Ann. Anim. Sci., Vol. 23, No. 1 (2023) 239–251 *DOI: 10.2478/aoas-2022-0071*

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

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Abstract

Black soldier larva meal (BSFM) seems to be a competitive protein and fat source for widely used fish meal (FM) and fish oil. Because of the still present problem of overfished seas and oceans for feed purposes, even a low substitution of the mentioned feed materials could have a positive impact on the environment. Due to the specificity of salmon metabolic processes, with particular attention to smoltification and the various requirements for nutrients related to individual stages, it is important to implement similar dietary inclusion levels of innovative feed materials in different life stages of Atlantic salmon. Thus, a holistic approach was undertaken in the cycle of two studies carried out: on fries and pre-smolts. This study aims to evaluate the effect of FM replacement by full-fat BSFM in Atlantic salmon pre-smolt diets on growth performance, feed utilization, somatic indices, histomorphology of intestines, colorimetric assessment and raw meat quality, as well as environmental sustainability. The following groups were applied: CON – without addition of full-fat BSFM and with 30% FM; BSFM5 – with 5% addition of full-fat BSFM and 27.1% FM; BSFM10 – with 10% full-fat BSFM and 24.3% FM; and BSFM15 – with 15% addition of full-fat BSFM and 21.3% FM. The present study showed satisfactory results of BSFM inclusion up to 15% as a replacement for FM in feeds for Atlantic salmon pre-smolts. The present study showed that BSFM is a suitable feed material for Atlantic salmon proper diet balancing and may be used to decrease FM content. The semi-technical application of BSFM containing diets confirmed their effectiveness during the growth phase of Atlantic salmon. According to our results, BSFM can be recommended as an alternative feed component in Atlantic salmon pre-smolt nutrition at levels up to 15%. Simultaneously, insect inclusion significantly improved the environmental sustainability of the rearing process.

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

Studies on the use of innovative feed materials in aquafeeds for salmonids have developed rapidly in recent years and have led to an increasing number of scientific contributions on this topic (De Santis et al., 2016; Gong et al., 2019; Tibbetts et al., 2020; Sørensen et al., 2021). At the same time, one of the most tested alternatives is insect-derived materials (Hoffmann et al., 2020, 2021; Mikołajczak et al., 2020; Terova et al., 2021), particularly black soldier fly meal (BSFM, *Hermetia illucens*) (Józefiak et al., 2019; Weththasinghe et al., 2021 a, b; Mikołajczak et al., 2022). Recently, the vast majority of BSFM utilization in Atlantic salmon (*Salmo salar*) nutrition has mainly included its implementation in post-smolts diets during the seawater growth phase, and the inclusion levels reach up to 15% (Belghit et al., 2019 a; Bruni et al., 2020; Li et al., 2020 a, b; Husein et al., 2021). As a feed material, BSFM contains 30–58% crude protein and 40% crude fat (Nogales-Mérida et al., 2018). The requirements of Atlantic salmon before smoltification for crude protein are estimated between 50 and 60% of the diet and 18–30% for crude fat (Storebakken, 2009). BSFM seems to be a competitive protein and fat source for widely used fish meal (FM) and fish oil, even a low substitution of which could have a positive environmental impact, due to the still present problem of overfished seas and oceans for

*This work was supported by the funds of the National Centre for Research and Development, no. POIR.01.01.01-00-0828/15, titled InnSecta: Innovative Technology of Feedstuffs Production Based on Insect Biomass and the funds of Poznań University of Life Sciences.

feed purposes (Franco et al., 2021; Mohan et al., 2022). Moreover, the use of BSFM as an alternative feed material is supported by its mineral composition – it is a rich source of calcium (Palma et al., 2019); fatty acid profile – due to the high content of lauric acid, whose additional antimicrobial effect and positive influence on fish health have been previously confirmed (Skrivanova et al., 2007; Belghit et al., 2019 b); and the presence of chitin – which can positively affect the immune system (Gopalakannan and Arul, 2006; Askarian et al., 2012).

In nutritional experiments performed on fish, meat quality assessment should be one of the most important elements (Alfiko et al., 2022). While the inclusion of insect-derived materials in fish diets does not affect the appearance and colour assessment of the meat, it can have a significant influence on the fatty acid profile (Renna et al., 2017; Husein et al., 2021). Lipids are important determiners of taste; thus, the described effect may result in palatability. Some studies reported that a panel that conducted sensory testing of the fillets was unable to detect a significant difference between groups containing insects or without them (Sealey et al., 2011; Lock et al., 2016). According to scientific literature data based on surveys, a significant portion of consumers declare a positive attitude towards meat from farmed animals and fish fed insect meals and fats (Verbeke et al., 2015; Laureati et al., 2016; Mancuso et al., 2016; Popoff et al., 2017). Apart from the question of price, taste, microbiological quality and health influence, respondents also emphasized the importance of environmental sustainability (Verbeke et al., 2015; Szendrő et al., 2020) and even underlined that information about the environmental impact of feeding methods in aquaculture may influence consumer choice (Bazoche and Poret, 2016).

