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Black Soldier Fly (Hermetia Illucens) Meal as a Sustainable Alternative Protein Source in Aquafeeds: Effects on Growth Performance, Nutrient Utilization, and Gut Health of Pangasianodon Hypophthalmus

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ABSTRACT

The increasing demand for sustainable aquaculture production and the rising cost of conventional fishmeal have stimulated the search for alternative protein sources in fish feed. Black soldier fly (Hermetia illucens) meal has emerged as a promising insect-based ingredient due to its high nutritional value, sustainability and potential benefits for fish health. The present study evaluated the effects of graded dietary inclusion of BSF meal on growth performance, nutrient utilization, mineral digestibility, body composition, survival and intestinal morphology of Pangasianodon hypophthalmus fingerlings. A 60-day feeding trial was conducted using four experimental diets containing 0% (control), 5% (T1), 10% (T2), and 15% (T3) BSF meal as a partial replacement for fishmeal. The experiment followed a completely randomized design with three replicates per treatment. Dietary inclusion of BSF meal significantly improved fish performance, with the best results observed in fish fed the 10% BSF meal diet (T2). Fish in the T2 group achieved the highest final body weight (40.76 ± 1.62 g), weight gain (30.58 ± 1.41 g), and specific growth rate (2.56 ± 0.06% day^{-1}), along with the lowest feed conversion ratio (1.48 ± 0.03) compared with other treatments. Survival remained high across all dietary treatments, with the highest value observed in fish fed the 10% BSF meal diet (T2). Although survival differed statistically among treatments, the variation was relatively small (approximately 7 percentage points between the lowest and highest values), indicating that all diets supported good fish survival under the experimental conditions. Furthermore, T2 improved whole-body nutrient deposition, with the highest crude protein (60.4 ± 1.5%) and ash content (16.4 ± 0.5%) and the lowest lipid accumulation (10.6 ± 0.4%). Apparent digestibility coefficients were also significantly enhanced, with protein digestibility reaching 85.9 ± 1.3% and mineral digestibility reaching 74.3 ± 1.5% in the T2 group. Histological evaluation revealed improved intestinal development in T2-fed fish, showing increased villus height (502 ± 28 μm) and goblet cell density (18 ± 2 cells $field^{-1}$), indicating enhanced intestinal absorptive capacity and mucosal health. However, increasing BSF meal inclusion to 15% reduced performance compared with the 10% inclusion level, suggesting that excessive supplementation may negatively affect nutrient utilization. BSF meal inclusion influenced survival responses, although all dietary treatments maintained high survival rates, suggesting that the tested diets were suitable for maintaining fish health under the experimental conditions

INTRODUCTION

Aquaculture has become an increasingly important source of animal protein, but its continued expansion depends on the availability of nutritionally adequate and economically sustainable feeds. Fishmeal remains an important protein ingredient in aquafeeds because of its high digestibility and favorable amino acid profile; however, fluctuations in availability, increasing cost, and pressure on marine resources have encouraged the search for alternative protein sources. Insect-derived ingredients have attracted particular interest because they can provide substantial amounts of protein and lipid while converting low-value organic materials into useful biomass (Acutis et al., 2012). Among the insect species being investigated for aquafeeds, the black soldier fly (*Hermetia illucens*) has emerged as one of the most promising candidates for partial replacement of conventional fishmeal (Makkar, etal., 2014).

Black soldier fly larvae meal (BSFLM) contains a relatively high concentration of protein together with lipids, minerals, essential amino acids, and several potentially functional compounds (Mendes et al., 2024). Nevertheless, its nutritional value is influenced by larval developmental stage, rearing substrate, processing method, degree of defatting, and the level incorporated into the diet (Rahman et al., 2024). Consequently, the response of fish to BSFLM has varied considerably among species and experimental conditions. In rainbow trout, partially defatted *H. illucens* meal has been incorporated at dietary levels as high as approximately 40% without major impairment of growth under appropriate formulations (Renna et al., 2017). Such findings indicate that BSFLM has considerable potential as an aquafeed ingredient, although the optimum inclusion level cannot be generalized across species.

