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Evaluation of superworm (*Zophobas morio*) larvae meal as a fish meal substitute in juvenile stellate sturgeon (*Acipenser stellatus*) diets

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ABSTRACT

This study aimed to evaluate the effects of dietary inclusion of superworm (*Zophobas morio*) larvae meal (SWM) on growth performance, hematological parameters, blood biochemical parameters, proteolytic enzyme activity, and body composition in juvenile stellate sturgeon (*Acipenser stellatus*). A total of 120 juvenile fish (initial body weight: 28.08 ± 0.13 g) were randomly assigned to four dietary treatments in triplicate groups. Experimental diets were formulated to replace 0% (control), 10% (SWM10), 20% (SWM20), and 30% (SWM30) of fish meal with SWM, maintaining isonitrogenous (45.27% protein) and isoenergetic (19.50 MJ/kg) profiles. Fish were hand-fed to apparent satiation three times daily over an 8-week period. Growth performance data indicated that up to 10% SWM inclusion did not significantly affect final weight, weight gain, or condition factor compared to the control ($p > 0.05$). However, 20% and 30% inclusion levels resulted in significantly reduced growth performance ($p < 0.05$). Hematological analysis showed that WBC counts were significantly higher in all SWM-fed groups (16.2–19.3 $\times 10^3$/mm^3) compared to the control (13.9 $\times 10^3$/mm^3), with neutrophil percentages also elevated (16.3–17.2% vs. 13.7% in the control) ($p < 0.05$). No significant differences were observed in RBC count, hemoglobin, hematocrit, MCV, MCH, or MCHC ($p > 0.05$). Biochemical results showed that triglyceride levels were significantly elevated in the SWM30 group ($p < 0.05$), while cholesterol levels were significantly lower compared to the control ($p < 0.05$). Serum glucose level remained unaffected across treatments ($p > 0.05$). Proteolytic enzyme analysis indicated a significant reduction in trypsin activity in fish fed the SWM30 diet ($p < 0.05$). Whole-body composition analysis revealed that increasing SWM inclusion significantly decreased carcass protein, moisture, and ash contents, while lipid content increased, with the highest fat level observed in the SWM30 group ($p < 0.05$). Overall, the findings suggest that superworm larvae meals can be included in the diet of juvenile stellate sturgeon at levels up to 10% fishmeal replacement without adverse effects on growth, hematological parameters, blood biochemistry, or proteolytic enzyme activity.

1. Introduction

The aquaculture industry relies heavily on fishmeal (FM) as a key protein ingredient in aquafeeds (Drosdowech et al., 2024; Gatlin and Wu, 2025). This dependence is primarily due to FM's high protein content, balanced amino acid and fatty acid profiles, excellent digestibility, and strong palatability (Abdel-Tawwab et al., 2020; Sun et al., 2025). However, declining wild fish stocks have intensified the demand for FM. Consequently, its rising cost and limited availability have rendered it an unsustainable protein source, placing significant financial pressure on many rural fish farmers (Adeoye et al., 2019; Nephale et al., 2024).

In recent years, the high cost and growing demand for FM have prompted an urgent search for non-conventional, cost-effective protein alternatives that offer comparable quality and nutritional value (Das et al., 2024). Numerous studies have examined plant-based proteins as

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potential substitutes in aquafeeds (Aas et al., 2019; Ytrestøyl et al., 2015; Das, 2023; Hossain et al., 2024). However, many of these plant-derived protein sources directly compete with food supplies for both humans and livestock, raising concerns about their long-term sustainability (Das et al., 2024; Gil et al., 2024; Singh et al., 2025). This ongoing challenge highlights the importance of exploring alternative protein sources that are not only nutritionally robust and cost-effective but also practical for small-scale and rural aquaculture operations (Nephale et al., 2024; Iheanacho et al., 2025).

In this context, insect meals have attracted growing interest in aquaculture research due to their promising nutritional profiles, environmental sustainability, and recent regulatory approval for use in aquafeeds (Fantatto et al., 2024; Roccatello et al., 2024). Insects are considered an eco-friendly protein source due to their rapid growth, high reproductive efficiency, minimal land and water requirements, and their capacity to convert organic waste into biomass (Kumar et al., 2021; Hancz et al., 2024). Insect meal (IM) is particularly appealing because it provides a rich array of amino acids, lipids, minerals, vitamins, and energy (Barroso et al., 2014; Alejandro Ruiz et al., 2025).

The protein content of insect's ranges from 50% to 82% of dry matter, depending on the species and processing method (Rumpold, Schlüter, 2013; Li et al., 2017; Yadav et al., 2025). Notably, insect proteins have favorable amino acid profiles, especially in essential amino acids such as lysine, methionine, and leucine, nutrients often lacking in plant-based proteins (Gaylord and Barrows, 2009; Gorissen et al., 2018; Li et al., 2017). Interestingly, insects are also becoming part of the human diet in some countries (Orsi et al., 2019; Turan et al., 2025).

Despite their potential, the current production cost of insect meals remains higher than that of conventional protein sources, which limits their broader market competitiveness (Koeleman, 2014; Chidozie Ogwu, 2025). Nevertheless, various insect species including mealworms, crickets, grasshoppers, and black soldier fly larvae have been successfully evaluated as FM replacements in the diets of a wide range of aquaculture species, including food fish (e.g., rainbow trout (*Oncorhynchus mykiss*), African catfish (*Clarias gariepinus*), Jian carp (*Cyprinus carpio*), and Atlantic salmon (*Salmo salar*) and ornamental fish (e.g., goldfish (*Carassius auratus*), and guppy (*Poecilia reticulata*) (Belghit et al., 2019; Hu et al., 2020; Perera and Bhujel, 2022; Mohan et al., 2022; Gebremichael et al., 2023; Das, 2023).

Among these, superworms (*Zophobas morio*), the larval form of the darkling beetle, are commonly reared as feed for birds and reptiles due to their high nutritional value (Rumbos and Athanassiou, 2021; Vasilopoulos et al., 2024). These larvae are naturally larger than commercially used mealworms, reaching 5–6 cm in length at the end of their larval stage (Hosseini Shekarabi et al., 2021). They are relatively easy to rear and can be grown on various organic waste substrates, including garden and vegetable residues (Harsányi et al., 2020). Recently, superworm larvae have been identified as a valuable source of protein and lipids for feeding ornamental and exotic animals such as lizards, frogs, birds, koi fish, and other insectivorous species (Jabir et al., 2012; Prachom et al., 2021). These larvae are rich in crude protein (44%–47% of dry matter) and lipids (40%–41% of dry matter), and contain essential amino acids, fatty acids, and antimicrobial peptides (Jabir et al., 2012; Nederlof et al., 2017; Soon et al., 2018; Benzertiha et al., 2020). Previous studies have reported successful use of superworm meal (SWM) as a FM replacement in the diets of several species, including Nile tilapia (*Oreochromis niloticus*) (Jabir et al., 2012), juvenile rainbow trout (Hosseini Shekarabi et al., 2021), Asian sea bass (Prachom et al., 2021), sea trout (Mikołajczak et al., 2020), and juvenile goldfish (Das et al., 2024).

To date, however, the use of insect meals in the diet of stellate sturgeon (*Acipenser stellatus*) has not been investigated. The stellate sturgeon is of particular conservation and commercial significance: it is listed as Critically Endangered in the wild and is an important species for caviar and meat production, which has led to the development of aquaculture programmes both for commercial production and for conservation/restocking purposes (Bronzi et al., 2011; Anderson et al., 2022). As such, dietary improvements that reduce reliance on FM while maintaining growth, health and reproductive potential have direct relevance to both sustainable aquaculture and species conservation. Therefore, the present study was conducted to evaluate the effects of dietary replacement of FM protein with SWM on growth performance, hematological parameters, body chemical composition, blood biochemical parameters, and proteolytic enzyme activity in juvenile stellate sturgeons.

2. Materials and methods

2.1. Ethical information

All animal procedures were performed following the ethical standards set by the institution and in compliance with the guidelines outlined in Directive 2010/63/EU. Also, all animal-related procedures followed the ethical standards outlined in the ARRIVE guidelines, ensuring the welfare of animals used in scientific research.