Due to the specificity of salmon metabolic processes, with particular attention to smoltification and the various requirements for nutrients related to the stage of the individuals, or even other tolerance for specific feed materials depending on age, it is important to implement similar experimental diets in different life stages of Atlantic salmon as a continuation of the two treatments. The current study aims to present the results of our second experiment with Atlantic salmon presmolts reared on a semitechnical scale. The scientific goals were to assess the possibility of dietary application of BSFM at the levels of 5, 10, and 15% and the effect on growth performance, feed utilization, selected nutriphysiological traits, and meat quality 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 addition of BSFM to Atlantic salmon diets will not disturb growth parameters, feed utilization, and designated gastrointestinal tract (GIT) 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). Insect biomass 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 is based on the ratio of amino acids to total protein content with N × 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) × 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 × 6.25 basis,

6.25 was the traditionally used Kp,

5.12 was the Kp calculated for BSFL.

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

Table 1. Chemical composition and amino acid profile of insect larval meal and fishmeal 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 = fishmeal (Brown fishmeal, 62% total protein, 16% fat, Agro-Fish, Kartoszyno, Poland).

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

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 addition of full-fat BSFM and with 30% FM; BSFM5 – with 5% addition of full-fat BSFM and 27.1% FM; BSFM10 – with 10% full-fat BSFM and 24.3% FM; BSFM15 – with 15% addition of full-fat BSFM and 21.3% 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 diets of experiments on pre-smolts is presented in Table 2.

Table 2. Ingredient composition of the experimental diets in Atlantic salmon pre-smolts

Ingredients (g/kg)	CON	BSFM5	BSFM10	BSFM15
FM[1]	300	271	243	213
BSFM[2]	0	50	100	150
Blood cells[3]	95	95	95	95
Yeasts	60	60	60	60
DDGS	50	50	50	50
Soybean meal[4]	90	90	90	90
Wheat gluten[5]	120	120	120	120
Wheat meal	120	109	97	88
Fish oil[6]	129	119	109	98
Lecithin[7]	10	10	10	10
Premix[8]	15	15	15	15
Vitamin mix[9]	1	1	1	1
Choline chloride	2	2	2	2
Chalk	5	5	5	5
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 30% of FM; BSFM5 = 5% addition of full-fat BSFM and 27.1% of FM; BSFM10 = 10% addition of full-fat BSFM and 24.3% of FM; BSFM15 = 15% addition of full-fat BSFM and 21.3% of FM.

[1]Brown fishmeal, 62% total protein, 16% fat, Agro-Fish, Kartoszyno, Poland.

[2]Dried black soldier fly larvae, HiProMine S.A., Poland.

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

[4]Solvent extracted 45% crude protein, 1.8% crude lipid;

[5]Gluvital, Cargill, Poland.

[6]Agro-Fish, Kartoszyno, Poland.

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

[8]Premix (g kg^{-1}): vitamin A, 1,000,000 IU; vitamin D_3, 200,000 IU; vitamin E, 1.5 g; vitamin K, 0.2 g; vitamin B_1, 0.05 g; vitamin B_2, 0.4 g; vitamin B_{12}, 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.

[9]Vitamin mix (g kg^{-1}): vitamin A, 50,000 IU; vitamin D_3, 5000 IU; vitamin E, 30.0 mg; vitamin C, 100.0 mg.

Biological material analyses

Experimental diets and whole fish bodies 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. 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 macroelements (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, while the chemical composition of the whole fish body can be found in Table 4.

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

Nutrients	CON	BSFM5	BSFM10	BSFM15
% of DM				
crude protein	45.00	45.01	45.04	45.04
crude fat	20.00	20.05	20.08	20.00
nitrogen-free extract[1]	19.40	19.41	19.28	19.37
ash	9.26	9.05	8.83	8.59
crude fibre	1.53	1.85	2.18	2.52
Ca	0.97	0.98	0.99	1.00
P	0.99	0.96	0.92	0.89
gross energy (MJ/kg)	21.77	21.79	21.79	21.77
energy/protein ratio	48.37	48.41	48.37	48.34
Amino acid profile (% of crude protein)				
arginine	4.14	4.09	4.03	3.98
histidine	2.31	2.33	2.35	2.37
lysine	4.34	4.33	4.31	4.29
tryptophan	0.78	0.75	0.72	0.69
phenylalanine + tyrosine	4.77	4.86	4.94	5.02
methionine + cysteine	1.79	1.77	1.76	1.74
threonine	3.02	3.03	3.05	3.06
leucine	6.20	6.17	6.13	6.09
isoleucine	3.47	3.45	3.43	3.42
valine	4.75	4.75	4.74	4.74

FM = fish meal; BSFM = black soldier fly larvae meal; CON = control group, without the addition of full-fat BSFM and with 30% FM; BSFM5 = 5% addition of full-fat BSFM and 27.1% FM; BSFM10 = 10% addition of full-fat BSFM and 24.3% FM; BSFM15 = 15% addition of full-fat BSFM and 21.3% FM.
[1]Nitrogen-free extract = dry matter % – (crude protein % + crude fat % + crude fibre % + ash %).

