – intestinal share in total gastrointestinal tract length; PL/GIT – proximal intestine share in total gastrointestinal tract length; DL/GIT – distal intestine share in total gastrointestinal tract length; no statistically significant differences were observed among the groups ($P \geq 0.05$)

Digestibility trial

During the digestibility of nutrients trial, no significant differences among the groups in terms of crude protein and crude fat were reported. The results of digestibility of nutrients of Atlantic salmon fry are presented in Figure 1.

Somatic indices

In the case of somatic indices of Atlantic salmon fry, there were no significant differences in CF, VSI or HIS among the groups. The results are presented in Table 6. Similarly, in terms of gastrointestinal tract indices, including GIT/FTL, IL/FTL, PL/FTL, DL/FTL, IL/GIT,

PL/GIT, and DL/GIT, no significant differences were reported. The results of those indices are presented on the graphs in Figures 2 and 3.

Gut histomorphology

Experimental groups did not show any significant differences in comparison to the control group. All histomorphology results are presented in Table 7.

Environmental sustainability assessment

In terms of environmental sustainability parameters, a decrease in the FCE value was observed only in the BSFM15 group ($P = 0.0111$). In the case of FMU, all groups with insects showed decreased values compared to CON ($P = 0.0017$), while FOU values of insect groups did not differ from CON ($P = 0.0261$). Additionally, BSFM5 and BSFM10 were significantly different compared to CON, and a decrease was observed ($P = 0.0070$). All results of the environmental sustainability parameters are presented in Table 8.

Discussion

The basic chemical composition of full-fat black soldier fly larvae meal (BSFM) and fish meal (FM) showed lower crude protein content and higher crude fat and crude fibre levels in insect meal. These findings are in agreement with several scientific papers (Belghit et al., 2018; Nogales-Mérida et al., 2018; Józefiak et al., 2019; Basto et al., 2020; Rawski et al., 2020). The higher content of crude fibre can be explained by the occurrence of chitin in insect exoskeletons due to its chemical structure, which qualifies it as a polysaccharide. Simultaneously, chitin can contain non-amino acid nitrogen bonds in its structure. According to suggestions of previous researchers (Finke, 2007; Gasco et al., 2018), protein overestimation would occur due to the presence of chitin in insect biomass. Additionally, the overestimation will reach a higher level as the inclusion of insect meal in fish diets increases (Rawski et al., 2020). Therefore, it is crucial to perform calculations of the crude protein conversion ratio (Kp) to compensate for differences in the content of nutrients. The current study confirmed the foregoing statement of Janssen et al. (2017), who assessed Kp for insect meals in the range from 4.76 to 5.60, depending on crude protein quality and purification. The abovementioned correction in the nitrogen to protein conversion ratio allowed us to calculate isonitrogenous diets for Atlantic salmon. No differences were observed in the chemical composition of diets, which could potentially affect and disturb the proper growth performance of fish. Substantial dissimilarities were reported in the crude fibre content only, which seems to be justified. Additionally, according to Storebakken (2009), the requirement for calcium has not been established in Atlantic salmon. However, the level of total phosphorus in the diet, depending on the literature, was estimated at 6 g/kg (Ketola, 1975) or even

10–11 g/kg for juvenile salmon (Åsgård and Shearer, 1997). Therefore, the diets designed for the experiment meet the requirements in terms of mineral composition.

The data focused on the pellet quality of aquafeeds with insect material inclusion are still limited. However, our findings are in line with Rawski et al. (2020) – an increase in the share of BSFM in Atlantic salmon diets led to increasing results of sinking velocity (SV), volume increase (VI), and expansion ratio (ER), with a simultaneous decrease in pellet density (PD). In contrast, Weththasinghe et al. (2021 a) reported that higher inclusion of full-fat BSFM meal or paste led to a decrease in ER, while the SV did not show any differences among the groups. In turn, Irungu et al. (2018) reported that an increase in insect inclusion in aquafeeds led to higher pellet bulk density. The main reason for the observed results, which was suggested previously (Rawski et al., 2020), is that the usage of full-fat insect meals provided better oil dispersed in the feed during extrusion. However, it is important to emphasize that in the present study, the percentage values of crude fat in BSFM and FM were quite similar, oscillating at approximately 16%. Simultaneously, in the mentioned study, the higher ER and SV positively related to a decrease in PD could be explained by increasing pore formation in the pellet structure. A similar effect in the case of SV was previously observed in pellets with higher DDGS inclusion (Kannadhasan et al., 2009). Additionally, Liu et al. (2021) reported that the addition of wheat flour leads to a more porous pellet texture due to expansion, which affects the water stability of feed. Therefore, this might be the reason for the much higher SV in groups with the addition of BSFM, since BSFM was characterized by a significantly higher crude fibre proportion.