2.2. Experimental diet preparation

Superworm larvae were sourced from Caspian Eel Aquaculture Company (Guilan, Iran). Upon arrival, the larvae were cleaned, oven-dried, and ground into a fine powder for use in experimental diets. SWM was used to replace fish meal (FM) at four inclusion levels: 0% (control), 10%, 20%, and 30%. These graded inclusion levels were chosen to span low-to-moderate replacement rates commonly tested for *Z. morio* and other insect meals, and to allow direct comparison with previous studies that reported effects on growth, digestibility and enzyme activity at similar ranges (e.g., Jabir et al., 2012; Hosseini Shekarabi et al., 2021). All diets were formulated to be isonitrogenous (45% crude protein), isolipidic (15% crude fat), and isocaloric (~19 MJ kg^{-1} gross energy); formulations were calculated using Microsoft Excel to ensure nutrient balance. Gross energy was calculated using standard physiological fuel values (protein = 23.6 MJ kg^{-1}, lipid = 39.5 MJ kg^{-1}, nitrogen-free extract = 17.2 MJ kg^{-1}) and the formula GE = (Protein × 23.6) + (Lipid × 39.5) + (NFE × 17.2). The ingredients were thoroughly mixed and pelleted using a commercial meat grinder fitted with a 2 mm die. Pellets were dried at 60 °C for 24 h, cooled, and stored in airtight plastic bags at –2 °C (short-term storage) until use. The proximate composition of the primary protein sources is presented in Table 1, while the proximate composition of the formulated experimental diets is shown in Table 2 was determined according to AOAC. (2010) methods. Diet formulations were designed to meet the reported essential amino-acid requirements of juvenile sturgeon based on available literature (Hung, 2017).

2.3. Experimental fish and design

An 8-week feeding trial was conducted at the Sturgeon Research Station in Chaboksar, Guilan Province, Iran. Before the start of the trial, juvenile stellate sturgeon were acclimated for 14 days in experimental

Table 1
Proximate composition (% dry matter basis) of superworm meal and fish meal used in experimental diets.

Nutritional composition (%)	Superworm meal	Fish meal
Crude protein	46.59 ± 0.87	64.00 ± 1.28
Crude lipid	32.2 ± 0.54	10.00 ± 1.02
Crude ash	2.18 ± 0.08	13.3 ± 1.14
Moisture	3.17 ± 0.28	5.90 ± 0.36

Values are means ± SD (n = 3). FM was produced from Caspian Sea sprat (*Clupeolella* sp.) and obtained from Yeganeh Khazar Company (Iran).

Table 2
Ingredient composition and proximate biochemical composition of the experimental diets containing different levels of superworm meal (SWM).

Experiment diets				
Ingredients (%)	**SWM0**	**SWM10**	**SWM20**	**SWM30**
Fish meal[a]	40	36	32	28
Superworm larvae meal[b]	0	5.5	11	16.5
Wheat flour	10	10	10	10
Wheat gluten	13	13	13	13
Soybean meal[c]	20	20	20	20
Fish oil	5	4.5	4	3.5
Canola oil	5	4.5	4	3.5
Vitamins Premix[d]	1	1	1	1
Minerals Premix[e]	1	1	1	1
Lecithin	1	1	1	1
Molasses	2	2	2	2
Antifungal	0.2	0.2	2.0	2.0
Bentonite	8.1	1.3	1	0.9
Crude protein	45.27	45.27	45.27	45.28
Crude lipid	25.15	62.15	15.79	15.76
Moisture	6.25	6.19	6.13	6.07
Crude Ash	8.57	8.16	7.75	7.34
Gross energy (MJ kg^{-1})	19.31	19.48	19.59	19.63

Gross energy (MJ kg^{-1}) was calculated using physiological fuel values of 23.6 MJ kg^{-1} for protein, 39.5 MJ kg^{-1} for lipid, and 17.2 MJ kg^{-1} for nitrogen-free extract (NFE).
Values are expressed on a dry matter basis and presented as mean ± SD (n = 3).
[a] Fishmeal (FM) derived from Caspian Sea sprat (*Clupeolella* sp.), obtained from Yeganeh Khazar Company (Iran)
[b] Caspian eel aquaculture company (Guilan, Iran)
[c] Vahdat Gilan Animal, Poultry and Aquatic Feed Company (Rasht, Guilan)
[d] Science Laboratories Co. (Qazvin, Iran) including (g kg^{-1}): A (1600000 IU), D3 (400000 IU), E (40), K3 (2), B1 (6), B2 (8), B3 (12), B5 (40), B6 (4), B9 (2), B12 (0.008), H2 (0.24), C (60), Inositol (20), Biotin (0.2)
[e] Science Laboratories Co. (Qazvin, Iran) including (g kg^{-1}): Iron (6 g), Zinc (10 g), Selenium (0.02 g), Cobalt (0.1 g), Copper (6 g), Manganese (5 g), Iodine (0.6 g), Choline chloride (6 g)

tanks and fed the control diet during this period. Following acclimation, 120 healthy juvenile stellate sturgeons with an average initial body weight of 28.08 ± 0.13 g and length of 24.28 ± 0.35 cm were randomly assigned to 12 circulars fibreglass tanks (500 L capacity), with 10 fish per tank. Ten fish were selected per 500-L tank to maintain a low stocking density that preserves water quality and animal welfare, while allowing for three independent tank replicates per treatment. Each dietary treatment (0%, 10%, 20%, and 30% SWM) was tested in triplicate. Fish were fed three times daily (08:00, 12:00, and 18:00) at a feeding rate of 3% of body weight per day, with a predetermined ration to ensure equal feed consumption across treatments. Tanks were siphoned daily to remove uneaten feed and fecal matter, maintaining optimal water quality. At the end of the 56-day feeding trial, fish were sampled in a strict chronological sequence: capture, anaesthesia, blood collection, euthanasia, and intestinal collection.

2.4. Water quality monitoring

Water quality parameters were monitored daily to maintain optimal rearing conditions. The mean water temperature was 18.54 ± 0.27 °C, pH was 8.09 ± 0.12, and dissolved oxygen concentration averaged 6.89 ± 0.14 mg L^{-1}, total ammonia (NH_3/NH_4^+), nitrite (NO_2^-), total alkalinity (as $CaCO_3$) and water hardness were monitored weekly using standard kits. All measured values remained within the recommended ranges for sturgeon culture (Chebanov et al., 2011): alkalinity 100 mg L^{-1}, un-ionized ammonia < 0.01 mg L^{-1}, nitrite < 0.1 mg L^{-1}.

2.5. Growth performance

Fish growth was monitored throughout the experiment by periodic weighing on days 14, 28, 42, and 56 of the feeding trial. Before each weighing event, fish were fasted for 24 h to minimize variation due to gut content. During intermediate samplings (days 14, 28, and 42), fish were gently netted and anesthetized using clove powder at a concentration of 100 mg L^{-1} for approximately 2–3 min, until loss of equilibrium was observed, to reduce handling stress (Pastaki et al., 2025). All fish were weighed individually using a digital balance (±0.01 g accuracy) and immediately returned to their respective tanks after full recovery from anesthesia. At the final sampling (day 56), in addition to body weight, the total length (TL, cm) of each fish was measured using a measuring board. Growth performance and feed utilization parameters were calculated using the following standard formulae (Abdollahpour et al., 2020):

$$\text{Weight gain (WG) (g)} = BWf - BWi$$

$$\text{Body weight index (BWI)} = [(BWf - BWi) / BWi] \times 100$$

$$\text{Specific growth rate (SGR)} = (Ln(BWf) - Ln(BWi)) \times 100 / n$$

$$\text{Feed conversion ratio (FCR)} = FI / (BWf - BWi)$$

$$\text{Condition factor (CF)} = [(BWf / TL^3)] \times 100$$

$$\text{Protein efficiency ratio (PER)} = (BWf - BWi) / PI$$

BWi is the initial body weight (g), BWf is the final body weight (g), TL is the final total length (cm), FI is the feed intake (g), PI is the protein intake, and n is the number of rearing days.

2.6. Blood sampling

Following the 56-day feeding trial, three fish were randomly selected from each tank and individually weighed prior to sampling. To minimize stress and ensure animal welfare, the fish were anesthetized using clove powder at a concentration of 100 mg L^{-1} in aerated water for approximately 2–3 min, until loss of equilibrium was observed. Blood was collected from the caudal vein using sterile 2 mL syringes and immediately divided into two portions. A portion of blood was transferred to Eppendorf tubes containing heparin as an anticoagulant for haematological analyses (e.g., complete blood count). The remaining blood was collected in plain Eppendorf tubes without anticoagulant, allowed to clot at room temperature for 30–40 min, and then centrifuged at 1500 *g*, 10 min to obtain serum. The separated serum was used for blood biochemical analyses (Jafari et al., 2019). All samples were carefully handled to avoid hemolysis and preserve sample integrity. The collected blood samples were transported to the laboratory in insulated containers filled with dry ice to maintain stability and prevent degradation during transit, ensuring the reliability of both hematological analyses. Following blood collection, the same fish were humanely euthanized using an overdose of clove powder (500 mg L^{-1}) in aerated water, and the digestive tract was then collected for proteolytic enzyme assays.