Studies conducted specifically in striped catfish (Pangasianodon hypophthalmus) have further demonstrated this variability. Sudha et al. (2022) evaluated replacement of fishmeal protein with BSFLM at 0, 20, 40, 60, 80, and 100%. Growth and feed utilization remained comparable with the control up to 60% fishmeal replacement, corresponding to approximately 174 g BSFLM/kg diet, whereas complete replacement reduced growth and feed efficiency. More recently, Haider et al. (2024) investigated diets containing 30% and 60% BSFL meal, both with and without exogenous protease, and demonstrated that processing and enzyme supplementation can substantially influence protein digestibility and growth responses. Ardra et al. (2024) similarly investigated organic-acid-fermented BSFL meal at 25–100% fishmeal replacement in P. hypophthalmus, emphasizing the potential role of processing in improving utilization of insect-derived protein. Together, these studies show that BSFLM can replace a substantial proportion of fishmeal, but they also indicate that biological responses depend strongly on inclusion level and processing strategy (Acutis et al., 2012).

One of the principal nutritional limitations associated with BSFLM is chitin, a structural polysaccharide present in the insect exoskeleton. Chitin concentrations vary with developmental stage and analytical method, and BSF larvae and prepupae have been reported to contain approximately 1–9% chitin on a dry-matter basis. At moderate concentrations chitin and its derivatives may have functional effects, whereas excessive dietary chitin can interfere with nutrient digestion and reduce nutrient availability in fish. Other antinutritional factors also require consideration. Low concentrations of phytate and other antinutritional compounds have been detected in some BSFL meals, and their occurrence may depend partly on the substrates used to rear the larvae and subsequent processing. The rearing substrate is also important from a feed-safety perspective because BSF larvae can accumulate certain heavy metals when exposed to contaminated substrates. Experimental work has demonstrated substrate-dependent transfer of metals such as cadmium, lead, and zinc, highlighting the importance of controlling the quality of the organic material used for BSF production (Erokhin et al., 2015). These nutritional and safety considerations may partly explain why increasing BSFLM inclusion does not always result in proportional improvements in fish performance.

Pangasianodon hypophthalmus is an economically important freshwater aquaculture species because of its rapid growth, tolerance of intensive culture conditions, and high commercial demand. Although previous studies have evaluated relatively high levels of BSFLM replacement, fermented BSFLM, and BSFLM combined with digestive enzymes in this species, information remains comparatively limited regarding the response to low graded dietary inclusion levels of conventional BSF meal, particularly when growth performance is evaluated together with protein and mineral digestibility, whole-body composition, survival, and quantitative intestinal morphology. This distinction is important because low-to-moderate inclusion may provide nutritional or functional benefits while limiting the adverse effects associated with higher concentrations of indigestible insect components.

Pangasianodon hypophthalmus (Pangasius) is one of the important aquaculture species for freshwater farming in South Asia. It is widely grown owing to its fast growth rate, market demand and its nutritional value by consumers. From the ecological point of view pangasius is a key species in polyculture systems due to its capability of efficient utilization of natural food within the water column (Ismat et al 2026). *Pangasianodon hypophthalmus* also known as Pangasius is one of the most farmed aquaculture species with a resilience to various challenging environmental conditions hence its contribution to food security, nutrition, income generation, and livelihoods (Lock et al., 2016). The species has wide physiological tolerance and is able to feed optimally at low levels of trophic through becoming very effective in intensive farming. Pangasius is described as having a high market demand, economical and sustainable as well as low impact on the environment (Hoque et al., 2021).

Its reproductive cycle is not very long, and fish reach market size of 1–1.5 kg in 8–12 months due to the high rates of growth. Pangasius is an omnivorous fish that can survive at high-density stocking and low dissolved oxygen and salinity environments. Due to this, it is an economically viable crop among the farmers and a stable and nutritious protein source to the consuming population of the world. The nutritional value of Pangasius can be altered depending on the rearing environment and diet, but as a decent 100 g portion of Pangasius fillet, it has around 17 g of protein and 5.6 g of fat (Khan, 2020). Besides, it is well-known that *Pangasianodon hypophthalmus* is an excellent source of nutrients, providing not only high-quality protein but also important minerals (iron), low fat content, and other potential health benefits. *Pangasianodon hypophthalmus* is particularly significant since it contains omega-3 fatty acids, which are known to protect cardiovascular disorders and are therefore heart-friendly foods. These fatty acids have been known to decrease the level of low-density lipoprotein (LDL) cholesterol, reduce inflammation, reduce blood pressure, and improve lipid profiles generally leading to a lower risk of cardiovascular diseases when used in a balanced diet. It is the fastest growing area in the agriculture industry. Aquaculture is less suited to plant diets because of anti-nutrient factors and non-starch polysaccharides (Gutasi, 2021).

