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ORIGINAL PAPER

The live fossil and nutritional novelty – the effects of insect meal on growth performance, feed utilisation, fish development and trophic environmental sustainability in Atlantic sturgeon (*Acipenser oxyrhynchus*) juveniles production

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ABSTRACT. This study investigates the inclusion of partially defatted black soldier fly (*Hermetia illucens*) meal (BSFM) in the diet of Atlantic sturgeon (*Acipenser oxyrhynchus*), evaluating its effects on feed physical properties, growth performance, feed efficiency, sustainability, and organosomatic and morphometric indices. The experiment examined the replacement of fish meal (FM) with BSFM at inclusion levels of 15%, substituting 43.75% of FM and 30%, substituting 87.5% FM. While BSFM incorporation influenced certain physical properties of the feed, it had no effect on the fish survival rate or growth performance. Key growth parameters, including body weight, weight gain, specific growth rate, percentage weight gain, feed conversion ratio, and protein efficiency ratio, did not change compared to the control group. Moreover, the reduction in FM use contributed to improved aquaculture environmental sustainability. While significant differences were observed in hepatosomatic index, no such differences were found in condition factor, viscerosomatic index, relative gastrointestinal tract length, or fin indices. These findings suggest that BSFM represents a viable alternative protein source in the conservative aquaculture of Atlantic sturgeon.

Introduction

An Atlantic sturgeon (*Acipenser oxyrhynchus*) is one of the largest and most endangered fish species in Europe (Hilton et al., 2016). The last records about this species in Poland are dated back to the 1970s in the Vistula River; currently, it is recognised as extinct in the wild in the country (Kolman et al., 2011). Its reintroduction projects are based on hatchery-captive raised fish (Hilton et al., 2016). Thus, maintenance and nutrition protocols must be developed for successful species propagation and further restocking.

When modern fish feeding strategies developed, the over-exploitation of pelagic fishes and the replacement of fish meal (FM) and fish oil (FO) in aquafeeds became the priority (Tacon and Metian, 2015). The main purpose of this action is to expand aquaculture production capacity independent from marine-derived resources to remain competitive and sustainable. However, it should also be applied to conservative aquaculture to reach its low environmental footprint (Quang et al., 2022). Regarding captive-raising fish for restocking, there is a noticeable problem with adapting individuals to the natural environment. At the initial point during early

rearing, fish live in an environment different from their natural ecosystem in which the species has evolved (Anderson et al., 2022). Due to the above-mentioned facts, aquatic animals raised in hatcheries often perform poorly after releasing to natural conditions compared to their wild counterparts. While numerous factors could contribute to this discrepancy, a recurring observation is that artificial rearing conditions lead to maladaptive behaviours for survival in the wild (Näslund, 2021). The issue of foraging inefficiencies is often attributed to the lack of recent exposure to natural prey. In the wild, the food items encountered by released animals are typically unfamiliar, which can reduce their ability to forage on them effectively (Sundström and Johnsson, 2001). It is possible to improve rearing conditions and better equip individuals for survival in the wild through ex-situ strategies. These approaches often include 'training' techniques, such as sensory stimulation, pre-release conditioning, and incorporating natural elements into the hatchery environment (Kleiman, 1989) or modified feed application to avoid unwanted fish swimming behaviour (Gebauer et al., 2021). For the initial feeding of sturgeon fry, natural food sources such as insects and other invertebrates including chironomids, and oligochaetes are commonly used (Vedrasco et al., 2002). Extensive research is essential to develop feeds that yield positive results in fry, juveniles, and adults. This information suggests that insect biomass products represent one of the most important alternatives for conventionally used protein and energy components. Among the most promising are raw materials from *Hermetia illucens* (Józefiak et al., 2019; Rawski et al., 2020, 2021; Mikołajczak et al., 2023; Mashood et al., 2025). Olfaction contributes significantly to the ability of the Acipenseridae to sense its environment and is a crucial for feeding (Edwards, 2022). The olfactory system in fish is highly sensitive, capable of detecting substances at concentrations as low as nano- or picomolar levels. It is one of the most distant sensory systems within these animals. Smell plays a vital role in triggering behaviours linked to essential life functions, including not only feeding but also reproduction, defence, and migration of sturgeons (Døving, 1986).