2.7. Determination of hematological parameters

Red blood cell (RBC) and white blood cell (WBC) counts were determined using a Neubauer hemocytometer chamber following the standard method described by Abdollahpour et al. (2021). For differential leukocyte counts, blood smears were prepared, stained, and examined under a light microscope to identify and quantify the various types of white blood cells, as outlined by Jafari et al. (2019). Hemoglobin concentration (Hb) was measured using the cyanomethemoglobin method, a widely accepted and accurate technique for quantifying hemoglobin in fish blood. Hematocrit (Hct), representing the percentage of red blood cells in whole blood, was assessed using the microhematocrit method by centrifuging blood samples in capillary tubes, as per Zhao et al. (2018). For differential leukocyte analysis, thin blood smears were carefully prepared, air-dried, fixed in absolute methanol, and subsequently stained with May Giemsa solution. The stained slides

were then examined under a light microscope, and leukocytes were identified and categorized into lymphocytes (LYM), neutrophils (NEU), monocytes (MON), and eosinophils (EOS), based on cellular morphology, as described by Abdollahpour et al. (2021). Furthermore, red blood cell indices, including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), were calculated using standard hematological formulas. These indices provide important insights into red blood cell size and hemoglobin content, which are critical indicators of the physiological and nutritional status of fish. The calculation methods followed those described by Chung et al. (2021), and were as follows:

$$\text{MCH (pg)} = \text{Haemoglobin (g/L)} \times \text{Red blood cell } (10^{12}/\text{L})$$

$$\text{MCHC (g/L)} = \text{Haemoglobin (g/L)} / \text{Haematocrit (\%)}$$

$$\text{MCV (fl)} = \text{Haematocrit} \times 10 / \text{Red blood cell } (10^{12}/\text{L})$$

These hematological assessments are commonly used in aquaculture research to evaluate fish health, detect physiological stress, and assess the impacts of dietary interventions.

2.8. Determination of blood biochemical parameters

Serum total protein (TP) concentration was assessed using the Biuret colorimetric method, which relies on peptide bonds forming a colored complex with copper ions in an alkaline medium. Measurements were conducted at 540 nm using a commercially available assay kit (product number: 1500028; detection range: 0.5–15 g dL^{-1}) (Abdollahpour et al., 2024). Glucose (Glu) levels in serum were quantified via an enzymatic colorimetric approach based on the glucose oxidase–peroxidase (GOD–POD) method, employing a diagnostic kit (product number: 1500017; detection range: 5–400 mg dL^{-1}). Additionally, serum triglycerides (TG) and total cholesterol (CHOL) were measured using enzymatic colorimetric techniques with specific commercial kits TG (product number: 1500012; range: 5–700 mg dL^{-1}) and CHOL (product number: 1500010; range: 5–500 mg dL^{-1}) according to the manufacturer's instructions (Pars Azmoon, Karaj, Iran). All biochemical readings were performed using the same T80 UV/VIS spectrophotometer (PG Instruments Limited, United Kingdom). These biochemical assays were conducted in accordance with standard clinical procedures and supported by previous studies (Jafari et al., 2020; Abdollahpour et al., 2020), ensuring consistency and reliability in evaluating the fish's physiological response to the dietary treatments.

2.9. Proteolytic enzyme assay and body chemical composition analysis

Three fish per tank were used for sequential sampling 24 h after the final feeding. Fish were first anesthetized using clove powder (100 mg L^{-1}), and blood was collected from the caudal vein. Immediately after blood collection, the same individuals were humanely euthanized using an overdose of clove powder (500 mg L^{-1}) in aerated water, ensuring rapid loss of consciousness and cessation of opercular movements, in accordance with ethical guidelines (Abdollahpour et al., 2025a).

The digestive tracts (stomach and intestine) were immediately removed for proteolytic enzyme assays. Entire digestive tracts were homogenized in nine volumes (w/v) of ice-cold 100 mM Tris–HCl buffer solution. Homogenates were centrifuged at 20,000 × g and 4 °C for 15 min to separate soluble enzyme fractions. The resulting supernatants were aliquoted and stored at –80 °C until enzymatic assays were performed. Pepsin activity was measured by incubating gastric supernatant with 0.5% hemoglobin in 0.1 M glycine–HCl buffer at pH 2.0 at 25 °C for 30 min; the reaction was stopped with 0.5 mL of 20% trichloroacetic acid, and liberated peptides were quantified spectrophotometrically at 280 nm. Trypsin activity was determined using the chromogenic substrate BAPNA (N-benzoyl L-arginine p-nitroanilide) with reaction mixtures containing 100 μL enzyme extract and 700 μL of 1.25 mM BAPNA in 0.2 M Tris–HCl (pH 8.4), incubated at 37 °C for 30 min. The reaction was terminated with 800 μL of 30% acetic acid, and released p-nitroaniline was measured at 410 nm (Peña-Marín et al., 2021; Solovyev et al., 2023). Chymotrypsin activity was assayed based on hydrolysis of benzoyl tyrosine ethyl ester in 44.4 mM Tris–HCl containing 55.5 mM $CaCl_2$ (pH 7.8) at 25 °C, and absorbance changes were measured at 256 nm. All enzyme assays were performed using the T80 UV/VIS spectrophotometer (PG Instruments Limited, United Kingdom).

Following intestinal sampling, the same fish were processed for whole-body chemical composition analysis. Protein, lipid, moisture, and ash contents were analyzed following AOAC. (2010) procedures. Moisture content was determined by oven-drying at 105 °C to constant weight, and ash content by incineration at 550 °C for 6–8 h in a muffle furnace. Crude lipid was quantified using Soxhlet extraction with ether as the solvent, and crude protein was calculated from total nitrogen (N × 6.25) using the Kjeldahl method (Jia et al., 2023). This sequential sampling approach allowed multiple physiological and biochemical analyses to be performed on the same individuals, minimizing the total number of fish used while complying with ethical standards.

2.10. Statistical analysis

All statistical analyses were carried out using SPSS software (version 27.0.1; IBM Corp., Armonk, NY, USA). To ensure the reliability and robustness of the dataset, preliminary tests were performed to validate key assumptions of parametric analysis. The Kolmogorov–Smirnov test was used to assess the normality of data distributions, while Levene's test evaluated the homogeneity of variances across groups. Once these assumptions were confirmed, the data were subjected to one-way analysis of variance (ANOVA) to identify significant differences among the treatment groups. When the ANOVA indicated statistical significance ($p < 0.05$), Turkey's post hoc multiple comparison test was applied to determine specific differences between group means. Because replication was at the tank level ($n = 3$ per treatment), all inferential tests were performed on tank means. To improve interpretability given the relatively low replication, we report effect sizes and 95% confidence intervals for main comparisons, and where appropriate present means ± standard error (SE) alongside means ± standard deviation (SD). Initial body weight was tested for homogeneity across tanks before analysis and, if relevant, was included as a covariate in ANCOVA or as a fixed covariate in mixed-effects models to control for baseline differences. When ANOVA assumptions were borderline, results were confirmed using linear mixed-effects models with tank included as a random effect. Results are presented as means ± standard deviation (SD), and a significance level of 95% confidence ($p < 0.05$) was used throughout the analysis.

3. Results

3.1. Growth performance

The growth pattern of juvenile stellate sturgeon over the 56-day trial revealed that while initial weights were similar across all groups, significant differences in average body weight were evident from day 28 onward (Fig. 1). Specifically, on days 28, 42 and 56, fish fed the control diet (SWM0) had significantly greater mean body weight than fish fed SWM10 and SWM20 ($p < 0.05$), whereas fish fed SWM30 exhibited the lowest mean body weight and was significantly lower than the other groups ($p < 0.05$) (Fig. 1). By day 56, these differences remained statistically significant ($p < 0.05$). Growth performance and feed utilization results of juvenile stellate sturgeon after 56 days are presented in Table 3. Significant differences ($p < 0.05$) were observed in FW ($P = 0.010$), WG ($P = 0.007$), BWI ($P = 0.048$), and FCR ($P = 0.045$) among the dietary treatments. Fish fed the control diet (SWM0) exhibited the highest FW (71.95 ± 1.34 g), WG (43.75 ± 42.33 g), and

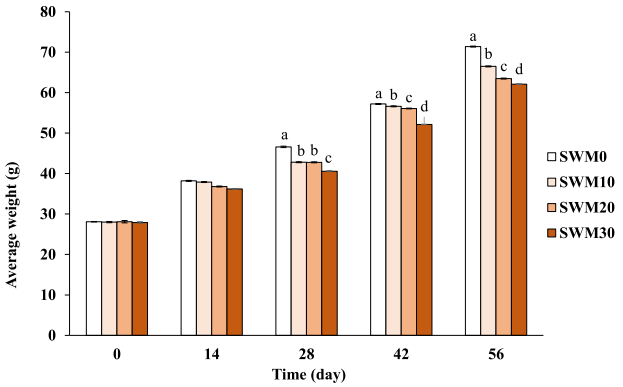

Fig. 1. Average body weight (g) of juvenile stellate sturgeon fed diets containing 0% (SWM0), 10% (SWM10), 20% (SWM20), and 30% (SWM30) superworm meal over a 56-day feeding trial. Values are presented as mean ± SE (n = 3). Different letters at each time point indicate statistically significant differences among dietary treatments ($p < 0.05$): Day 28 (P-value: 0.014), Day 42 (P-value: 0.023), and Day 56 (P-value: 0.010).