Black soldier fly (*Hermetia illucens*) belongs to the family Stratiomyidae and is ecologically important. It was native to North America, but has since spread, due in part to its growing use by researchers and by the local community. The adult is large, about 15–20 mm in length, with an exclusive appearance of black coloration on their wings. BSF has a friendly nature and is considered harmless, as it does not transmit diseases to humans or other organisms, which is also one of the important characteristics of friendly natures (Kim et al., 2021). *H. illucens* is a complete insect, and exists in four life stages: egg, larva, pupa and adult. Larvae are the most interesting, economically important and most valuable in waste management and composting and are called black soldier fly larvae (BSFL) (Sealey et al., 2011). After females hatch eggs in organic matter, the larvae feed on organic waste for 14–21 days. The larvae then pupate for 5–10 days.

Previous studies have evaluated relatively high levels of BSF larvae meal in striped catfish and other aquaculture species. For example, BSFL meal has been tested at considerably higher inclusion or fishmeal-replacement levels in *P. hypophthalmus*, including 30% and 60% dietary inclusion in a recent feeding trial. However, responses to BSF meal depend on inclusion level, diet formulation, processing conditions, and the ability of fish to utilize insect-derived nutrients. Higher inclusion may also increase the dietary contribution of chitin and other poorly digestible components, which can influence protein utilization. Therefore, rather than attempting near-total replacement of fishmeal, the present study was designed to evaluate a graded low-to-moderate inclusion range of BSF meal. The control diet contained 30% fishmeal, and BSF meal was incorporated at 5%, 10%, and 15% while fishmeal was correspondingly reduced to 25%, 20%, and 15%. Thus, the highest BSF treatment represented a 50% replacement of the fishmeal component of the control diet, while the remaining major dietary ingredients were maintained at constant levels. The 5% increments were selected to permit evaluation of a progressive dietary response and to identify an inclusion level that could improve nutrient utilization and intestinal health without requiring extensive alteration of the basal formulation. Accordingly, the present 60-day feeding trial evaluated diets containing 0%, 5%, 10%, and 15% BSF meal in *Pangasianodon hypophthalmus* fingerlings. We hypothesized that moderate BSF meal inclusion would improve growth performance, nutrient and mineral utilization, survival, body composition, and intestinal morphology, whereas increasing the inclusion level beyond the optimum would reduce these benefits.

MATERIALS AND METHODS

The present study was conducted to evaluate the effects of black soldier fly (*Hermetia illucens*) meal supplementation on growth performance, nutrient utilization, intestinal morphology, and mineral utilization of *Pangasianodon hypophthalmus* fingerlings. The feeding trial was conducted at the Fish Nutrition Laboratory, Department of Fisheries and Aquaculture, University of Okara, Pakistan.

Ethical Statement

The experimental procedures involving *Pangasianodon hypophthalmus* fingerlings were conducted in accordance with the ethical guidelines provided by the University of Okara for the care and use of experimental animals. All procedures were performed under controlled laboratory conditions, and necessary measures were taken throughout the experiment to minimize handling stress and ensure the welfare of experimental fish.

Fish Collection, Acclimatization and Experimental Conditions

Healthy fingerlings of *Pangasianodon hypophthalmus* were obtained from the Government Fish Seed Hatchery, Head Balloki, Pakistan. Before the initiation of the feeding experiment, fish were transported to the Fish Nutrition Laboratory and acclimatized under laboratory conditions for two weeks. During the acclimatization period, fish were maintained in glass aquaria with a water holding capacity of 50 L and supplied with continuous aeration through an air pump to maintain adequate dissolved oxygen levels. Fish were fed a basal diet once daily during acclimatization until apparent satiation. Prior to the start of the feeding trial, fingerlings were treated with sodium chloride (NaCl; 5 g L^{-1}) to reduce ectoparasite load and prevent fungal infection following the method described by Hussain et al. (2017). After acclimatization, fish were randomly distributed into experimental tanks according to the dietary treatments. The initial body weight of fish was recorded before the beginning of the feeding experiment.

Experimental Diet Formulation

The black soldier fly (*Hermetia illucens*) meal used in the present study was obtained from [supplier/source] and was prepared from dried BSF larvae. The meal was incorporated into experimental diets without additional processing. The proximate composition of BSF meal was determined before diet formulation, including dry matter, crude protein, lipid, crude fibre, ash, and gross energy contents (Table 2).