Findings suggest that olfactory sensory deprivation during the captive rearing may influence early larval and juvenile post-releasing development, potentially leading to low-performance phenotypes in hatchery programs. Thus, the release of fish raised in non-natural environments and fed with FM-based diets could result in a mismatch between fish and the environment in which they are introduced (Edwards, 2022). In this context, it should be noted that insects are often a natural component of predatory and omnivorous fish diets (Tran et al., 2015). Increased feed palatability with insect meal supplementation has been observed in several species, likely due to the presence of specific aromatic compounds, favourable free amino acid profiles, and the lower molecular weight of insect proteins (Ng et al., 2001; Rawski et al., 2020; Terrey et al., 2021; Kowalska et al., 2022). It turns out that due to the feeding ecology of sturgeons, insect-based meals can be used as natural food substitutes and feed intake stimulants because of a strong attraction to the scent or flavour of insects (Rawski et al., 2020). That is why *H. illucens* meal may be used as sensory stimulation that could prepare Atlantic sturgeon to forage in the natural environment, ameliorate cognitive learning skills, and enrich behaviour in aquatic animals reared for restocking. During the increasing research interest in applying alternative feed components, it is essential to consider not only productivity but also fish welfare and its condition, physical properties of feeds, nutritional values, and environmental sustainability of parental and stocking material rearing. In the literature, there are studies available on the use of feeds containing black soldier fly meal (BSFM) in the nutrition of sturgeons, mainly *Siberian and Russian sturgeon* (Ushakova et al., 2020; Gasco et al., 2017; Caimi et al., 2020a;b; Rawski et al., 2020; 2021). Although no information was found on the use of this component in the nutrition of Atlantic sturgeon, this is likely the first report on the impact of using this meal in the nutrition of *Acipenser oxyrhynchus*.

Therefore, this study aimed to evaluate the effectiveness of insect meals in the diet of juvenile Atlantic sturgeon, focusing on fish growth performance, overall fish health, somatic and fin indices and to develop suitable and environmentally sustainable feeds for practical application in producing fingerlings with optimal growth, health, and survival potential. The hypothesis was formulated stating that the replacement of FM with BSFM at inclusion levels of 15%, substituting 43.75% of FM and 30%, substituting 87.5% FM in the diet of juvenile Atlantic sturgeon would not negatively affect its growth performance, overall health, and somatic and fin indices while contributing to the development of environmentally sustainable feed formulations optimised for rearing high-quality fingerlings of this species.

Material and methods

Ethics statement

Experiments involving live animals were conducted in strict accordance with the guidelines set by the National Ethics Commission (Warsaw, Poland). All research personnel were trained in animal care, handling, and euthanasia techniques. Fish health, welfare and environmental conditions in the experimental tanks were monitored twice a day through visual observation of the animals' behaviour and assessment of water quality parameters, including oxygen saturation, temperature, and water flow. At the end of the experiment, 180 fish, 60 per treatment (10 fish per tank) were euthanized to collect tissue samples and measure internal organ morphology. Fish were anesthetized with an overdose of tricaine methane sulfonate (MS 222, 300 mg/l) via prolonged immersion (Topic Popovic et al., 2012). Following sedation, the animals were decapitated in accordance with the American Veterinary Medical Association Guidelines for the Euthanasia of Animals (Leary et al., 2013). All fish were not fed for 24 h prior to dissection. In compliance with Polish law and the EU directive (2010/63/EU), the experiments conducted in this study did not require approval from the Local Ethical Committee for Experiments on Animals in Poznań.

Insect partially defatted meals and diets preparation

The nutritive values of *Hermetia illucens* partially defatted larvae meal and FM used in the experiments are presented in Table 1. *H. illucens* meal was produced at HiProMine S.A., Robakowo, Poland. The BSFL feed was normalised in terms of dry matter (DM) content by the addition of wheat middling (17%) to a fresh vegetable and fruit mix consisting of apples (15%), carrots (50%), potatoes (15%), and cabbage (20%) and established at 22% DM. Fresh vegetable and fruit pre-consumer waste was ground (2000 rpm, 55 kW; HPM milling system, Robakowo Poland) to allow passage through a 2-mm screen and offered *ad libitum* to BSFL. Substrates were not contaminated by any animal products in accordance with an EC regulation (no 1069/09). At the prepupal stage (10th day of rearing), larvae were harvested, sieved through a 3-mm screen, and washed with water on a drum separator at 90 °C for 10 min (HPM cleaning system, Robakowo, Poland). A batch of BSFL was dried for meal production at 130 °C for 1 h and then at 80 °C for 23 h until a constant weight in an air flow dryer (HiProMine S.A., Poznań, Poland).

Table 1. Nutritive value and amino acid profile of *Hermetia illucens* partially defatted larvae meal and fish meal used in the experiment

Nutrient	Fish meal	*Hermetia illucens* partially defatted meal[1]
% of dry matter		
crude protein	65.47	54.29
crude fat	16.30	12.05
crude fibre	0.90	10.05
crude ash	15.79	11.31
nitrogen free extract	1.54	12.30
Amino acid, g/100 g of crude protein		
aspartic acid	7.79	8.61
glutamic acid	11.74	10.24
serine	3.87	3.98
glycine	11.38	5.03
histidine	2.08	2.55
arginine	6.05	4.31
threonine	3.70	3.72
alanine	6.67	7.28
proline	5.93	5.27
tyrosine	2.55	5.60
valine	4.03	5.65
isoleucine	3.30	3.95
leucine	5.57	6.23
phenylalanine	3.27	3.74
lysine	5.71	5.04

[1] *Hermetia illucens* partially defatted meal is estimated on the empirical nitrogen-to-protein conversion factor (Kp = 5.44)

Then, it was partially defatted using thermo-mechanical processing and the defatting efficiency was evaluated according to the internal analytical standards. BSFM was stored at 4 °C before use for feed preparation.