BWI (157.02 ± 28.75%), which were significantly greater ($p < 0.05$) than those in the SWM20 and SWM30 groups, but not significantly different ($p > 0.05$) from the SWM10 group. The best FCR (2.02 ± 0.08) was also observed in the control group, which was significantly lower ($p < 0.05$) than that in the SWM30 group (5.75 ± 1.26), but showed no significant difference compared to the SWM10 and SWM20 treatments ($p > 0.05$). No significant differences ($p > 0.05$) were detected among treatments in CF (P = 0.112), SGR (P = 0.058), or PER (P = 0.566). Survival rate (SR) did not differ significantly among treatments (P = 0.163)), although a trend was observed with survival decreasing from 100% in the control group (SWM0) to 80 ± 11.5% in the SWM30 group, 95 ± 2.9% in SWM10, and 85 ± 2.9% in SWM20 (Table 3).

3.2. Hematological parameters

Among the measured parameters, WBC counts (P = 0.000) and neutrophil percentages (P = 0.028) were significantly affected ($p < 0.05$) by dietary treatments. WBC counts were significantly higher ($p < 0.05$) in all SWM-fed groups compared to the control (SWM0), with the highest value recorded in the SWM30 group (19.3 ± 0.09 ×10 ^{3}/mm^3) and the lowest in the control group (13.9 ± 0.01 ×10 ^{3}/mm^3). Similarly, neutrophil percentages were significantly elevated ($p < 0.05$) in the SWM-fed groups (16.33–17.21%) compared to the control (13.67 ± 2.02%). In contrast, no significant differences ($p > 0.05$) were observed among treatments in RBC count (P = 0.748), Hb (P = 0.979), Hct (P = 0.950), MCV (P = 0.113), MCH (P = 0.290), MCHC (P = 0.359), lymphocyte percentage (P = 0.494), monocyte percentage (P = 0.750), or eosinophil percentage (P = 0.193).

3.3. Serum biochemical parameters

The results demonstrated that dietary inclusion of SWM at 20% and 30% led to a significant reduction in serum total cholesterol levels compared with the control (SWM0) and SWM10 groups (P = 0.011; Fig. 2A). This suggests a potential cholesterol-lowering effect associated with high inclusion levels of insect-derived protein. Conversely, the same 30% SWM inclusion resulted in a significant increase in serum triglyceride concentrations compared to the control treatment (P = 0.032; Fig. 2 B), indicating a shift in lipid metabolism or energy storage dynamics due to changes in dietary lipid composition. On the other hand, no statistically significant differences were observed among treatments in serum glucose levels (P = 0.821; Fig. 2 C). The total serum protein concentration of juvenile stellate sturgeon was significantly affected by the dietary inclusion levels of SWM (Fig. 2 D). Fish fed the control diet (SWM0) exhibited the highest total protein concentration (4.65 g dL^{-1}), which was significantly greater (P = 0.000) than that observed in the SWM10 (2.39 g dL^{-1}), SWM20 (2.33 g dL^{-1}), and SWM30 (2.45 g dL^{-1}) groups. No significant differences were observed among the SWM10, SWM20, and SWM30 treatments.

3.4. Proteolytic enzymes activity

Among the enzymes evaluated, trypsin activity showed a significant response to the varying levels of SWM in the diet (P = 0.017; Fig. 3A). The SWM0 group exhibited the highest trypsin activity, which was significantly greater than that of the SWM20 and SWM30 groups ($p < 0.05$). Fish fed the 10% SWM diet also showed significantly higher trypsin activity than those fed 20% and 30% SWM ($p < 0.05$), whereas

Table 3
Growth performance and feed efficiency of juvenile stellate sturgeon (*Acipenser stellatus*) fed diets containing different levels of superworm larval meal over an 8-week feeding trial.

Experimental diets					
Parameters	**SWM0**	**SWM10**	**SWM20**	**SWM30**	**P-value**
Initial weight (g)	28.10 ± 3.68^a	28.00 ± 1.41^a	28.10 ± 1.27^a	27.90 ± 0.57^a	
Final weight (g)	71.95 ± 1.34^a	66.49 ± 6.66^{ab}	63.49 ± 1.79^b	62.10 ± 1.27^b	0.010
WG (g)	43.75 ± 2.33^a	38.49 ± 5.25^{ab}	35.39 ± 3.06^b	34.50 ± 0.71^b	0.007
BWI (%)	157.02 ± 28.75^a	137.17 ± 8.36^{ab}	126.53 ± 11.97^b	125.33 ± 0.58^b	0.048
CF	0.21 ± 0.02	0.19 ± 0.00	0.21 ± 0.01	0.20 ± 0.01	0.112
SGR (%/day)	1.78 ± 0.21	1.63 ± 0.09	1.54 ± 0.14	1.53 ± 0.00	0.058
FI (g/fish)	87.0 ± 0.0	87.0 ± 0.0	87.0 ± 0.0	87.0 ± 0.0	-
FCR	2.02 ± 0.08^b	2.57 ± 0.29^{ab}	4.17 ± 0.53^{ab}	5.75 ± 1.26^a	0.045
PER	1.55 ± 1.15	1.99 ± 0.27	1.83 ± 0.16	1.79 ± 0.04	0.566
SR	100 ± 0.00	95 ± 2.9	85 ± 2.9	80 ± 11.5	0.163

Weight gain (WG, g) = BWf – BWi
Body weight index (BWI, %) = [(BWf – BWi) / BWi] × 100
Specific growth rate (SGR, % day^{-1}) = [(Ln BWf – Ln BWi) × 100] / n
Feed intake (FI, g/fish) = total feed intake
Feed conversion ratio (FCR) = FI / (BWf – BWi)
Condition factor (CF) = (BWf / TL^3) × 100
Protein efficiency ratio (PER) = (BWf – BWi) / PI
Survival rate (SR, %) = (final number of fish / initial number of fish) × 100
Where: BWi = initial body weight (g), BWf = final body weight (g), TL = final total length (cm), FI = feed intake (g), PI = protein intake (g), n = number of farming days.
Values are presented as mean ± SD (n = 3). Different superscript letters within the same row indicate statistically significant differences among treatments ($p < 0.05$).

Table 4
Blood hematological parameters of juvenile stellate sturgeon (*Acipenser stellatus*) fed diets containing different levels of superworm (TM) meal after 56 days of feeding.

Experimental diet					
Parameters	**SWM0**	**SWM10**	**SWM20**	**SWM30**	**P-value**
RBC (10^6/mm^3)	0.77 ± 0.03	0.74 ± 0.06	0.72 ± 0.07	0.71 ± 0.08	0.748
WBC (10^3/mm^3)	13.9 ± 0.01^b	18.7 ± 0.02^a	18.9 ± 0.05^a	19.3 ± 0.09^a	0.000
Hb (g/dL)	5.51 ± 0.42	5.15 ± 0.58	4.80 ± 0.65	4.79 ± 0.89	0.979
Hct (%)	27.47 ± 1.21	28.67 ± 1.20	27.37 ± 1.97	27.08 ± 2.49	0.950
MCV (fl)	342.00 ± 3.54	354.00 ± 4.58	349.00 ± 2.64	352.33 ± 4.63	0.113
MCH (pg/cell)	70.33 ± 0.14	71.26 ± 1.07	69.13 ± 0.86	70.73 ± 0.44	0.290
MCHC (g/dL)	20.42 ± 0.32	19.84 ± 0.49	20.34 ± 0.03	20.05 ± 0.01	0.359
Lymphocytes (%)	82.00 ± 3.50	77.66 ± 0.88	78.66 ± 2.02	77.00 ± 1.15	0.494
Neutrophils (%)	13.67 ± 2.02^b	17.00 ± 0.57^a	16.33 ± 0.88^a	17.21 ± 1.15^a	0.028
Monocytes (%)	5.00 ± 0.50	5.66 ± 1.20	5.00 ± 0.57	4.33 ± 0.88	0.750
Eosinophils (%)	0.00 ± 0.00	0.00 ± 0.50	0.33 ± 0.06	1.00 ± 0.57	0.193

RBC, red blood cells; WBC, white blood cells; Hb, hemoglobin; Hct, hematocrit.
Mean corpuscular hemoglobin (MCH, pg) = Hb (g/L) × 10 / RBC (10^{12}/L)
Mean corpuscular hemoglobin concentration (MCHC, g/L) = Hb (g/L) / Hct (%) × 100
Mean corpuscular volume (MCV, fl) = Hct (%) × 10 / RBC (10^{12}/L)
Values are presented as mean ± SD (n = 9). Different superscript letters within the same row indicate statistically significant differences among treatments ($p < 0.05$).

no significant differences were observed between the SWM20 and SWM30 groups ($p > 0.05$). These results indicate that both the absence of SWM and low-level inclusion (10%) maintained higher trypsin activity, while higher SWM inclusion (20–30%) may reduce intestinal proteolytic capacity. In contrast, pepsin and chymotrypsin activities were not significantly affected by dietary treatment ($p > 0.05$; Fig. 3 B, C), suggesting that these enzymes are less sensitive to variations in dietary protein sources within the tested range of SWM inclusion.