Table 4. Chemical composition of the whole body of Atlantic salmon pre-smolts fed with experimental diets

Nutrients	CON	BSFM5	BSFM10	BSFM15
Dry matter (%)	32.56	31.32	32.70	31.06
Crude protein (%)	18.83	18.41	18.61	18.48
Crude fat (%)	11.64	11.29	11.91	10.41
Ash (%)	2.11	2.13	2.04	2.30

FM = fish meal; BSFM = black soldier fly larvae meal; CON = control group, without the addition of full-fat BSFM and with 30% FM; BSFM5 = 5% addition of full-fat BSFM and 27.1% FM; BSFM10 = 10% addition of full-fat BSFM and 24.3% FM; BSFM15 = 15% addition of full-fat BSFM and 21.3% FM.

Animal handling and growth performance trial

Atlantic salmon pre-smolts were maintained at the Experimental Station of Feed Production Technology and Aquaculture in Muchocin. Water was collected from a natural watercourse, flowed through a system of filters filled with thick gravel, and then sent to two equalizing tanks (each with a capacity of 5 m^3), where it was aerated (Hiblow HP200, Hiblow, Japan) and distributed to the tanks through a system of pipes. Water parameters were recorded daily throughout the entire 60-day experiment. The temperature ranged from 14.2°C to 16.3°C, the concentration of oxygen ranged from 7.8 mg to 8.4 mg L^{-1}, and the photoperiod was maintained at 16:8 (light:dark). Fish were fed using automatic belt feeders (12 h discharge time, FIAP Fishtechnik GmbH, Germany). The feed ratio was based on a feeding chart designed for Atlantic salmon, taking into consideration the average body weight and water temperature (NRC, 2011). The trial was carried out in three replications (tanks) per diet and 25 fish per tank with a water capacity of 260 L. Fish mortality and health condition were monitored daily. All animals were weighed at 10-day intervals. To calculate the growth performance and feed utilization parameters, the following formulas were used:

1. Final Body Weight (FBW):

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

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):

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

6. Specific Growth Rate (SGR):

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

7. Survival Rate (SR):

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

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 = 3 tanks per group (20 fish/tank, 60 fish/group).

Sample collection and somatic indices

On the last day of the experiment, all fish were weighed individually, and 6 animals from each tank were euthanized and decapitated for dissection and sampling for further analysis. Measurements of the carcass, liver, spleen, total intestines, proximal intestines, distal intestines, GIT, and total fish length were recorded to calculate numerous indices:

1. Condition Factor (CF):

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

2. Hepatosomatic Index (HSI):

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

3. Relative GIT Length (GIT/FTL):

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

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

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

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

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

6. Carcass Share (CS):

$$CS\ (\%) = (\text{final carcass weight (g)})/(\text{final body weight (g)}) \times 100$$

7. Spleen Share (SS):

$$SS\ (\%) = (\text{final spleen weight (g)})/(\text{final body weight (g)}) \times 100$$

Gut histomorphology

At the end of the experiment, 30 fish were sampled from each treatment. Samples of the proximal and distal portions of the GIT 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 an addition of glycerol. The prepared samples were analysed using an AnMN-800 F microscope (OPTATECH, 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. The muscular layer thickness was defined as the average of five measurements per fish.

Colorimetric assessment and raw meat quality

During the last day of the experiment, 18 fish from each treatment were randomly chosen to take images of fresh fillets. A space with a natural daylight source was prepared. All images were taken using the Color Analysis Smartphone App v. 7.0.0. (Research Lab Tools), which allowed us to record L (lightness), a (redness index), and b (yellowness index). The RGB, chroma, and hue values were recorded based on ImageJ v.1.53 analyses.

The pH value was measured using a combined glass and reference electrode (pH 100 L; VWR International, Leuven, Belgium). Measurements were recorded in the meat of nine randomly selected fish three times: in fresh fillets directly after slaughter, after 24 h in cooled fillets, and the last time after cooking.