Chemical analyses of the BSFM, FM and feeds were performed before diet formulation in the chemical laboratory of the Department of Animal Nutrition (Poznan University of Life Sciences) as previously described in detail by Rawski et al. (2020). Analysis of the amino acid composition of the proteins was performed to assess the amount of protein and amino acids, as well as to establish the conversion factor (Kp), which was calculated according to Rawski et al. (2020):

$$\mathrm{Kp} = \left(\frac{\text{total amino acid content}}{\text{total protein content based on N} \times 6.25}\right) \times 6.25.$$

Accordingly, in the presented experiment, based on the analysis of BSFM, it was calculated as:

$$544 = \left(\frac{468.9}{538.1}\right) \times 6.25,$$

where: 468.9 g − sum of amino acids in 1000 g of BSFM DM; 538.1 g − total protein content in 1000 g of BSFM calculated on an N × 6.25 basis; 6.25 − traditionally used Kp; 5.44 − Kp calculated for BSFL.

In total, three diets were formulated: the control diet (CON) contained 300 g/kg of FM. In two experimental treatments, 150 and 300 g/kg of *H. illucens* partially defatted meal was introduced (H15, H30, respectively). Diets were prepared using extrusion processing with a twin-screw warm extruder at the Experimental Station of Feed Production Technology and Aquaculture in Muchocin of the Poznan University of Life Sciences. The temperature settings for the extruder's cylinder were 90 °C in the zone where pressure builds and 120 °C in the high-pressure zone, with the head temperature set at 130 °C. The screw operated at a speed of 52 rpm, and the nozzle used had a diameter of 3 mm. During postproduction, FO was added by vacuum coating (Rollermac BA 15 FR aut. Pomati Group S.R.L, Codogno, Italy). The compositions, nutritive values and amino acid profile of the experimental diets are presented in Tables 2 and 3.

Table 3. Nutritive value and amino acid profile of the experimental diets applied in the growth trial with *Acipenser oxyrhynchus* juveniles

Group	CON	H15	H30
Nutrient, % dry matter			
crude protein	46.0	45.9	45.8
crude fat	11.5	11.6	11.5
nitrogen free extract	26.5	25.7	25.4
crude ash	9.0	8.9	9.1
crude fibre	0.7	1.3	2.5
phosphorus	0.8	0.8	0.8
calcium	1.3	1.3	1.3
gross energy, MJ/kg	20.4	20.1	20.2
E/P, kJ/g of crude protein	44.34	43.79	44.10
Amino acid, g/100 g of crude protein N x 6.25			
arginine	4.29	4.24	4.18
histidine	2.36	2.38	2.40
lysine	4.78	4.76	4.75
tryptophan	0.78	0.75	0.72
phenylalanine+tyrosine	4.74	4.82	4.90
methionine+cystine	1.78	1.77	1.75
threonine	2.83	2.84	2.86
leucine	6.19	6.15	6.11
isoleucine	3.39	3.38	3.36
valine	4.67	4.66	4.65

CON – control feed, BSFM – black soldier fly meal, H15 – experimental feed with 150 g/kg partially defatted BSFM, H30 – experimental feed with 300 g/kg partially defatted BSFM, E/P – gross energy to crude protein ratio

Feed physical properties

All feed analyses were conducted at a temperature of 21 °C and controlled air humidity below 60% (Toyotomi, YD-C312, Nagoya, Japan). The expansion ratio (ER) was measured as the ratio of pellet diameter to extrusion matrix perforation diameter. The analysis was performed on 20 pellets from each production batch using an electronic caliper (Yato, YT-7201, Shanghai, China) with an accuracy of 0.01 mm. ER was calculated according to the formula used in the scientific literature (Khater et al., 2014), which was previously used for extruded pellets containing *H. illucens* meal and for evaluating the use of other animal-derived feed materials (Das et al., 1993; Irungu et al., 2018):

$$\text{ER (\%)} = \left(\frac{\text{pellet diameter (mm)}}{\text{matrix diameter (mm)}}\right) \times 100.$$

Sinking velocity (SV) was measured according to a modified version of the protocol (Das et al. 1993). A water column with a height of 1050 mm was filled to 1000 mm. Twenty randomly selected pellets from each experimental feed group were individually dropped into the water. The time taken to reach the bottom of the water column was measured with an accuracy of 0.1 s. The following formula was used for calculations:

$$\text{SV (s/1000 mm)} = \frac{\text{sinking time (s)}}{\text{water column height (mm)}}.$$

The bulk density analysis of the pellet (PD) was conducted using a modified procedure described by Irungu et al. (2018). A total of 3 randomly taken 100 ml feed samples (W) were weighed using a scale (RADWAG PS 06.R2), and the density was expressed in g/dm³ according to the following formula:

$$\text{PD (g/l)} = \frac{\text{sample weight (g)}}{\text{l}}.$$

Fish rearing conditions

The experiment was performed in an experimental recirculation aquaculture system (eRAS). A growth trial was carried out using 1800 juvenile Atlantic sturgeons (6 replicates per treatment) with an average body weight of 10.3 ± 1.4 g. The fish were acclimatised to the experimental conditions for a week and randomly divided into 18 fibreglass laminate tanks, each with a diameter of 150 cm and a height of 50 cm. The volume of water in a single tank was 680 dm³ (147 fish/m³). All tanks were equipped with individual water inlets and bottom drains. Each tank represented a separate replication, and their arrangement was designed to ensure the most uniform distribution of surrounding environmental factors. The experiment was conducted over 50 days; during the entire experimental period, the mean daily water temperature was 20 ± 2 °C, the mean level of oxygen dissolved in water was 8.50 mg/dm³ (WTW Multi Line P4 with an optical oxygen sensor, WTW, FDO 924, 99, Weilheim, Germany), the mean pH of water was 8.10