3.5. Body chemical composition analysis

The results of body biochemical composition in juvenile stellate sturgeon at the end of the experiment are presented in Fig. 4. Increasing dietary replacement with SWM significantly decreased carcass protein, moisture, and ash, while lipid content increased ($p < 0.05$). The control group showed the highest protein content, which was significantly different from the 20% and 30% replacement groups ($P = 0.000$; Fig. 4A), but not from the 10% group ($p > 0.05$). Lipid content increased with SWM inclusion, with the 30% group showing the highest fat level, significantly higher than the control and 10% groups ($P = 0.018$; Fig. 4B), but similar to the 20% group ($p > 0.05$). Moisture content was highest in the control, significantly higher than all SWM groups ($P = 0.000$; Fig. 4C). Ash content also peaked in the control group, significantly differing from the 30% replacement group ($P = 0.005$; Fig. 4D), while no significant differences were observed for the 10% and 20% groups ($p > 0.05$).

4. Discussion

The increasing reliance on FM as the primary protein source in aquafeeds has raised significant concerns regarding sustainability, ecological impact, and long-term economic feasibility. Recent reviews and experimental studies have evaluated multiple FM alternatives, including plant by-products, terrestrial animal by-products, microbial biomass and insect meals and report that low-to-moderate inclusion of these alternatives can often maintain growth and physiological status while improving sustainability metrics (Shahzad et al., 2020; Jafari et al., 2022; Tabassum et al., 2021; Hussain et al., 2024). The depletion of wild fish stocks and the fluctuating availability of fishmeal highlight the need for sustainable aquafeed alternatives. Insects, particularly superworm meals, offer a promising, eco-friendly protein source due to their high nutritional value and potential for scalable, low-impact production. Integrating such alternatives can enhance the sustainability and resilience of aquaculture. In the present study, the inclusion of SWM in the diet of stellate sturgeon juveniles had a significant impact on growth performance parameters. Fish in the control group (FM-based diet) achieved the highest FW, WG, and BWI, while those fed the highest inclusion level of SWM (30%) exhibited the lowest performance in these metrics. Notably, growth performance declined significantly when SWM inclusion exceeded 10%, suggesting that high levels of SWM may negatively affect growth, possibly due to nutritional or physiological limitations. FCR was also adversely affected by higher SWM inclusion, with fish in the 30% SWM group showing significantly elevated FCR values, indicating reduced feed efficiency compared to the control. These findings align with the hypothesis that SWM, despite its nutritional value, may contain components that impair digestibility or nutrient absorption at higher inclusion levels. One possible explanation for the observed decline in growth performance is the presence of anti-nutritional factors inherent to insect meals, such as chitin. Chitin, a structural polysaccharide found in insect exoskeletons, is known to reduce nutrient bioavailability by decreasing the retention time of feed in the digestive tract and limiting the interaction between digestive enzymes and feed components (Barroso et al., 2014). Mechanistically, chitin can act both as a physical barrier reducing enzyme access to substrate and as a modulator of gut transit time; furthermore, because many teleosts lack high endogenous chitinase activity, chitin particles may persist in the gut, sequester digestive enzymes or nutrients, and alter mucosal surface interactions, thereby reducing apparent digestibility. Such effects have been documented in several studies examining FM replacement with insect meals, including SWM (Makkar et al., 2014; Magalhães et al., 2017; Xiao et al., 2018; Bruni et al., 2018; Fontes et al., 2019). In particular, Belghit et al. (2018) reported that certain insect-based meals could suppress the activity of proteolytic enzymes, further supporting the idea that reduced digestive efficiency may underlie the diminished growth observed at high SWM inclusion levels. This suppression may occur via reduced expression or secretion of pancreatic proteases (trypsinogens) and brush-border peptidases, or via increased presence of protease inhibitors or physical sequestration of proteases by indigestible fractions; assessing gene expression of trypsinogen, chymotrypsinogen and aminopeptidases would help clarify these mechanisms.

In addition to antinutritional factors such as chitin, differences in essential amino-acid (EAA) composition between SWM and FM are a likely contributor to the reduced growth and feed efficiency observed at higher SWM inclusion levels. Recent work has demonstrated that full-fat *Z. morio* meals have lower concentrations of several key EAAs most notably methionine, lysine and threonine, when compared with conventional FM (Asimaki et al., 2025). Because our diets were formulated to be isonitrogenous but did not include supplemental crystalline amino acids, progressive replacement of FM with full-fat SWM probably reduced the supply of limiting EAAs on a protein-equivalent basis and thereby constrained protein accretion and growth. At the cellular level,

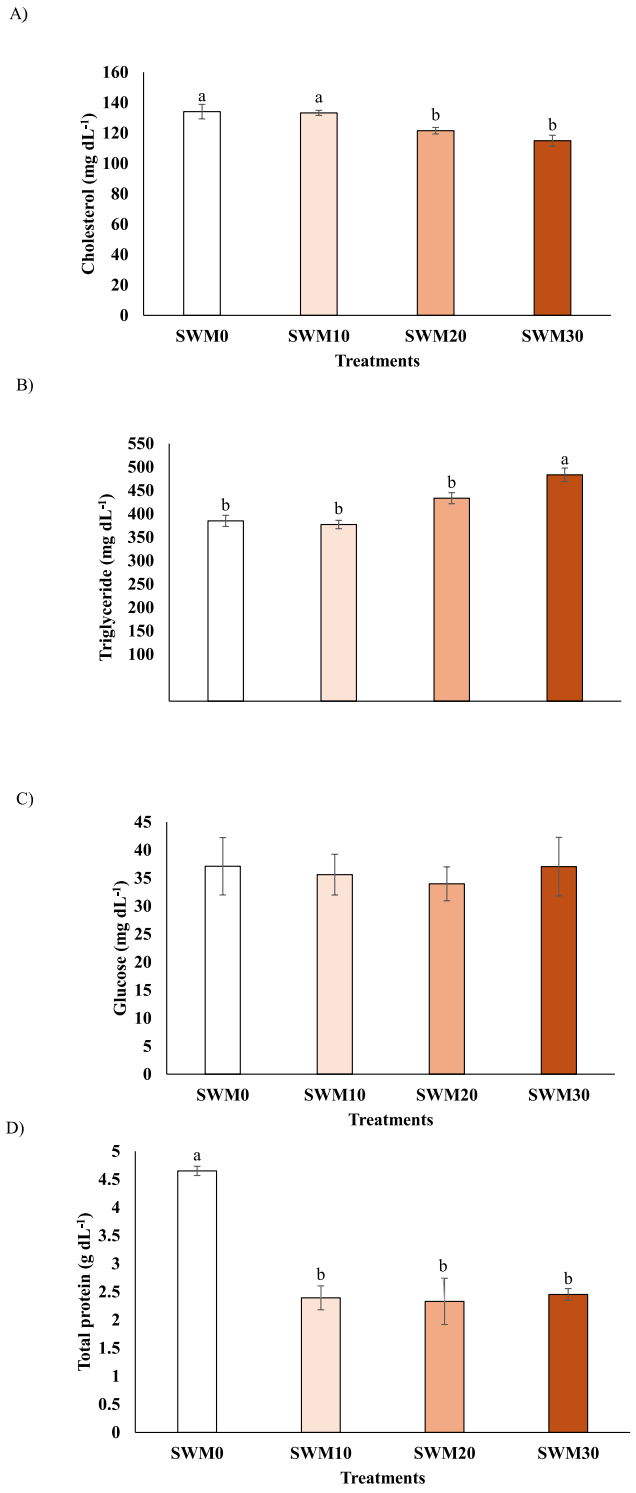

Fig. 2. Blood biochemical parameters of juvenile stellate sturgeon fed diets containing 0% (SWM0), 10% (SWM10), 20% (SWM20), and 30% (SWM30) superworm meal after 8 weeks of feeding. Values are presented as mean ± SD (n = 9). Different superscript letters indicate statistically significant differences among treatments ($p < 0.05$). A) Cholesterol (P-value: 0.011), B) Triglyceride (P-value: 0.032), C) Glucose (P-value: 0.821), D) Total protein (P-value: 0.000).