Four experimental diets were formulated to evaluate the effect of graded inclusion levels of BSF meal on the performance of *P. hypophthalmus* fingerlings. The dietary treatments were, Control diet (T0): 0% BSF meal, T1: 5% BSF meal, T2: 10% BSF meal and T3: 15% BSF meal. BSF meal was incorporated into the diets by partially replacing conventional fish meal. Feed ingredients were obtained from commercial sources and their proximate compositions were determined using standard analytical procedures (Mueller-Harvey, 2004). The diet composition is presented in Table 1.

Preparation of Experimental Pellets

All feed ingredients were finely powdered to obtain a uniform particle size suitable for fingerling feeding. The powdered ingredients were mixed thoroughly for 10–20 minutes using an electric mixer to ensure homogeneous distribution of nutrients. After mixing the dry ingredients, oil was gradually incorporated while continuous mixing was maintained. Chromic oxide (Cr_2O_3) was added at 1% concentration as an inert marker for digestibility analysis. Water (approximately 10–15%) was gradually added to prepare a dough-like mixture. The prepared dough was processed through a laboratory pelletizer/extruder to produce sinking pellets suitable for *P. hypophthalmus* fingerlings. The prepared diets were dried, packed separately, and stored under appropriate conditions until use in the feeding trial.

Experimental Design and Feeding Trial

The feeding experiment was conducted for 60 days. A completely randomized design was followed with four dietary treatments and three replicates per treatment. Each experimental treatment was assigned to three aquaria, and each aquarium contained ten fish. Therefore, a total of 12 experimental aquaria were used for the feeding trial. Fish were fed twice daily (morning and afternoon) at a feeding rate of 5% of their live body weight per day. The daily feed ration was adjusted according to periodic weight measurements of fish. Feed was provided manually, and uneaten feed was collected approximately two hours after feeding using siphoning. The collected feed residues were removed from the tanks to maintain water quality and were considered during feed utilization calculations. Fish fecal material was collected carefully from each aquarium using siphoning while avoiding breakage of fecal strands to minimize nutrient loss. The collected fecal samples from each replicate were pooled, dried, ground, and stored for subsequent chemical analysis.

Table 1. Ingredient composition (%) of experimental diets containing graded levels of black soldier fly meal (*Hermetia illucens*).

Ingredients (%)	Control (T0)	T1 (5% BSF)	T2 (10% BSF)	T3 (15% BSF)
Fish meal	30.0	25.0	20.0	15.0
Black soldier fly meal	0.0	5.0	10.0	15.0
Soybean meal	20.0	20.0	20.0	20.0
Wheat flour	11.0	11.0	11.0	11.0
Corn gluten meal	10.0	10.0	10.0	10.0
Maize gluten meal	12.0	12.0	12.0	12.0

Rice polish	12.0	12.0	12.0	12.0
Vegetable oil	2.0	2.0	2.0	2.0
Vitamin premix	1.0	1.0	1.0	1.0
Mineral premix	1.0	1.0	1.0	1.0
Chromic oxide (Cr_2O_3)	1.0	1.0	1.0	1.0
Total (%)	**100**	**100**	**100**	**100**

Water Quality Monitoring

Water quality parameters were monitored on a daily basis throughout the experimental period to maintain optimal environmental conditions for *Pangasianodon hypophthalmus* fingerlings. Temperature, pH, and dissolved oxygen were recorded regularly and maintained within the suitable range for fish growth. To minimize the accumulation of metabolic wastes and maintain water quality, a scheduled partial replacement of aquarium water with fresh water was carried out at regular intervals during the feeding trial. In addition, continuous aeration was provided, and uneaten feed and fecal materials were removed through siphoning to reduce organic matter accumulation. These management practices helped minimize the risk of nitrogenous waste accumulation under the experimental conditions. Although ammonia and nitrite concentrations were not directly measured in the present study, all experimental tanks were maintained under identical management conditions, and water quality parameters remained stable throughout the feeding period. Temperature, pH, and dissolved oxygen (DO) were measured regularly using a Jenway pH meter (Model 3510) and dissolved oxygen meter (Model 970).

Table 2. Proximate composition of feed ingredients used for experimental diets (Dry matter basis).