(WTW Multi Line P3 pH meter, WTW, Weilheim, Germany), the mean conductivity of water was 784 µS (HM Digital Inc., EC-3, Redondo Beach, CA, USA), the mean content of ammonia was 0.03 mg/dm^3, the mean content of NO_2 was 0.13 mg/dm^3, and the mean content of NO_3 was 13.7 mg/dm^3 (Merck MColortest cat no. 1144230002, 1144240001, and 111170.0001, Merck Darmstadt, Germany). The photoperiod was maintained at 16 h of light and 8 h of darkness throughout the experiment, with light occurring from 07:00 to 23:00.

Growth performance, feed utilization, organosomatic and morphometric indices

To reduce stress caused by contact with personnel, the animals were fed by automatic band feeders (12 h discharge time, FIAP Fishtechnik GmbH., Ursensollen, Germany). A feed dose was given to the fish according to their body weight and consumption on the previous days. The fish mortality and health condition were monitored daily. For feed intake control and accuracy, fish were weighed every 10 days, and the feeding rate was corrected according to body weight and water temperature, as described by Hung et al. (1993). The effects of the diets were evaluated based on fish biomass, feed intake, diet utilisation, and dissection of internal organs. Equations recommended by Hardy and Barrows (2002) were used to calculate growth performance. The calculated parameters included final body weight (FBW), body weight gain (BWG), specific growth rate (SGR), relative body weight gain (PWG), feed intake (FI), feed conversion ratio (FCR), protein efficiency ratio (PER), and survival rate (SR). The formulas used in the experiment are presented below:

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

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

$$\text{SGR}\left(\frac{\%}{\text{day}}\right) = \left(\frac{\ln \text{final body weight} - \ln \text{initial body weight}}{\text{number of feeding days}}\right) \times 100.$$

$$\text{PWG (\%)} = \left(\frac{\text{final body weight (g)} - \text{initial body weight)}}{\text{number of fish in the tank}}\right);$$

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

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

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

$$\text{SR (\%)} = \left(\frac{\text{final number of live fish}}{\text{initial number of live fish}}\right) \times 100.$$

Somatic indices used to assess the impact of diet on internal organs and fish condition were analysed following the methodology outlined by Piccolo et al. (2017). The indices included: CF – condition factor, VSI – viscerosomatic index, HSI – hepatosomatic index and RGL – relative gastrointestinal tract (GIT) length. They were calculated according to the formulas presented below:

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

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

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

$$\text{RGL (\%)} = \frac{\text{total GIT length (mm)}}{\text{fish total length (mm)}} \times 100.$$

To determine the relative lengths of the fins, the following fin indices were calculated: relative pectoral fin length (PI), relative dorsal fin length (DI), and relative pelvic fin length (VI) according to the methodology described by Bosakowski and Wagner (1994):

$$\text{PI} = \frac{\text{pectoral fin length (mm)}}{\text{total fish length (mm)}} \times 100;$$

$$\text{DI} = \frac{\text{dorsal fin length (mm)}}{\text{total fish length (mm)}} \times 100;$$

$$\text{VI} = \frac{\text{pelvic fin length (mm)}}{\text{total fish length (mm)}} \times 100.$$

Trophic environmental sustainability

The trophic environmental sustainability indicators of fish farming were calculated based on raw data regarding growth and feed utilisation according to scientific literature (Rawski et al., 2020; Stejskal et al., 2020). Feed conversion efficiency (FCE) was used as a fish growth index of each feed. It provides the reverse of the FCR, indicating the amount of fish

weight gain per kg of feed consumed. This was calculated using the formula described by Sunde et al. (2004):

$$\text{FCE (g/g)} = \frac{\text{body weight gain (g)}}{\text{feed intake (g)}}.$$

The relative utilisation of marine-derived feed materials (FM and FO) – FM use (FMU) and FO use (FOU) – was calculated using the following formulas:

$$\text{FMU}\left(\frac{\text{g}}{\text{kg}}\text{of fish gain}\right) = \left(\frac{\text{fish meal share in the diet}\left(\frac{\text{g}}{\text{kg}}\right)}{\text{fish intake}\frac{\text{g}}{\text{kg}}\ \text{weight gain (g)}}\right) \times 100;$$

$$\text{FOU}\left(\frac{\text{g}}{\text{kg}}\text{of fish gain}\right) = (\text{fish oil share in the diet (g/kg)} \times \left(\frac{\text{feed intake (g)}}{\text{body weight gain (g)}}\right).$$