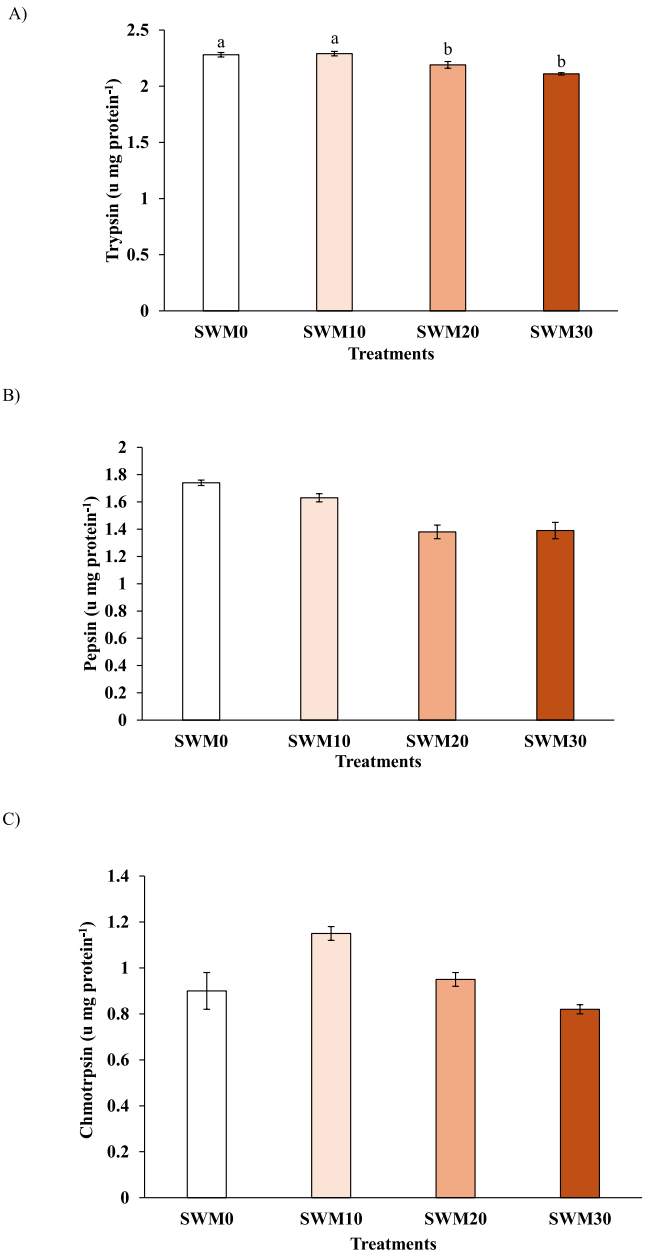

Fig. 3. Proteolytic enzyme activity in juvenile stellate sturgeon fed diets containing 0% (SWM0), 10% (SWM10), 20% (SWM20), and 30% (SWM30) superworm meal after 56 days. Values are presented as mean ± SD (n = 9). Different superscript letters indicate statistically significant differences among treatments ($p < 0.05$). A) Pepsin (P-value: 0.177), B) Trypsin (P-value: 0.017), C) Chymotrypsin (P-value: 0.667).

inadequate supply of essential amino acids can down-regulate translational and anabolic pathways (e.g., mTOR signaling) and reduce rates of muscle protein synthesis, while simultaneously increasing amino-acid catabolism; these shifts reduce net protein deposition and can increase substrate flux toward gluconeogenesis or lipogenesis. Although the experimental diets were formulated to be isolipidic, the inclusion of full-fat SWM increases the contribution of lipids from the ingredient itself, which slightly reduces the proportion of high-quality protein sources such as fishmeal. Consequently, the concentration of essential amino acids in the protein fraction can be diluted at high inclusion levels, even if total crude protein remains similar across diets; Asimaki et al. (2025) note that these differences are more pronounced with full-fat than with defatted insect meals. Comparative studies also indicate that defatted insect meals often permit higher and more effective inclusion rates than full-fat meals because defatting concentrates protein and EAAs and reduces excess dietary lipid factors that influence palatability, nutrient balance and metabolic responses (Karapanagiotidis

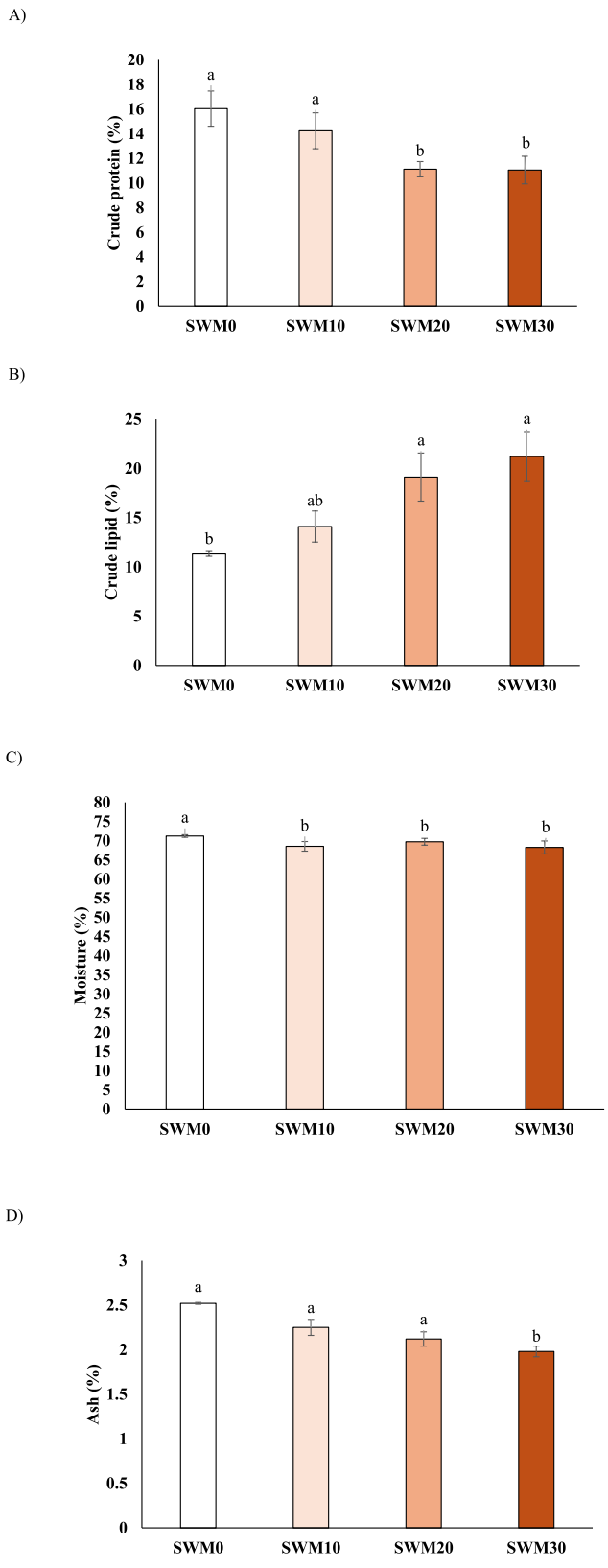

Fig. 4. Proximate body composition of juvenile stellate sturgeon fed diets containing 0% (SWM0), 10% (SWM10), 20% (SWM20), and 30% (SWM30) superworm meal after 8 weeks of feeding. Values are presented as mean ± SD (n = 6). Different superscript letters indicate statistically significant differences among treatments ($p < 0.05$). A) Crude protein (P-value: 0.000), B) Crude lipid (P-value: 0.018), C) Moisture (P-value: 0.000), D) Ash (P-value: 0.005).