Ingredient	Dry Matter (%)	Crude Protein (%)	Lipid (%)	Crude Fibre (%)	Ash (%)	Gross Energy (kcal/g)	Carbohydrate (%)
Fish meal	92.5	65.2	9.8	0.5	15.1	4.67	9.4
BSF meal	93.0	42.1	28.4	7.2	7.5	5.43	14.8
Soybean meal	89.0	44.0	1.5	6.8	6.5	4.10	41.2
Wheat flour	87.5	11.5	1.2	2.5	0.6	3.82	84.2
Corn gluten meal	90.0	60.5	2.1	1.8	1.5	4.55	34.1
Maize gluten meal	89.5	22.0	3.5	8.5	5.2	4.20	60.8
Rice polish	88.0	12.5	14.2	9.8	8.5	4.35	55.0
Vegetable oil	99.8	0.0	99.8	0.0	0.0	9.50	0.2

Growth Performance Analysis

Growth performance of *Pangasianodon hypophthalmus* fingerlings was evaluated throughout the 60-day feeding trial. Fish from each experimental tank were weighed at 15-day intervals (days 15, 30, 45, and 60) to monitor growth changes. Before weighing, fish were gently handled to minimize stress. The final body weight was recorded at the end of the feeding experiment. Growth performance parameters were calculated using the following formulas:

Weight Gain (WG)

WG (g) = Final weight(g)−Initial weight(g)

Weight Gain % (WG%)

Weight Gain (%) = [(Final Weight − Initial Weight) / Initial Weight] × 100

Feed Conversion Ratio (FCR)

FCR = Feed Consumed (g) / Weight Gain (g)

Specific Growth Rate (SGR %)

SGR (%) = 100 × (ln Wf − ln Wi) / Number of Trial Days

Where Wf = final body weight and Wi = initial body weight.

Formation of Pellets

All feed ingredients were finely ground and passed through a 0.5 mm sieve to obtain a uniform particle size suitable for *Pangasianodon hypophthalmus* fingerlings. The required quantities of each ingredient were weighed according to the experimental diet formulation and thoroughly mixed for 10–20 minutes using an electric mixer to ensure uniform distribution of nutrients. Vegetable oil was gradually incorporated during mixing, followed by the addition of chromic oxide (Cr_2O_3) at 1% inclusion level as an inert marker for digestibility analysis. Water (approximately 10–15%) was gradually added to form a dough-like mixture. The prepared feed dough was processed through a laboratory pelletizer/extruder to produce sinking pellets suitable for fingerling feeding. The pellets were dried under controlled conditions, packed separately, and stored in airtight containers until use in the feeding trial (Sealey et al., 2011).

Feeding Protocol and Sample Collection

P. hypophthalmus fingerlings were fed twice daily (morning and afternoon) at a feeding rate of 5% of their body weight per day throughout the 60-day feeding trial. The daily feed ration was divided equally between the two feeding periods and adjusted according to periodic changes in fish biomass. Each treatment group was assigned to three replicate aquaria, with ten fish stocked per aquarium. After two hours of feeding, uneaten feed was carefully collected by siphoning to minimize feed wastage and contamination. The collected feed residues were removed from the tanks and considered during feed intake calculations. Fecal samples were collected from each aquarium by siphoning every two hours while avoiding breakage of fecal strands to minimize nutrient loss. The collected fecal material from each replicate was pooled, dried, ground, and stored for subsequent chemical analysis and digestibility estimation.

Chemical Analysis of Feed and Feces

Feed ingredient, experimental diet and feces samples were collected using a mortar and pestle and routine analysis (Pigden et al., 1980) carried out. Crude fat was extracted by the petroleum ether method described by Sánchez-Muros et al. (2014) followed by the measurement of crude fiber by loss on ignition of dried lipid-free residues of sample after digesting with 1.25-percent H 2 SO4 & 1.25-percent NaOH and Gross energy by use of Oxygen Bomb Calorimeter.

Dry Matter Estimation

One gram of feed and excrement was placed in a weight Petri dish and placed in the oven at 105 °C for 12 hours. The sample was cooled in a desiccator for five minutes, followed by weighing. Once again the sample was placed in an oven for 1 or 2 hours until it was down to a consistent weight. To calculate the percentage of moisture and dry matter the following formula was used.

$$\text{Moisture (\%)} = [(W1 - W2) / W1] \times 100$$

$$\text{Dry Matter (\%)} = 100 - \text{Moisture (\%)}$$

Where W_1 = weight of dish + sample before drying; W_2 = weight of dish + sample after drying