The fish-in fish-out (FIFO) ratio was calculated using the following formula:

$$\text{FIFO} = \left(\frac{\text{level of fish meal in the diet (g/kg)} + \text{level of fish oil in the diet (g/kg)}}{\text{yield of fish meal from wild fish (g/kg)} + \text{yield of fish oil from wild fish (g/kg)}}\right) \times \left(\frac{\text{feed intake (g)}}{\text{body weight gain (g)}}\right).$$

where: the yield of FM from wild fish was assumed to be 225 g/kg of fresh fish weight and the yield of FO from wild fish was assumed to be 50 g/kg of fresh fish weight (Crampton et al., 2010; Stejskal et al., 2020). The value of FIFO ≤ 1 was assumed to be environmentally sustainable, i.e., not causing higher fish removal than production (Tacon and Metian, 2008; Naylor et al., 2009; Jackson, 2009; Quang et al., 2022).

Statistical analysis

In the case of feed physical properties, n/treatment is given in the methodological descriptions for each parameter. For growth performance, feed utilization and trophic environmental sustainability the experimental unit was the tank n/treatment (n/treatment = 6). In the case of organosomatic and morphometric indices, the experimental unit was the fish thus, n/treatment = 60. All obtained data for growth performance, feed physical properties, sustainability and fin indices were tested for normal distribution using the Kolmogorov-Smirnov test. Analysis of variance homogeneity (ANOVA) was conducted using Bartlett's test. The significance of differences between groups was determined by Duncan's multiple range test at a significance level of $P \leq 0.05$. The calculations were performed using SAS 9.4 software (Cary, NC, USA). The following general model was used:

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

where: Yi – observed dependent variable; μ – overall mean; αi – effect of insect inclusion; δij – random error (according to Rawski et al. (2016).

Results

Fish and *Hermetia illucens* meal, experimental feed composition and physical properties

The chemical compositions of *H. illucens* partially defatted meal and FM are given in Table 1. BSFL meal was characterised by a lower crude protein, fat and ash content than that of FM. However, the crude fibre and nitrogen-free extract levels were higher. The same extrusion procedure was successfully used to produce feed for all treatments. The experimental diets are reported in Tables 2 and 3 in terms of composition and nutritive value. They were isoprotein and isoenergetic and met the nutritional requirements of juvenile sturgeons (Webster and Lim, 2002). The crude protein (45.8–46.0%), crude fat (11.5–11.6%), crude ash (8.9–9.1%) as well as gross energy (20.1–20.4 MJ/kg) levels were comparable among treatments. The levels of FM replacement in experimental feeds reached 43.75% for H15 and 87.5% for H30 treatments.

The physical properties of experimental feeds are shown in Table 4. The longest feed particles were recorded in H15 ($P < 0.0001$) in comparison to control (CON) and H30 groups. The H30 feed had lower expansion ratio values compared to CON and H15 ($P < 0.0001$). The bulk density was the highest in the control feed, and a further increase in the BSFM proportion caused a significant decrease in pellet density.

Growth performance, feed utilisation, organosomatic and morphometric indices

Throughout the experiment, no fish mortality was recorded, and no significant differences in individual body mass were observed between the groups. The final individual body weight of the juvenile Atlantic sturgeon varied between 42.6

Table 4. Feed physical properties of experimental diets containing partially defatted BSFM

Group	CON	H15	H30	SEM	*P*-value
Parameters					
pellet length, mm	7.11[b]	10.72[a]	6.67[b]	0.281	<0.0001
expansion ratio, %	38.98[a]	37.63[a]	30.13[b]	0.916	<0.0001
sinking velocity, s/100 cm	12.96[b]	18.72[a]	18.38[a]	0.580	<0.0001
bulk density, g/dm^3	65.96[a]	62.67[b]	57.99[c]	0.634	<0.0001

BSFM – black soldier fly meal, CON – control feed, H15 – experimental feed with 150 g/kg partially defatted BSFM, H30 – experimental feed with 300 g/kg partially defatted BSFM, SEM – standard error of the mean; $P < 0.05$ means that the data is significantly different; [abc] – means with different superscripts are significantly different at $P < 0.05$

and 43.6 g ($P = 0.86$), with the BWG increase ranging from 30.5 to 31.6 g per fish ($P = 0.97$). The percentage weight gain (PWG) was not different among the treatments oscillating from 250 to 263% ($P = 0.84$). The specific growth rate (SGR) of the fish reached very similar values across all experimental groups ($P = 0.47$). Among all treatments, there were no significant differences in FCR ($P = 0.97$) and protein efficiency ratio ($P = 0.87$) as well (Table 5).

Among all treatments, there were no significant differences in condition factor ($P = 0.77$), viscerosomatic index ($P = 0.34$) and relative GIT length ($P = 0.82$). However, significant differences between the experimental groups were observed in the hepatosomatic index. The highest HSI was recorded in H15 treatment, it was not different from the control group but it was significantly higher than in H30 group ($P = 0.05$). Among all treatments, there were no significant differences in relative pectoral fin length (PI) ($P = 0.15$), relative dorsal fin length (DI) ($P = 0.27$) and relative pelvic fin length (VI) ($P = 0.62$) (Table 5).