The cooking procedure was performed at 100°C for 10 minutes. The fillet share, cooled fillet and cooking efficiency were calculated according to the following formulas:

1. Fillet Share (FS):

$$FS\ (\%) = (\text{final fillet weight (g)}/\text{final body weight (g)}) \times 100$$

2. Cooled Fillet (CFL):

$$CFL\ (\%) = (\text{cooled fillet weight (g)}/\text{final fillet weight (g)}) \times 100$$

3. Cooking Efficiency (CE):

$$CE\ (\%) = (\text{fillet weight before cooking (g)}/\text{fillet weight after cooking (g)}) \times 100$$

Environmental sustainability assessment

The environmental sustainability assessment was calculated based on raw data for growth performance and feed utilization parameters. The relative usage of marine-derived feed materials – fish meal (FMU) and fish oil (FOU) – was calculated according to two formulas:

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

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

The fish-in-fish-out ratio (FIFO) was calculated as a practical measure of the quantity of live fish from capture fisheries required for each unit of farmed fish produced, as follows:

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

where the yield of fishmeal 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:

$$Yi = \mu + \alpha i + \delta ij$$

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

Results

Growth performance trial

In the experiment conducted on Atlantic salmon pre-smolts no significant differences were found in FBW, BWG, FI, SGR, SR, or PER among the groups. However, in group BSFM15, an increase in FCR and a decrease in LER compared to CON were reported (P = 0.0195 and P = 0.0114, respectively). The results of growth performance and feed utilization of Atlantic salmon pre-smolts are presented in Table 5.

Somatic indices

In the case of somatic indices, there were no significant differences in CF, HSI, CS, and SS among the groups (Table 6). Similarly, in terms of GIT indices, including GIT/FTL, PL/FTL and DL/FTL, no significant differences were reported. The results of those indices are presented in the graphs in Figure 1.

Gut histomorphology

All parameters represent the proximal intestine and showed significant differences among the groups. A decrease in villus height was observed among all groups fed BSFM diets (P<0.0001). In the case of villus width, only BSFM15 did not differ from the CON; moreover, BSFM5 showed a decrease, while in BSFM10, an increase in villus width was recorded (P<0.0001). In turn, CON and BSFM10 did not differ in terms of villus area; however, BSFM5 and BSFM15 showed a decrease in this item (P<0.0001). The muscular layer thickness increased with the inclusion of insects in the diets (P<0.0001).

Table 5. Growth performance, survival rate and feed utilization parameters of Atlantic salmon pre-smolts fed with experimental diets

Parameter	CON	BSFM5	BSFM10	BSFM15	SEM	P value
IBW (g)	201.2	201.0	202.0	201.1	0.3728	0.8180
FBW (g)	473.6	468.2	462.5	460.5	2.4136	0.2110
BWG (g)	272.4	267.2	260.5	259.4	2.4358	0.1910
PWG (%)	135.4	132.9	129.5	124.0	1.4618	0.1010
SR	98.7	98.7	98.7	97.3	0.5946	0.8590
FI (g)	6613	6610	6627	6610	18.0278	0.9900
FCR	0.98 b	0.99 b	1.02 ab	1.03 a	0.0078	0.0195
SGR	1.43	1.41	1.38	1.38	0.0091	0.1930
PER	2.23	2.19	2.13	2.08	0.0238	0.0678
LER	6.47 a	6.43 a	6.09 a	5.18 b	0.1641	0.0114

FM = fish meal; BSFM = black soldier fly larvae meal; CON = control group, without the addition of full-fat BSFM and with 30% FM; BSFM5 = 5% addition of full-fat BSFM and 27.1% FM; BSFM10 = 10% addition of full-fat BSFM and 24.3% FM; BSFM15 = 15% addition of full-fat BSFM and 21.3% FM; IBW – initial fish body weight; FBW – final fish body weight; BWG – fish body weight gain; PWG – percent of fish body weight gain; SR – fish survival rate; FI – feed intake; FCR – feed conversion ratio; SGR – specific growth rate; PER – protein efficiency ratio; LER – lipid efficiency ratio.

a–b – values within a row with different letters differ significantly at $P<0.05$. Values are presented as the means (n = 3/treatment), pooled standard error of the mean, SEM.

Table 6. Somatic indices of Atlantic salmon pre-smolts fed with experimental diets

Parameter	CON	BSFM5	BSFM10	BSFM15	SEM	P value
CS (%)	90.3	90.6	90.2	91.3	0.2936	0.5370
SS (%)	0.17	0.17	0.15	0.17	0.0049	0.4530
CF	0.79	0.84	0.78	0.86	0.0191	0.3630
HSI (%)	1.06	1.12	1.03	1.08	0.0206	0.4690

FM = fish meal; BSFM = black soldier fly larvae meal; CON = control group, without the addition of full-fat BSFM and with 30% FM; BSFM5 = 5% addition of full-fat BSFM and 27.1% FM; BSFM10 = 10% addition of full-fat BSFM and 24.3% FM; BSFM15 = 15% addition of full-fat BSFM and 21.3% FM; CS – carcass share; SS – spleen share; CF – condition factor; HSI – hepatosomatic index.
Values are presented as the means (n = 18/treatment), pooled standard error of the mean, SEM.