In the present study, the addition of 15% BSFM affected body weight gain (BWG), percent body weight gain (PWG), feed conversion ratio (FCR), protein efficiency ratio (PER) and lipid efficiency ratio (LER). However, 5 and 10% BSFM inclusion did not affect growth performance or feed utilization parameters. Compared to Weththasinghe et al. (2021 b), feed intake presented lower values than control in groups including defatted BSFM (accounting for approximately 15% of diet) and *H. illucens* exoskeleton (accounting for approximately 7% of diet); however, this outcome did not negatively affect the FCR results. Additionally, the FCR in the group with the inclusion of full-fat BSFM (more than 20% of diet) did not differ from the control group and simultaneously showed the highest values in final fish body weight (FBW). Belghit et al. (2018) reported that even a high amount of defatted BSFM inclusion (60% of diets) did not affect FBW, FCR, FI, PER, or LER. In contrast, 25% substitution of FM by full-fat BSFM led to higher values in FCR and lower FBW, which is in line with our findings (Weththasinghe et al., 2021 a). Similar results were observed by Fisher et al. (2020), where the inclusion of BSFM at the level of 30% significantly decreased the BWG and increased the FCR of Atlantic salmon pre-

smolts. The most common explanation in the scientific literature for the effect of insect addition in fish diets on growth performance and feed utilization is the presence of chitin (Belghit et al., 2018; Fisher et al., 2020; Mikołajczak et al., 2020; Hoffmann et al., 2021 a; Weththasinghe et al., 2021 a) due to crude protein overestimation (Finke, 2007). However, to minimize this effect and avoid distorting the results, a corrected Kp value was applied.

Despite the fact that crude protein and crude fat digestibility were lowest in the group with 15% BSFM inclusion, the differences were not statistically significant. Our findings are in agreement with Weththasinghe et al. (2021 a). Previously stated reductions in PER values could be an outcome of numerical digestibility results. According to Marono et al. (2015), the presence of chitin in insects is negatively correlated with protein digestion. This is probably due to the reduced availability of protein for protease enzymes by chitin (Henry et al., 2015) or decreased activity of these enzymes (Belghit et al., 2018). In fact, Atlantic salmon is characterized by a low capacity for chitin digestibility (Olsen et al., 2006). This chemical compound can also be responsible for the numerically lower crude fat digestibility. By analysing the fatty acid profile of previous studies by our research team, it can be concluded that lauric acid can comprise approximately 46% of all estimated fatty acids (Rawski et al., 2020). Antecedent consideration highlights that lauric acid can be a satisfactory energy source for salmonids by simple oxidation, which results in low tissue deposition (Renna et al., 2017; Belghit et al., 2019 b). This statement explains the reduction in LER results, and limited lipid retention simultaneously affects lower crude fat digestibility.

No effect on the condition factor of Atlantic salmon fries was found among the groups. This result is in line with Belghit et al. (2018). However, no changes to the hepatosomatic index (HSI) and viscerosomatic index (VSI) were observed in our research, which is in contrast to the abovementioned study, where increasing inclusion of BSF meal and oil led to higher HSI and VSI results. This observation was previously pointed out in the scientific literature focused on insect-derived material addition in salmonid juvenile and fingerling diets (Hoffmann et al., 2020; Mikołajczak et al., 2020; Fawole et al., 2021). The reason for fat storage in the visceral cavity and liver of fish may depend on the fatty acid composition in the diets (Huang et al., 2016). Due to the possibility of metabolic disorders indicated by carbohydrates and lipids, the presence of oxidized fat and vitamin deficiency (A, D, E, and K) the HSI should not exceed 1–2% (Munshi and Dutta, 1998). Therefore, the only result in terms of liver condition was elevated HSI in the group with a 5% BSFM addition. However, Hoffmann et al. (2021 a) reported that excellent fish conditions can be observed even with a very high (over 3.48) hepatosomatic index without a negative effect on liver histomorphology.