Crude Protein Estimation

The crude protein of the feed and feces sample was considered using a method created by Micro Kjeldahl. K2 SO4, FeSO4, and CuSO4 were combined in a 90:3:7 ratio. Then, 30 ml of H2SO4, 5g digestion mixture, and 1g dried sample were combined to a Kjeldahl flask. The Kjeldahl flask was warmed in a heated plate until it changed to crystal clear and translucent and greenish in color. The content of the mixture underwent digestion and was then cooled followed by a dilution with distilled water in a 250 ml flask. This diluted 10 ml. of a 10 mm H 2O solution and 10 ml. of 40 percent NaOH were then added to the micro Kjeldahl apparatus and steam-distilled. To capture the ammonia which was released, 10 milliliters of a 2% solution of boric acid were added to which, a drop of methyl red indicator was added. The ammonia was collected near to two minutes after the color of the indicator had changed to golden yellow. A titration of the ammonia in the boric acid solution was then performed against 0.1 N H2SO4. The following calculation was used to determine the sample's percentage of N2.

$$\text{N2 (\%)} = [(\text{Volume of } H_2SO_4 \times \text{Normality} \times 0.014 \times 250) / (\text{Weight of Sample} \times 10)] \times 100$$

Crude protein was then derived as:

$$\text{Crude Protein (\%)} = N_2 \text{ (\%)} \times 6.25$$

Where,

Given standard volume of 0.1N H2SO4 used for neutralizing ammonia = 0.014 ml

If digestion mixture is 250 = Dilution

•100 = for percentage of N2

10 = Volume of digestion and diluted sample used

The amount of crude protein present in the sample was determined by using the formula below.

•Crude protein (%) = N2 × 6.25

Chromic Oxide

The chromic oxide concentration of the ash samples of the experimental meals and faecal samples were measured by UV-VIS 2001 spectrophotometer with the help of acid digestion method (Divakaran et al., 2002) at 350 nm.

Calculation of Nutrient Digestibility

The apparent digestibility coefficients (ADC%) of dietary nutrients were calculated using chromic oxide (Cr_2O_3) as an inert dietary marker. Chromic oxide concentrations in experimental diets and fecal samples were determined, and nutrient digestibility was calculated according to the following equation (Divakaran et al., 2002):

ADC (%) of experimental diets was calculated by the following formula:

$$\text{ADC (\%)} = 100 \times [100 - (Cr_2O_3 \text{ in feed} / Cr_2O_3 \text{ in feces} \times \text{Nutrient in feces} / \text{Nutrient in feed})]$$

Intestinal Histology and Morphometric Analysis

At the end of the feeding trial, intestinal samples were collected from randomly selected fish from each experimental treatment for histological evaluation. The anterior intestine was selected for analysis because it represents the primary site of nutrient digestion and absorption in fish. The intestinal tissues were carefully removed and immediately fixed in 10% neutral buffered formalin for preservation. After fixation, samples were processed through graded ethanol dehydration, cleared in xylene, and embedded in paraffin wax. Tissue sections of approximately [4–5 μm] thickness was prepared using a microtome and stained with hematoxylin and eosin (H&E) for general intestinal morphology evaluation. Histological examination was performed using a light microscope equipped with an image analysis system. Villus height and goblet cell density were measured from well-oriented intestinal sections. Villus height was measured from the base of the villus to the tip, while goblet cells were counted per defined microscopic field. Measurements were obtained from [number] villi/fields per fish and averaged for each individual. The mean values from sampled fish were used for statistical comparison among dietary treatments. Villus height and goblet cell density were selected as primary morphometric indicators because they provide important information regarding intestinal absorptive capacity, epithelial integrity, and mucosal function in response to dietary changes.

STATISTICAL ANALYSIS

The experimental data were subjected to one-way analysis of variance (ANOVA) to evaluate the effects of different dietary inclusion levels of black soldier fly meal on growth performance, nutrient digestibility, and other measured parameters. When significant differences were detected among treatments, Tukey's honestly significant difference (HSD) test was applied for multiple mean comparisons. Statistical significance was considered at $P < 0.05$. All statistical analyses were performed using CoStat Statistical Software (Version 6.303, PMB 320, Monterey, CA, 93940, USA).