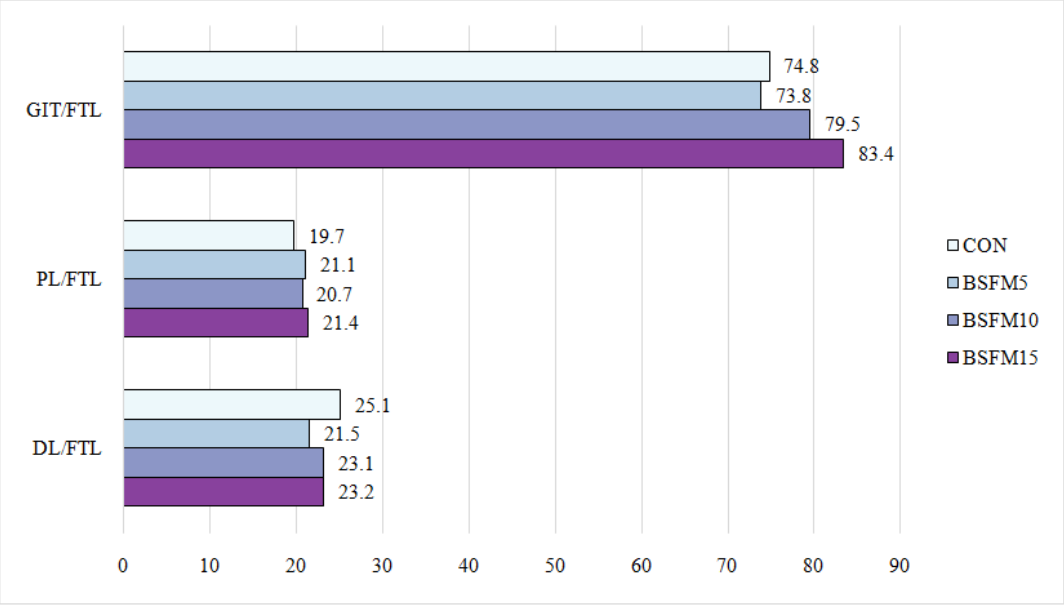

Figure 1. The effect of experimental diets on the percentage of length of individual parts of the gastrointestinal tract in the total body length of Atlantic salmon pre-smolts. FM = fish meal; BSFM = black soldier fly larvae meal; CON = control group, without the addition of full-fat BSFM and with 30% FM; BSFM5 = 5% addition of full-fat BSFM and 27.1% FM; BSFM10 = 10% addition of full-fat BSFM and 24.3% FM; BSFM15 = 15% addition of full-fat BSFM and 21.3% FM; GIT:FTL – relative gastrointestinal tract length; PL:FTL – relative proximal intestine length; DL:FTL – relative distal intestine length; no statistically significant differences were observed among the groups ($P\geq0.05$)

Table 7. Histomorphology of proximal and distal intestines of Atlantic salmon pre-smolts fed with experimental diets

Parameter	CON	BSFM5	BSFM10	BSFM15	SEM	P value
Proximal intestine						
villus height (µm)	781.04 a	592.20 b	605.56 b	530.18 b	18.5074	<0.0001
villus width (µm)	123.24 b	109.00 c	145.73 a	112.34 bc	2.6153	<0.0001
villus area (mm^2)	301.80 a	199.57 b	276.51 a	188.55 b	8.7725	<0.0001
muscular layer thickness (µm)	105.81 c	131.19 b	151.28 ab	154.79 a	4.4038	<0.0001
Distal intestine						
villus height (µm)	535.69	513.08	513.08	580.69	12.5467	0.2220
villus width (µm)	128.64 b	152.59 a	138.76 ab	144.58 ab	2.8854	0.0253
villus area (mm^2)	215.56	245.29	232.03	270.25	8.1266	0.1070
muscular layer thickness (µm)	222.08	217.27	248.84	220.76	6.0209	0.2260

FM = fish meal; BSFM = black soldier fly larvae meal; CON = control group, without the addition of full-fat BSFM and with 30% FM; BSFM5 = 5% addition of full-fat BSFM and 27.1% FM; BSFM10 = 10% addition of full-fat BSFM and 24.3% FM; BSFM15 = 15% addition of full-fat BSFM and 21.3% FM.

a–c – values within a row with different letters differ significantly at P<0.05. Values are presented as the means (n = 30/treatment for villus height, villus width and villus area; n = 15/treatment for muscular layer thickness), pooled standard error of the mean, SEM.