Trophic environmental sustainability sustainability

There were no significant differences in FCE ($P = 0.55$) and in relative FO use (g/kg of fish gain) ($P = 0.57$). The relative FM usage per kg of fish gain decreased with the increasing inclusion of BSFM in the diets ($P < 0.0001$). In comparison with the CON group, the FMU per kg of fish gain was decreased by 43.52% in H15 and 87.32% in H30. Among all treatments, a FIFO ratio higher than 1 was present only in CON group. With the increasing share of BSFM in the diet, FIFO values decreased by 39.42% (H15) and 79,81% (H30) compared to CON ($P < 0.0001$) (Table 6).

Table 5. Survival rate, growth performance, feed utilization, somatic and fin indices of Acipenser oxyrhynchus juveniles fed experimental diets containing partially defatted BSFM

Group	CON	H15	H30	SEM	*P*-value
Survival rate, %	100	100	100	-	-
Growth performance and feed utilisation					
final body weight, g	43.6	42.6	42.7	2.652	0.86
body weight gain, g	31.6	30.6	30.5	1.694	0.97
percent of body weight gain, %	263.3	253.2	250.6	14.167	0.84
specific growth rate, %/day	2.15	2.10	2.09	0.146	0.47
feed conversion ratio	0.82	0.82	0.83	0.097	0.97
protein efficiency ratio	3.07	3.04	3.0	0.192	0.87
Somatic indices					
condition factor	0.28	0.28	0.28	0.002	0.77
viscerosomatic index, %	4.46	4.44	4.16	0.071	0.34
hepatosomatic index, %	2.41[ab]	2.48[a]	2.21[b]	0.031	0.05
relative GIT length, %	68.3	68.4	66.7	2.821	0.82
Fin indices					
pectoral fin length, %	10.24	9.53	9.10	0.438	0.15
relative dorsal fin length, %	6.98	7.47	7.25	0.264	0.27
relative pelvic fin length, %	7.64	7.94	7.85	0.331	0.62

BSFM – black soldier fly meal, GIT – gastrointestinal tract, CON – control feed, H15 – experimental feed with 150 g/kg partially defatted BSFM, H30 – experimental feed with 300 g/kg partially defatted BSFM, SEM – standard error of the mean; $P < 0.05$ means that the data is significantly; [abc] – means with different superscripts are significantly different at $P < 0.05$

Table 6. Trophic environmental sustainability of *Acipenser oxyrhynchus* production with the use of experimental diets containing partially defatted BSFM.

Group	CON	H15	H30	SEM	*P*-value
Parameter					
FCE	1.22	1.22	1.20	0.007	0.55
FMU	262.4[a]	148.2[b]	33.27[c]	22.709	<0.0001
FOU	24.95	24.70	24.60	0.133	0.57
FIFO	1.04[a]	0.63[b]	0.21[c]	0.082	<0.0001

FCE – feed conversion efficiency (g/g), FMU – relative fish meal use (g/1 kg of fish gain), FOU – relative fish oil use (g/1 kg of fish gain), FIFO – fish-in fish-out ratio, BSFM – black soldier fly meal, CON – control feed, H15 – experimental feed with 150 g/kg partially defatted BSFM, H30 – experimental feed with 300 g/kg partially defatted BSFM, SEM – standard error of the mean; $P < 0.05$ means that the data is significantly; [abc] – means with different superscripts are significantly different at $P < 0.05$

Discussion

The adaptation of juvenile Atlantic sturgeons reared in captivity to natural conditions is a key element for the successful restoration of this species (Bogacka-Kapusta et al., 2012). It is important to develop effective feeds. Prior to introducing novel feed components such as defatted *H. illucens* meal