et al., 2023; Asimaki et al., 2025). Therefore, while chitin and other antinutrients remain plausible mechanisms, an EAA imbalance, particularly relative deficiencies of methionine and lysine arising from substitution with full-fat SWM without AA supplementation, likely contributed materially to the depressed growth, elevated carcass lipid and altered serum lipid profiles observed at ≥ 20–30% replacement in the present study. Direct quantification of diet amino-acid profiles and targeted supplementation (e.g., methionine, lysine) would be the most direct approach to test this hypothesis and to disentangle EAA limitation from chitin/physical effects. We recommend that future experiments include direct amino-acid profiling of diets, consider targeted supplementation of limiting EAAs (e.g., methionine, lysine), and evaluate defatted SWM as an alternative to full-fat meals to improve nutrient balance and allow higher safe inclusion rates (Karapanagiotidis et al., 2023; Asimaki et al., 2025). Our findings are consistent with those of Hosseini Shekarabi et al. (2021), who observed impaired growth performance in rainbow trout when SWM replaced FM at levels exceeding 30%. Similarly, Jabir et al. (2012) reported significant variations in growth in Nile tilapia fed diets containing different SWM levels compared to FM-based controls. However, other studies present contrasting evidence. For instance, Das et al. (2024) observed enhanced growth in goldfish fed SWM-based diets even at replacement levels up to 100%, a finding not supported by the present study. Likewise, Alves et al. (2021) found no significant differences in growth performance of Nile tilapia across increasing levels of SWM inclusion. Such discrepancies across studies could be attributed to multiple factors, including species-specific digestive physiology and tolerance to insect-based ingredients, the developmental stage of the fish, the quality and composition of the IM (influenced by rearing substrate), and processing methods applied to the insect material. Additionally, variations in the types of insect species used may also play a role in determining feed utilization efficiency and overall growth outcomes, highlighting the complexity of integrating insect meals into aquafeeds (Alves et al., 2021). In summary, while SWM shows potential as an alternative protein source, its inclusion must be carefully optimized to avoid adverse effects on fish performance. Further research is warranted to refine insect processing methods, enhance digestibility, and better understand species-specific responses, ultimately supporting the sustainable and effective use of insect meals in aquaculture.

In general, body composition is influenced by various physiological, biotic, and abiotic factors. Several factors, such as fish species, water temperature, body weight, feeding regime, and diet formulation, can affect and alter body composition (Desai and Singh, 2009). Even among individuals of the same fish species under identical conditions, variations in body composition may occur. However, the primary determinant of fish body composition is undoubtedly linked to diet and nutritional intake (Breck, 2014). The analysis of the carcass chemical composition of juvenile stellate sturgeon in the present study showed that increasing the levels of SWM replacement in the diet resulted in significant differences in protein, lipid, moisture, and ash contents compared to the control group. Specifically, with increasing replacement levels up to 30%, carcass protein, moisture, and ash contents decreased, while lipid content increased. Supporting these findings, Hosseini Shekarabi et al. (2021) reported that increasing the replacement of SWM in place of FM in the diet of rainbow trout led to reductions in protein, moisture, and ash contents, and an increase in lipid content. Similarly, Alves et al. (2021) observed that replacing FM with SWM up to 30% in the diet of Nile tilapia resulted in decreased protein and increased carcass fat levels. In contrast, Muin et al. (2017) reported that replacing FM with black soldier fly larvae meal in the diet of Nile tilapia did not significantly affect carcass protein content, which contradicts the findings of the present study.

Researchers believe that the reduction in body protein content may be due to increased chitin levels with higher SWM inclusion in the diet. In monogastric animals, chitin remains undigested, and its elevated consumption can reduce the retention time of feed in the intestine and

limit enzyme contact with nutrients, thereby impairing the availability and digestibility of certain macronutrients, such as proteins (Bruni et al., 2018; Xiao et al., 2018; Fontes et al., 2019). Belghit et al. (2018) also reported that higher IM content leads to a reduction in leucine aminopeptidase activity, an important brush border enzyme that breaks peptides into amino acids in the anterior and mid-intestine, where the majority of protein digestion and absorption occurs.

In addition to the factors discussed above, it is important to note that replacement of FM with full-fat SWM changed the source and profile of dietary lipids supplied to the fish, and this most likely contributed substantially to the increased body lipid deposition. Although experimental diets were formulated to be isolipidic, progressive substitution with full-fat SWM shifted a larger fraction of total dietary fat to insect-derived lipids, which are characterized by relatively high saturated fatty-acid content and an elevated n-6/n-3 ratio and relatively low levels of long-chain n-3 PUFA (e.g., EPA and DHA) compared with typical marine FM lipids (Barroso et al., 2014; Finke, 2002). Such a change in lipid quality can promote lipogenesis and fat storage in fish by providing a high proportion of readily depositable saturated fatty acids and by reducing the dietary supply of LC-n-3 PUFA that support oxidative metabolism and membrane functions. Therefore, even when diets are isolipidic by proximate analysis, differences in lipid origin and fatty-acid composition, rather than crude lipid level by itself, can explain the elevated carcass fat observed with increasing SWM inclusion. This mechanism is consistent with previous reports showing that IM with high full-fat content tend to increase carcass lipid and alter tissue fatty-acid profiles, and that defatted insect meals or balancing of dietary LC-PUFA (e.g., by fish oil supplementation) can attenuate this effect (Karapanagiotidis et al., 2023; Asimaki et al., 2025). Accordingly, practical strategies to reduce excess fat deposition when using SWM include using defatted SWM, adjusting supplemental fish oil to restore EPA/DHA and the n-3/n-6 balance, or limiting the inclusion level of full-fat SWM unless amino-acid and lipid imbalances are corrected.

Hematological parameters are important indicators of the physiological status and overall health of fish (Maita, 2007; Alghada et al., 2023). In the present study, the inclusion of SWM in the diets of juvenile stellate sturgeon resulted in an observable increase in WBC counts. This elevated WBC response suggests an activation of the immune system, possibly triggered by dietary components in the SWM. Such an increase in WBCs is often interpreted as a physiological adaptation to stress or a protective immune response (Das et al., 2006). Therefore, the rise in WBC levels in SWM-fed fish may reflect the immunostimulatory potential of insect-based protein sources. These findings are in agreement with previous research using insect meals and, more specifically, with recent studies on *Zophobas morio*: Henry et al. (2022) reported that both full-fat and defatted SWM can modulate innate immune indicators (including increased neutrophil percentage and elevated complement-associated bacterial killing) in gilthead seabream (*Sparus aurata*), and Prachom et al. (2021) found that defatted SWM did not adversely affect growth or most hematological parameters in Asian sea bass (*Lates calcarifer*), although some parameters (e.g., haematocrit) changed with higher inclusion levels. These findings are in agreement with previous research, such as that by Okore et al. (2018) and Taufek et al. (2018), who reported elevated WBC counts in African catfish fed alternative insect-based meals like cricket (*Gryllus bimaculatus*) and fruit fly pupae (*Drosophila melanogaster*).

Another important hematological parameter is neutrophil count, which provides valuable insight into inflammatory processes and the innate immune response in fish. Neutrophils are key defense cells that play a critical role in maintaining physiological homeostasis and combating infection (Flajnik and Du Pasquier, 2004). These cells are among the first responders during an immune challenge, migrating rapidly to infection or inflammation sites (Kourtzelis et al., 2017). In this study, the number of circulating neutrophils increased significantly in fish fed SWM-enriched diets compared to the control group. This neutrophilia is consistent with the observations of Henry et al. (2022), who reported increased neutrophil percentages in seabream fed certain SWM-containing diets, and suggests that dietary SWM may stimulate innate immune activity. However, while moderate increases in neutrophils can indicate enhanced immune readiness, excessive or sustained neutrophilia may also reflect immune stress or subclinical inflammation; thus, the immunological significance of the present changes requires cautious interpretation and would benefit from challenge tests to determine whether the observed modulation confers enhanced disease resistance. Species differences and the lipid/protein form of SWM (full-fat vs. defatted) appear to mediate divergent hematological responses across studies, which likely explain why some trials report minimal hematological impact (e.g., Prachom et al., 2021) while others report clear immunomodulatory effects (e.g., Henry et al., 2022).

This finding suggests that the dietary inclusion of SWM may stimulate the innate immune response, likely due to bioactive compounds or structural components present in insect meals. Alves et al. (2021) similarly observed heightened neutrophil levels in Nile tilapia fed diets containing insect proteins, reinforcing the current results. Henry et al. (2022) also reported neutrophilia in fish fed SWM, attributing this response to the potential immunogenicity of insect exoskeletons. These structures, rich in chitin, may mimic the structural patterns of parasitic organisms, thereby triggering an immune response even in the absence of a true infection. However, while a moderate increase in neutrophils can be interpreted as a sign of enhanced immune readiness, excessive or sustained neutrophilia might also be a marker of immune stress or an ongoing inflammatory response (Henry et al., 2022). Thus, careful consideration is required when evaluating the immunological effects of insect meals in aquafeeds to differentiate between beneficial stimulation and potentially adverse reactions. In summary, the observed changes in WBC and neutrophil counts suggest that SWM inclusion in stellate sturgeon diets can modulate immune parameters, likely through both nutritional and immunological pathways. These results highlight the dual potential of insect-based meals not only as sustainable protein alternatives but also as functional feed components capable of influencing fish immune responses.