Table 8. Colorimetric assessment and raw meat quality of Atlantic salmon pre-smolts fed with experimental diets

Parameter	CON	BSFM5	BSFM10	BSFM15	SEM	P value
Cie L	63.1	62.9	63.0	61.5	0.7819	0.8710
Cie A	7.08	7.76	6.51	7.21	0.3585	0.6860
Cie B	13.2	13.2	12.0	12.1	0.3055	0.3040
Chroma	15.1	15.5	13.8	14.2	0.4012	0.4430
Hue	24.6	23.6	24.7	23.5	0.5076	0.7650
R	174	175	172	169	1.9686	0.7830
G	148	147	148	144	2.0398	0.8910
B	130	129	132	128	2.2386	0.9590
Meat pH 0 h	6.60	6.60	6.66	6.67	0.0245	0.6070
Meat pH 24 h	6.38	6.39	6.40	6.50	0.0355	0.6900
Meat pH after cooking	6.37	6.44	6.38	6.50	0.0333	0.5290
Fillet share (%)	53.1	53.0	53.9	53.5	0.4118	0.8630
Cooled fillet (%)	97.1	97.2	97.3	97.3	0.1113	0.7960
Cooking efficiency (%)	91.7	92.3	91.4	92.8	0.4680	0.7610

FM = fish meal; BSFM = black soldier fly larvae meal; CON = control group, without the addition of full-fat BSFM and with 30% FM; BSFM5 = 5% addition of full-fat BSFM and 27.1% FM; BSFM10 = 10% addition of full-fat BSFM and 24.3% FM; BSFM15 = 15% addition of full-fat BSFM and 21.3% FM. Values are presented as the means (n = 18/treatment for colorimetric assessment; n = 9/treatment for cooking efficiency), pooled standard error of the mean, SEM.

Table 9. Environmental sustainability parameters of Atlantic salmon pre-smolts fed with experimental diets

Parameter	CON	BSFM5	BSFM10	BSFM15	SEM	P value
FCE (g/g)	1.02 a	1.00 ab	0.98 b	0.97 b	0.0078	0.0367
FMU (g/kg)	294.5 a	274.4 b	254.8 c	229.6 d	7.3546	<0.0001
FOU (g/kg)	126.6 a	118.7 b	111.6 c	101.4 d	2.8637	<0.0001
FIFO	1.53 a	1.43 b	1.33 c	1.20 d	0.0372	<0.0001

FM = fish meal; BSFM = black soldier fly larvae meal; CON = control group, without the addition of full-fat BSFM and with 30% FM; BSFM5 = 5% addition of full-fat BSFM and 27.1% FM; BSFM10 = 10% addition of full-fat BSFM and 24.3% FM; BSFM15 = 15% addition of full-fat BSFM and 21.3% 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 = 3/treatment), pooled standard error of the mean, SEM.

The distal intestine results did not show any significant differences in terms of villus height and area or muscular layer thickness. However, villus width showed an increase in BSFM5 (P = 0.0253). All gut histomorphology parameters are presented in Table 7.

Colorimetric assessment and raw meat quality

No effect of experimental diets on colorimetric assessment results was recorded during the study. The pH value of raw meat after slaughter varied between 6.60 and 6.67. After 24 h from the end of the experiment and

after cooking, the pH of the meat decreased slightly. The numerical differences between experimental groups in the fillet share were not wide, and the values were within a range of 53.0–53.9. All results are presented in Table 8.

Environmental sustainability assessment

In terms of environmental sustainability parameters, a decrease in the FCE value was observed in the BSFM10 and BSFM15 groups ($P = 0.0367$). However, all groups with insect addition presented decreases in FMU, FOU, and FIFO ($P<0.0001$ for all parameters). All results of the environmental sustainability parameters of Atlantic salmon pre-smolts are presented in Table 9.