into conventional aquaculture or commercial feed production, it is essential to evaluate their nutritional properties and technological applicability. Due to the lack of data on the use of BSFM in Atlantic sturgeon feeding, most of the obtained results were compared to the knowledge acquired in the experiments related to Siberian sturgeon. However, only a few studies not only assess the impact of feeds containing alternative ingredients on fish growth and physiological parameters but also evaluate the technological properties of the extrudates used (Irungu et al., 2018; Rawski et al., 2020; Weththasinghe et al., 2021). Thus, the experiment presented a holistic approach to the impact of BSFM use in Atlantic sturgeon nutrition. A key limitation in comparing study outcomes is the variability in the chemical composition of *H. illucens* meal, influenced by insect diet, genetic background, developmental stage, and processing methods (Oddon et al., 2024). This study used a partially defatted meal, which differs in chemical composition compared to the full-fat meal used in Rawski et al. (2020) experiment. The applied Kp factor for BSFM increased the accuracy of estimating the nutritional value of the feed by the reducing the overestimation in the total protein level. That occurs when using insect biomass materials are used; due to the presence of chitin, which, despite not being a protein, contains nitrogen (Janssen et al., 2017). From the perspective of feed production technology, the impact of insect meal on feed physical properties is a frequently overlooked topic, which complicates the assessment of innovative feeds for practical aquafeed production and water quality management – especially in recirculation of aquaculture systems (Yildiz et al., 2017). To assess the quality of the extrusion process, the length of the feed pellet was considered a measure of the degree of breakability, where the greater the length, the lower the breakability of the extrudate. The shortest length was observed in the H30 and CON groups, attributed to the possible uneven gelatinisation of starch. Noticeable is the significant increase in both the length and expansion of the H15 feed. It was found that the expansion ratio of the pellet increased with the higher inclusion of full-fat BSFM (Rawski et al., 2020), which contradicts the results obtained. Previous studies have also reported reduced pellet expansion with increasing BSFM inclusion (Irungu et al., 2018; Weththasinghe et al., 2021). This discrepancy can be attributed to the differing wheat gluten content in the experimental feeds. It has been shown that oil can be used to improve the gluten network and processing of gluten-based products with a denser network, and we hypothesised that the variation of disulfide bond and secondary structure of gluten with higher fat content results in gluten aggregation (Jia et al., 2019). H15 feed contained a higher proportion of FM compared to H30 feed, and since FM has a higher fat content, this contributed to improved feed expansion. However, during the extrusion process, the aggregation of gluten particles in the mixture needs further investigation and confirmation (Chen et al., 2021). In the study of Rawski et al. (2020) it was observed that the increasing inclusion of BSFM in diets reduced pellet bulk density and decreased sinking velocity, likely due to a greater pore formation within the pellet structure, which confirms the current results. Irungu et al. (2018) suggest that feeds with 9 to 27% BSFM inclusion will suit bottom-feeding fish, which is representative of the Atlantic sturgeon. The abovementioned authors explained that the moisture level of pre-extrusion materials may affect feed physical properties. However, in this study, changes in density should not be justified by different moisture levels since the moisture content was balanced across all experimental feeds. The literature often uses floatability analyses of extrudates (Irungu et al., 2018) to assess the pellet sinking time. In the mentioned study, decreasing floatability was noted with increasing levels of BSFM in the feeds, which contradicts the obtained results. This discrepancy is likely due to the lower crude fat content in the tested H15 and H30 feeds compared to the diets used in the Irungu et al. (2018) study, as the increase in fat content could be responsible for the decrease in pellet expansion and thus their floatability. Despite the decrease in pellet expansion and bulk density with the inclusion of insect meal, no noticeable impact on fish growth performance was observed. This is likely because the pellets maintained sufficient quality within the critical period between their release and consumption by the fish.

To our knowledge, no studies using BSFM as a protein source have been conducted with Atlantic sturgeon. Our results indicate that up to 87.5% of FM replacement by partially defatted BSFM can be included without impairing the survival rate and growth performance of this species. For all parameters related to growth performance and feed utilisation, such as BW, BWG, SGR, PWG, FCR, and PER, no differences were observed with 15% and 30% BSFM inclusion in the diets. This proves that the experimental feeds possess satisfactory physical properties and nutritional value for Atlantic sturgeon. The growth performance comparable with the control group – FM-based, can largely be attributed to the functional role of insect protein, which

includes antimicrobial peptides (AMPs) and fat composition, particularly the high content of lauric acid, along with the prebiotic properties of chitin (Moretta et al., 2020; Rawski et al., 2025). The results are contradictory to those of Caimi et al. (2020a), who found that defatted BSFM negatively impacted growth performance and feed utilisation parameters in Siberian sturgeon. It is important to note that the methodology employed in the present study differed from that used by Caimi et al. (2020a,b), which may partly explain the discrepancies observed in the results. While their trial lasted 118 days, the duration of the present experiment was 50 days. Furthermore, they conducted their research on Siberian sturgeon (*Acipenser baerii*), whereas the current study focused on Atlantic sturgeon (*Acipenser oxyrinchus*). The initial fish size also differed substantially, with a higher average BW reported in Caimi et al. (2020a,b), in addition to differences in feeding strategies between the two experiments. Moreover, in referred study sturgeons were maintained in suboptimal water temperature 13 ± 1 °C using flow-through system, while in our study fish were raised in RAS system characterised by water temperature of 20 ± 2 °C. These methodological differences could have influenced the outcomes and limit the direct comparability of results. Therefore, conducting further studies on the same species under standardized environmental and methodological conditions is recommended to improve consistency and reliability across research findings. Notably, the fish did not exhibit decreased feed intake during our experiment. As the authors themselves suggest in their study, the negative impact of defatted BSFM on growth performance and feed utilisation in fish could be due to the chitin content of the diets (Caimi et al., 2020a), as low levels of chitin could decrease feed consumption in fish, directly affecting all growth indicators (Kroeckel et al., 2012). However, the proven lack of negative impact of BSFM use in Atlantic sturgeon diets is consistent with the results obtained in the studies by Józefiak et al. (2019) and Rawski et al. (2020).