RESULTS

The present study evaluated the effects of graded dietary inclusion of BSF meal on growth performance, nutrient utilization, survival, body composition, intestinal morphology, and mineral digestibility of *P. hypophthalmus* fingerlings. The results demonstrated that dietary supplementation of BSF meal influenced several physiological and nutritional responses of fish. Among the tested dietary treatments, moderate inclusion of BSF meal (10%; T2) showed the most favorable outcomes, with improved growth performance, feed utilization efficiency, nutrient digestibility, mineral utilization, and intestinal morphological characteristics compared with the control and other inclusion levels. The effects of BSF meal supplementation on water quality parameters, growth performance, survival, body composition, apparent digestibility coefficients, and intestinal morphology are presented in the following sections. Fish were weighed at 15-day intervals primarily for monitoring growth progression and feed adjustment during the feeding trial. The statistical evaluation was performed using the final growth performance parameters obtained after completion of the 60-day feeding period.

Water Quality Parameters

Water quality parameters including temperature, pH, and dissolved oxygen (DO) were maintained within suitable ranges throughout the 60-day feeding trial. Table 3 presents the mean values of water quality parameters recorded in all experimental treatments. Water temperature showed no significant differences among dietary treatments ($P > 0.05$), with values ranging from 27.3 ± 0.6°C to 27.6 ± 0.5°C. Similarly, pH values remained stable among treatments, ranging from 7.3 ± 0.2 to 7.5 ± 0.3, and no significant variation was observed ($P > 0.05$). Dissolved oxygen concentrations ranged from 6.1 ± 0.4 to 6.3 ± 0.3 mg/L among experimental groups. Statistical analysis indicated no significant differences in DO levels among treatments ($P > 0.05$). Overall, water quality parameters remained consistent throughout the experimental period, indicating that observed differences in growth performance, feed utilization, body composition, and digestibility parameters were primarily associated with dietary treatments rather than environmental variations.

Table 3. Water quality parameters (temperature, pH, and dissolved oxygen) recorded during the 60-day feeding trial in *P. hypophthalmus* fingerlings fed with graded levels of black soldier fly meal (Mean ± SD).

Parameter	Control	T1 (5% BSF)	T2 (10% BSF)	T3 (15% BSF)
Temperature (°C)	27.4 ± 0.6	27.6 ± 0.5	27.5 ± 0.7	27.3 ± 0.6
pH	7.4 ± 0.2	7.5 ± 0.3	7.4 ± 0.2	7.3 ± 0.2
Dissolved oxygen (mg/L)	6.2 ± 0.3	6.1 ± 0.4	6.3 ± 0.3	6.2 ± 0.2

Growth Performance

The experimental diets had a significant impact on the growth performance of *P. hypophthalmus* fingerling. The parameters of growth measured were, initial body weight, final body weight, weight gain, specific growth rate (SGR), and the feed conversion ratio (FCR).

There was no significant difference (P >0.05) in the initial body weight of fish among treatment, thus, establishing equal distribution of the fingerlings before the feeding trial was carried out. Weights at baseline were 10.12 ± 0.45 g in the control group and 10.18 ± 0.41 in T2, showing that there was no heterogeneity in the experimental groups. After the 60-day feeding trial, treatment showed significant difference (P < 0.05) in final body weight. The final body weight (40.76 ±1.62 g) was highest in fish fed T2 (10% insect inclusion diet), followed by T1 (36.82 ± 1.45 g). The final weights of the control group (32.45 ± 1.23 g) and T3 (35.12 ± 1.37 g) were relatively lower and did not differ significantly from each other (P > 0.05). The positive effect of moderate inclusion (10%) of insect by-products in T2 suggests improved nutrient utilization and growth efficiency. Figure 1 illustrates this trend, with T2 clearly demonstrating superior growth performance.

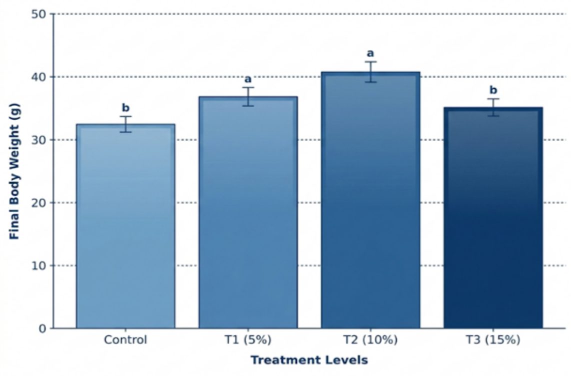

Figure 1. Effect of graded dietary inclusion levels of black soldier fly meal (BSF meal) on final body weight of P. hypophthalmus fingerlings after 60 days of feeding trial.