Discussion

A wide spectrum of results, both positive (Józefiak et al., 2019; Rawski et al., 2020, 2021) and negative (Roncarati et al., 2015; Dietz and Liebert, 2018; Dumas et al., 2018) effects of insect meal application in aquaculture, was observed over the last decade (Weththasinghe et al., 2022; Hua, 2021). However, due to insect biomass processing technology development, the products that were tested should not be compared without mentioning that fact. Thus, publications with the same product compared among species and stages of development are needed. Considering the two presented experiments conducted on Atlantic salmon, the following mode of action could be assumed. In the following study, the inclusion of BSFM in diets up to 15% did not affect growth performance. However, Atlantic salmon fries showed a physiologically lower tolerance to the BSFM content in feeds, which resulted in a decrease in final body weight in the mentioned group. In both cases, a 15% share resulted in higher FCR values, which from the livestock and aquaculture production perspective is an undesirable effect. These observations were not confirmed by nutrient digestibility or intestinal histomorphology results of Atlantic salmon fry experiments due to the lack of statistically significant differences. Nevertheless, the structure of the gastrointestinal tract of older individuals indicated a decrease in villus height in the proximal intestine, which may result in decreased protein absorption in this section, which could ultimately affect the abovementioned FCR results. Importantly and worth emphasizing, in the case of both experiments, no effect of diets on somatic indices or meat quality was found. However, one of the most important results of the conducted research is the confirmation of the environmental sustainability of insect-based feed. All groups with BSFM inclusion in the diets showed a positive effect on the FIFO ratio. The results suggest that in the nutrition of Atlantic salmon fries, it is optimal to use lower BSFM shares (up to 10%), while pre-smolts can tolerate a wider range (up to 15%) without significantly affecting growth performance. Moreover, the authors' research team has previously conducted experiments with the usage of insects in salmonid diets. An experiment using BSFM in brown trout nutrition showed no effect on growth performance or feed utilization and simultaneously improved the environmental sustainability parameters (Mikołajczak et al., 2022). In this experiment, a positive effect of diet on the hepatosomatic index was also found. Despite no differences in growth performance, crude protein and crude fat digestibility were reduced in some insect groups (especially with 20% BSFM in the diets). In turn, the usage of mealworm (*Tenebrio molitor*) and superworm (*Zophobas morio*) in sea trout nutrition did not affect the growth performance but decreased the protein efficiency ratio (Mikołajczak et al., 2020). The addition of superworm meal increased liver lipid values, which resulted in an increasing hepatosomatic index. No differences were observed in the histomorphological assessment of the intestines. Previous experiments indicate that insect meals may also modulate the microbiota of the gastrointestinal tract (Mikołajczak et al., 2020), as well as the biochemical blood parameters (Mikołajczak et al., 2020, 2022).

The results of the chemical analyses of the experimental diets confirmed the isonitrogenous, isolipidic and isoenergetic properties of the applied feeds. In terms of key nutrients, balanced diets should not disturb the proper growth performance of fish. The highest numerical differences were observed in the case of crude fibre, which increased with the higher share of BSFM in the diet. This can be explained by the chitin content, which qualifies it as a polysaccharide. Despite the fact that ash content decreased in all diets with insect inclusion, the calcium remained at a similar level. A decrease in phosphorus levels was recorded in the BSFM groups; however, the proper level of total phosphorus in the diet was maintained according to dietary requirements not exceeding 6 g/kg (Storebakken, 2009).

In the present study, the inclusion of BSFM in Atlantic salmon pre-smolt diets did not affect the final body weight (FBW), body weight gain (BWG), percent of body weight gain (PWG), feed intake (FI), specific growth rate (SGR), survival rate (SR), or protein efficiency ratio (PER). Nevertheless, a significant increase in the feed conversion ratio (FCR) was observed in the BSFM15 group. In the case of FCR, the recorded results are in agreement with Weththasinghe et al. (2021 b), where 20% inclusion of full-fat BSFM resulted in the highest FCR level. It should be noted that this effect was also accompanied by an increase in FBW, SGR, and FI, which was not observed in our study. In turn, the usage of full-fat BSFM at a level of 16% in Atlantic salmon diets resulted in a decrease in FCR and simultaneously did not show any differences in terms of FBW, SGR and FI compared to the control group (Weththasinghe et al., 2021 a). The initial body weight of the fish may contribute to these differences between the experimental results. Despite the fact that in the described studies the individuals are called pre-smolts, their body weight is only a dozen grams, while fish used in our study weighed approximately 200 g at the beginning. Considering stud-

ies on another farmed salmonid species – rainbow trout (*Oncorhynchus mykiss*), which had a higher initial body weight, the results are not so divergent. There were no significant differences in FBW, SGR, BWG or PER (Renna et al., 2017; Cardinaletti et al., 2019); however, FCR, even without significant differences, showed higher results with increasing dietary BSFM inclusion (Cardinaletti et al., 2019). Therefore, it is important during data comparison with the scientific literature to not only focus on the fish age determination or phase – fresh or saltwater but also, or even primarily, on body weight, which perhaps better describes their stage of development.

No effect on somatic indices, including carcass share (CS), spleen share (SS), condition factor (CF), or hepatosomatic index (HSI), was observed among the groups. The vast majority of scientific literature that also studied the effects of different insect diets on somatic indices of salmonids has focused mainly on HSI and VSI (Stenberg et al., 2019; Hoffmann et al., 2020; Hossain et al., 2021; Weththasinghe et al., 2021 b). Taking into account these data, as well as the established norms for HSI (Munshi and Dutta, 2017), the condition of the liver was not disturbed by either the control diet or any of the experimental groups. Despite the lack of literature data that would examine the influence of insect introduction in fish nutrition on changes in the spleen indices, the usage of BSFM in the diets of Atlantic salmon did not affect these organs compared to the control group. The results of the spleen share were slightly higher than those found in other studies. However, as the authors pointed out, smolts show lower values of spleenosomatic index, which indicate an emptying of the spleen blood cell reservoirs (Nordgarden et al., 2002). This process has probably not yet occurred in the case of our fish.