Assessing the health and condition of fish intended for restocking remains crucial. In our study, we analysed the condition factor, viscerosomatic and hepatosomatic indexes, GIT length as well as fin indices, and the results may provide valuable insights into the comprehensive impact of *H. illucens* meal in Atlantic sturgeon nutrition on all key parameters related to the quality of the produced juveniles. A statistically significant effect of increasing BSFM inclusion in the diets was observed only on the hepatosomatic index. However, differences were noted between the H15 and H30 groups. This effect can be explained by the different levels of lauric acid and AMPs in the experimental feeds. Various types of AMPs play a significant role in maintaining animal health, including that of fish, as they exhibit strong antibacterial, antifungal, and antiviral activities, which can significantly boost their immune system (Xia et al., 2021). Additionally, lauric acid has antibacterial and anti-inflammatory properties (Borrelli et al., 2021). It was noted that the increasing inclusion of BSFM in the diet could increase the hepatosomatic index, but these changes were not statistically significant (Józefiak et al., 2019; Rawski et al., 2020). The beneficial effects of AMPs and lauric oil, combined with a reduced proportion of FM in H30 with a higher fat content than *H. illucens* meal, led to a decrease in hepatosomatic indices due to reduced liver degeneration and lower levels of inflammation. However, it is important to note that no group showed statistically significant differences compared to the control group. This leads to the general conclusion that including insect meal in the feed does not impair fish health, as evidenced by the absence of differences in other parameters. Additionally, it should be noted that the hepatosomatic index analysis is a relatively rough measure and, despite its popularity in determining the health status of fish, a more detailed analysis of liver condition based on histological parameters and biochemical markers should be conducted. Future studies should include a broader assessment of Atlantic sturgeon health status, with particular emphasis on immune system parameters, which are critical for the success of restocking programs and the long-term survival of reared individuals in natural environments.

As an additional parameter indicating the proper development of Atlantic sturgeon juveniles fed feeds containing *H. illucens* meal, fin indices were distinguished. Fin erosion is an established indicator of fish welfare under aquacultural conditions (Adams et al., 2007). The causes of various types of fin erosion are attributed to maintenance conditions, pressure from other individuals, and the composition of the feed, including the content of minerals, vitamins, and nutrients (Klíma et al., 2013). To our knowledge, no studies have been conducted on the influence of BSFM on fin indices in sturgeon. However, it can be stated that the use of *Hermetia illucens* meal in Atlantic sturgeon diets did not worsen the ratio of fin length to total body length compared to the control group, positively impacting the overall health and condition of Atlantic sturgeons.

In conservative aquaculture systems, the assessment of alternative feed ingredients should integrate growth performance and comprehensive indicators of fish health and physiology with sustainability metrics. Considering the rapidly increasing global annual apparent consumption of aquatic animal foods per capita, the environmental impact of modern aquaculture is one of the most important factors. The most used ratio for assessing fish production sustainability (FIFO) (Jackson, 2009) was decreased by all experimental treatments compared to the CON. All results in the experimental groups containing BSFM were below 1.0, indicating that none of them resulted in higher fish capture than aquaculture production. It indicates that for carnivorous and omnivorous fish, FIFO optimisation can be achieved when the FM content in the diets is between 26–27% and the FCR is approximately 1.0 or lower (Rawski et al., 2021). Additionally, it is important to note that modern diets increasingly contain less FM and FO due to their low sustainability and high costs (Naylor et al., 2009). The obtained results are consistent with the research of Rawski et al. (2021). However, no differences between groups were found regarding FOU, which is evident due to using the same amount of FO in all feeds.

Based on the aforementioned information, the hypothesis was formulated that replacing FM with insect meal in the diet of juvenile Atlantic sturgeon would not negatively affect growth performance, overall health status, or somatic and fin indices. This hypothesis has been empirically validated, supporting the development of environmentally sustainable feed formulations for high-quality fingerling production. The study is a fundamental step in evaluating the effectiveness of diets that incorporate *H. illucens* larvae in the rearing of the Atlantic sturgeon. The obtained results provide a basis for further application tests to assess the practical implementation of this feeding strategy in aquaculture. Due to the short trial duration and small initial fish size (10.3–43.6 g), the findings are limited to early growth stages. Sturgeons at different sizes or under longer feeding regimes may respond differently. Further research is needed to assess long-term effects and determine optimal size for restocking, which was beyond the scope of this initial study. In subsequent stages, it will be essential to conduct behavioural studies to analyse the responses of reared individuals after their introduction into the natural environment. This will enable a comprehensive assessment of alternative protein sources' impact on the species' adaptive capabilities.

Conclusions

The obtained results confirm the potential of using partially defatted black soldier fly meal (BSFM) in the production of extruded feeds with high technological quality. The study demonstrated that partially defatted meal derived from *Hermetia illucens* larvae biomass is highly suitable for feeding juvenile Atlantic sturgeon, as it does not negatively affect growth performance, feed utilisation, or organosomatic and morphometric indices. Moreover, the findings indicate that replacing fish meal (FM) with BSFM in feeds allows for achieving a high level of environmental sustainability in conservative Atlantic sturgeon aquaculture. Based on the conducted research, it can be recommended that up to 30% of partially defatted *H. illucens* meal may effectively replace 87.5% of FM in the diets of juvenile Atlantic sturgeon.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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