Blood biochemical parameters are widely used as sensitive indicators for assessing the health, metabolic state, and physiological performance of fish. These measurements provide valuable insights into the organism's ability to adapt to nutritional variations and environmental stressors (Abdel-Rahim et al., 2023; Abdollahpour et al., 2025b). They can also signal early pathophysiological changes, making them essential tools for evaluating dietary interventions. In the present study, a notable increase in serum triglyceride levels was observed in fish receiving the diet with 30% SWM inclusion. This aligns with the findings of Valipour et al. (2019), who reported elevated triglycerides in rainbow trout fed insect-based diets. One possible explanation for this trend is a metabolic imbalance caused by inadequate availability of essential amino acids, which may impair liver function and promote lipid synthesis while reducing fatty acid oxidation (Rahmdel et al., 2018). Additionally, insect meals contain chitin and its derivative chitosan, both of which can influence lipid metabolism. Chitin's hydrophilic properties and ability to bind to proteins may interfere with lipid absorption in the gastrointestinal tract, potentially contributing to the observed increase in triglycerides (Tharanathan and Kittur, 2003; Xia et al., 2011). Therefore, the significantly elevated triglyceride levels in the 30% SWM group may reflect a disruption in hepatic lipid processing or absorption, suggesting a degree of hepatic stress or dysfunction at higher substitution levels. Cholesterol concentrations showed an inverse trend, with the highest levels recorded in control and 10% SWM groups, while significantly lower levels were observed in groups receiving higher levels of SWM. This reduction in serum cholesterol associated with higher SWM inclusion has been reported in previous studies (Magalhães et al., 2017; Sharifinia et al., 2023). Animal protein sources like FM typically contain higher cholesterol, which may explain the elevated levels in the control group. In contrast, insect meals due to their chitin content have been proposed to exert a cholesterol-lowering effect, likely through inhibition

of cholesterol absorption or synthesis (Tharanathan and Kittur, 2003). Regarding glucose, no significant differences were found among dietary treatments, suggesting that carbohydrate metabolism remained stable regardless of the SWM inclusion level. This is in agreement with findings reported by Zhou et al. (2018) and Valipour et al. (2019), where glucose homeostasis appeared unaffected by dietary IM inclusion. Significant changes in total serum protein were detected among the groups, indicating that protein metabolism and systemic protein regulation were affected by substituting FM with SWM. These results align with findings from Sankian et al. (2018) and Abdel-Tawwab et al. (2020), which demonstrated that moderate inclusion of alternative protein sources does not impair protein synthesis or degradation. However, substituting FM with SWM likely disrupted the supply of essential amino acids such as methionine and lysine required for hepatic albumin and globulin production, as reported by Brezas and Hardy (2020). Although it is known that alternative protein sources can influence enzyme activity related to protein metabolism (Jahanbakhshi et al., 2013), the absence of changes in serum total protein implies that the inclusion levels used in this study did not reach a threshold that would trigger such effects. In conclusion, the serum biochemical results suggest that moderate inclusion levels of SWM in stellate sturgeon diets can be metabolically tolerated without negative impacts on glucose or protein regulation. However, higher inclusion levels (e.g., 30%) may pose challenges to lipid metabolism, warranting further investigation into optimal inclusion rates and potential dietary supplements to mitigate such effects.

Assessing digestive enzyme activity remains a vital tool for evaluating the effects of novel dietary protein sources in aquaculture nutrition. The current study revealed a significant reduction in trypsin activity in fish receiving the diet containing 30% SWM, suggesting impaired protein digestion at higher inclusion levels. These results are in agreement with the findings of Hosseini Shekarabi et al. (2021), who also reported reduced trypsin activity in rainbow trout fed diets in which FM was partially replaced by superworm larvae meal. Similar trends have been observed in other species. For instance, reductions in digestive enzyme activity were reported when increasing levels of mealworm (*Tenebrio molitor*) and black soldier fly larvae (*Hermetia illucens*) meals were included in the diets of (*Argyrosomus regius*) and *Danio rerio*, respectively (Li et al., 2017; Hoffmann et al., 2020; Wang et al., 2021). These findings highlight a potential limitation of excessive insect meal inclusion, possibly due to factors such as antinutritional compounds like chitin, changes in nutrient availability, or alterations in gut microbiota that may influence enzyme production. However, not all studies have reported consistent outcomes. For example, Hoffmann et al. (2020) and Li et al. (2017) observed no significant differences in digestive enzyme activities when FM was replaced with insect meal in the diets of sea trout (*Salmo trutta*) and Jian carp, respectively. These discrepancies may reflect species-specific digestive physiology, differences in insect meal processing, or variations in experimental conditions, such as fish age, size, and rearing environment. In addition, recent studies using *Z. morio* meals highlight the role of meal form (full-fat vs. defatted), species and inclusion level in shaping digestive-enzyme responses. Asimaki et al. (2025) reported that, in gilthead seabream, graded inclusion of full-fat and defatted *Z. morio* did not alter trypsin, total alkaline proteases or pepsin activities, whereas lipase and α-amylase activities increased with higher insect inclusion, interpreted as a compensatory response to process the altered lipid and carbohydrate supply. Conversely, Lin et al. (2023) found in Pacific white shrimp (*Penaeus vannamei*) that intestinal trypsin activity first increased at low–moderate replacement levels but decreased significantly at high FM replacement (≥60%), while hepatopancreas lipase activity rose at higher inclusion levels. These reports support the view that proteolytic enzyme responses to SWM are not uniform: some species or experimental conditions show no change in trypsin activity, others show reduced intestinal trypsin at high inclusion, and lipase/amylase responses may increase as compensatory mechanisms. Consequently, the reduced trypsin activity observed here at 20–30% SWM is consistent with studies indicating decreased proteolytic capacity at high substitution levels in some species (Lin et al., 2023; Hosseini Shekarabi et al., 2021), whereas other work (Asimaki et al., 2025) suggests that proteolytic capacity can remain unchanged but that lipid/carbohydrate digestive demands increase. Taken together, these data emphasise that SWM form (full-fat vs defatted), species-specific digestive physiology, inclusion level, and diet processing must all be considered when interpreting enzyme activity results and when defining safe inclusion thresholds for SWM. Overall, the variability in enzymatic responses underscores the complexity of incorporating novel protein sources into aquafeeds. It also highlights the importance of tailoring inclusion levels to species-specific digestive capacities. Further research is warranted to elucidate the mechanisms underlying these variable responses, optimise processing methods for insect meals, and determine the most effective inclusion rates that support both digestive efficiency and growth performance across a range of aquaculture species.

5. Conclusion

The present study demonstrates that superworm meals can be used as a partial replacement for FM in the diet of juvenile stellate sturgeons without compromising key performance indicators. Specifically, dietary inclusion of SWM at levels up to 10% did not significantly affect growth performance, feed utilization, hematological profiles, serum biochemical parameters, or proteolytic enzyme activity. This level of inclusion maintained biological functionality and metabolic stability while promoting sustainable feed formulation. Notably, while growth performance and protein utilization declined at higher inclusion levels (particularly at 30%), the moderate inclusion (10%) of SWM supported comparable results to the control group. This suggests a biological threshold beyond which nutritional efficiency and digestive enzyme activity begin to decline, likely due to the presence of indigestible components such as chitin or imbalances in essential amino acids. The immunological responses observed particularly the increase in WBC and neutrophils highlight a potential immunostimulatory role of SWM, though further investigation is warranted to clarify whether these changes represent a beneficial immune modulation or a stress-induced reaction. Overall, these findings support the feasibility of incorporating SWM as a sustainable, alternative protein source in aquafeeds, contributing to reduced reliance on FM and promoting environmental stewardship in aquaculture. Future studies should explore optimal processing methods to reduce antinutritional factors in insect meals, as well as investigate species-specific tolerances and long-term impacts of insect-based diets across various developmental stages and cultured species.

CRediT authorship contribution statement

Arash Lebria: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Hadi Ershad Langroudi:** Visualization, Validation, Supervision, Resources, Project administration, Methodology. **Zabih Ollah Pajand:** Validation, Resources, Project administration, Methodology, Formal analysis. **Mirmasoud Sajjadi:** Visualization, Validation, Methodology, Formal analysis. **Hamed Abdollahpour:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

Authors declare no conflict of interest.

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Data availability

Data will be made available on request.

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