In the present study, no statistically significant differences were observed in the GIT/FTL, PL/FTL, or DL/FTL. However, a slight increase in PL/FTL was observed at the expense of DL/FTL. Because enzyme activity is highest in the proximal intestine, this effect could be considered positive because of the increasing digestion probability (Krogdahl et al., 2015). Nevertheless, the digestibility of nutrients was not the subject of this study, which was due to the difficulties associated with the collection of fish faeces, widely described in the literature (Austreng, 1978; Choubert et al., 1982; Allan et al., 1999). In the previous part of the presented experimental cycle conducted on Atlantic salmon fries, no significant differences were observed in the case of crude protein and crude fat digestibility; however, GIT somatic indices seemed to demonstrate opposite results, where DL/FTL numerically showed an increase in insect groups at the expense of PL/FTL.

In the present study, in all BSFM groups, a decrease in villus height in the case of proximal intestine histomorphology was observed; nevertheless, because BSFM10 showed the highest values in the case of villus width, this group was the only one that did not differ from the CON in the event of villus area. According to Renna et al. (2017), the dietary inclusion of partially defatted BSFM up to 40% did not induce morphological changes in the rainbow trout intestine. In turn, Dumas et al. (2018) reported that the content of partially defatted BSFM at a level of 26% led to a decrease in villus height in the anterior intestinal part, which is in line with our research. These changes in the structure of the intestinal villi can lead to a decrease in nutrient absorption (English et al., 2021). Importantly, in the aforementioned study, this effect was manifested in the growth performance – the group with the highest BSFM inclusion was also characterized by reduced BWG. Nevertheless, such observations were not recorded in our experiment.

In the distal intestine, villus width was higher in fish from the BSFM5 group. The rest of the BSFM groups did not differ from the CON group; however, a numerical increase in villus width was observed. In contrast, Dumas et al. (2018) reported no effect of BSFM inclusion on the posterior intestine of rainbow trout. According to Egerton et al. (2020), larger villi equate to larger enterocytes or an increase in their number, which provide a greater surface area for the absorption of nutrients and metabolites.

No significant differences in the colorimetric assessment of Atlantic salmon raw meat were observed in our study. These findings are in agreement with several scientific papers in which BSFM was introduced into salmonid diets (Renna et al., 2017; Secci et al., 2019; Bruni et al., 2020; Husein et al., 2021). In our research, the proportions of these parameters seem to be preserved and in line with the literature on Atlantic salmon (Secci et al., 2019; Bruni et al., 2020): a clear advantage of L and reduced shares in b and a, with b showing higher values. Simultaneously, in the case of raw meat pH, the results presented by scientific papers are not unequivocal. Bruni et al. (2020) reported much lower pH values than we obtained in our study – even at 6.08. In turn, Secci et al. (2019) presented values closer to 6.40. Moreover, our results indicate that the pH of the meat was quite stable even 24 h after the analyses and oscillated at approximately 6.40, which is in agreement with Renna et al. (2017).

Modern aquaculture diets for predatory fish such as Atlantic salmon showed a significant decrease in fish meal (FM) use from >60% in the 1990s to <20% in the 2010s (Ytrestøyl et al., 2015). Simultaneously, it has been reported that total FM replacement in salmon diets has negative consequences on production parameters (Davidson et al., 2018; Robaina et al., 2019). In the present study, FIFO values decreased with the increase in the BSFM share in the diets. Despite the fact that even with the highest inclusion of BSFM, the FIFO value still exceeded 1.0, these results are still significantly lower compared to the estimated FIFO of salmon feed in 2017 (Naylor et al., 2021). Nevertheless, data on the influence of various alternative protein sources on FIFO values and FM use in aquafeeds are still limited and need further consideration.

Conclusions

The present study showed that BSFM is a suitable feed material for Atlantic salmon proper diet balancing and may be used to decrease fish meal content. The semi-technical application of BSFM-containing diets confirmed their effectiveness during the growth phase of Atlantic salmon. According to our results, BSFM can be recommended as an alternative feed component in Atlantic salmon pre-smolt nutrition at levels up to 15%. Simultaneously, insect inclusion significantly improved the environmental sustainability of the rearing process.

Acknowledgment

We gratefully acknowledge the contributions and logistical support provided by Poznań University of Life Sciences. The authors would like to thank Silvia Nogales-Mérida, Sanan Talibov, Joanna Kowalska, Krzysztof Florczyk and Jan Banaszak for their contribution to the technical parts of the insect meal and feed preparation, the growth experiment, and data analysis.

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Received: 6 VI 2022
Accepted: 9 IX 2022