While the vast majority of recent scientific literature is focused on sea-water phase Atlantic salmon (Elia et

al., 2018; Belghit et al., 2019 a; Li et al., 2020), the comparison of obtained results to experiments conducted on fish of different ages is not satisfactory. In the present research, no effect on the relative length of gastrointestinal tract sections, villus width, height and area, or muscular layer thickness was observed. According to Demirci et al. (2021) the use of different dietary protein sources has morphometric effects on the rainbow trout digestive system. Li et al. (2019) reported that the usage of a diet wherein 85% of the protein was supplied by BSFM leads to an increasing share of mid and distal intestines and simultaneously provides a decrease in the prevalence of steatosis in pyloric caeca enterocytes of salmon. It is also conjectured that insect meals stimulate the regulation of T-cell activity and xenobiotic metabolism in the proximal and distal intestines. According to Mikołajczak et al. (2020), the inclusion of mealworm (*Tenebrio molitor*) and superworm (*Zophobas morio*) meals did not affect the histomorphology of sea trout. Additionally, Hoffmann et al. (2021 a) observed that the usage of mealworm meal at the levels of 30 and 40% sea trout diets led to a decrease in villus width and villus surface, as well as an increase in the thickness of the muscle membrane. In contrast, Rawski et al. (2021) showed that 20% inclusion of BSFM in Siberian sturgeon (*Acipenser baerii*) diets increased villus height, villus width and villus surface. In general, the changes in intestinal morphology may be the effect of the presence of antimicrobial peptides (AMPs) in insect meals, which modulate the microbial community of the gastrointestinal tract (Tang et al., 2008). It is crucial to emphasize that the gaps in the scientific literature regarding the influence of BSFM on intestine histomorphology should be filled with novel experiments, since knowledge about this area is limited.

FM is the main protein source used in aquaculture feeds (Naylor et al., 2021). In farmed salmon, FM dietary inclusion oscillates between 20% and 50% (Jannathulla et al., 2019). Additionally, more often, this feed material is considered a potential pathway to introduce microplastics to the food chain of aquaculture products (Gündoğdu et al., 2021). The most important parameter describing feed sustainability in the nutrition of different species is the fish-in-fish-out ratio (FIFO). In general, FIFO is the requirement of wild-caught fish for nutritive purposes (as feed materials – FM and fish oil) necessary to produce one kg of farmed animals (Jannathulla et al., 2019). However, this ratio interacts with FCR, so feed intake and hence body weight gain influence the final FIFO score. The results observed in our research illustrate this situation well. Due to the lower growth performance of fish in BSFM15, FIFO did not differ significantly from the CON. However, BSFM5 and BSFM10 presented a decrease in FIFO, which indicates the potential of insects as a sustainable protein source in Atlantic salmon fries. Depending on the year, the FIFO ratio for salmonids oscillates between 2.57 and 0.82, which is an effect of an increase in alternate protein source utilization (Jannathulla et al., 2019). Naylor et al. (2021) emphasized that salmon

is one of the critical species, where FIFO exceeded 1.0, and the ratio reached 1.87 in 2017. However, the effect of insect-derived feed material utilization on environmental sustainability is still a new research object, and the summary data do not distinguish between seawater and freshwater phases of Atlantic salmon, which makes it impossible to clearly compare these results. Therefore, the presented results are an important incorporation into scientific knowledge.

Conclusions

The present study showed satisfactory results of BSFM inclusion at the levels of 5% and 10% as a replacement for fish meal in feeds for Atlantic salmon fries. No negative effects on growth performance, feed utilization, protein and fat digestibility, or intestinal structure were recorded. Simultaneously, insect meal inclusion in Atlantic salmon fry diets significantly improved the environmental sustainability of the feed. However, due to the lack of positive results observed in growth performance and sustainability, the use of shares higher than 10% of BSFM in Atlantic salmon fries may not be recommended.

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