Weight Gain

The gain in weight showed a similar trend to the final body weight. T2 (30.58 ± 1.41 g) had the highest weight gain that was significantly higher (P < 0.05) compared to the control and T3 groups. The weight gain was also better in T1 (26.67 ± 1.32 g) than the control (22.33 ± 1.10 g). The control group registered the lowest weight gain (Table 4). These outcomes point to the fact that insect by-products dietary supplementation to 10 percent was effective in significantly improving growth performance, but further addition (15%) did not lead to further increase.

Table 4. Growth performance parameters of *P. hypophthalmus* fingerlings fed experimental diets containing graded levels of black soldier fly meal (BSF meal) for 60 days (Mean ± SD).

Parameter	Control	T1 (5%)	T2 (10%)	T3 (15%)
Initial weight (g)	10.12 ± 0.45	10.15 ± 0.39	10.18 ± 0.41	10.14 ± 0.44
Final weight (g)	32.45 ± 1.23^{d}	36.82 ± 1.45^{b}	40.76 ± 1.62^{a}	35.12 ± 1.37^{c}
Weight gain (g)	22.33 ± 1.10^{d}	26.67 ± 1.32^{b}	30.58 ± 1.41^{a}	24.98 ± 1.29^{c}
SGR (%/day)	2.15 ± 0.08^{d}	2.38 ± 0.07^{b}	2.56 ± 0.06^{a}	2.30 ± 0.09^{c}
FCR	1.82 ± 0.05^{d}	1.65 ± 0.04^{b}	1.48 ± 0.03^{a}	1.70 ± 0.06^{c}

Different superscripts within rows indicate significant differences (P < 0.05).

Specific Growth Rate (SGR)

There was a significant difference in specific growth rates among treatments (P < 0.05). T2 (2.56 ± 0.06 percent per day) had the highest SGR, and T1 (2.38 ± 0.07 percent per day) had the lowest. The lowest levels of SGR (2.15±0.08%/day) were found in the control group, intermediate levels in T3 (2.30±0.09%/day) as shown in Table 4. Increased feed efficiency and improved metabolic efficiency with dietary nutrients at 10 percent inclusion level is indicated by increased SGR in T2.

Feed Conversion Ratio (FCR)

Dietary treatments had a significant influence on the feed conversion ratio (P < 0.05). T2 (1.48±0.03) had the lowest (best) FCR which means it makes better use of its feed. T1 had a better FCR (1.65 ± 0.04) than the control (1.82 ± 0.05). T3 also exhibited moderate improvement (1.70 ± 0.06) and it was lower than T2. The fact that FCR decreased considerably at 10% inclusion indicates optimum levels of dietary nutrient digestibility and efficiency of conversion at this dietary level. The relationship between the inclusion level and FCR is inverse with the level reaching 10% as shown in Figure 2. In general, the parameters of growth performance showed a clear improvement with an increase in the level of insect by-product up to 10 percent, above which, a slight decrease in performance was observed with the increase in insect by-product inclusion to 15 percent. The best inclusion level in terms of final weight, weight gain, SGR, and lowest FCR was found to be 10% dietary inclusion level (T2), hence it was the best inclusion level as per the current experimental conditions.

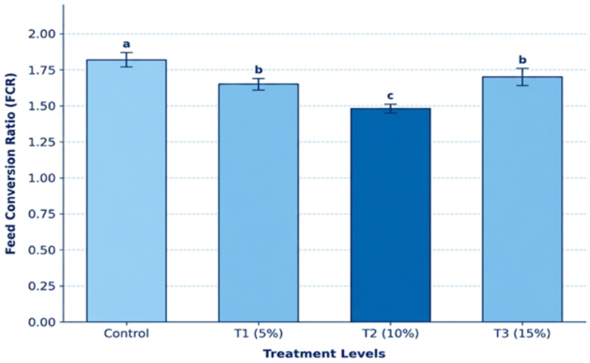

Figure 2. Effect of graded dietary inclusion levels of black soldier fly meal (BSF meal) on feed conversion ratio (FCR) of P. hypophthalmus fingerlings after 60 days of feeding trial.

Survival Rate

Table 5 shows the survival (%) of Pangasius fingerling fed on graded dietary inclusion levels of black soldier fly meal (BSF meal). The treatment differences were found to be significant ($P < 0.05$). T2 (95.6 ± 1.5%) showed the highest survival rate, followed by T1 (92.4 ± 1.8%) and T3. T1 and T3 were statistically similar to each other but significantly higher than the control group (88.3 ± 2.1%). The lowest survival rate was observed in the control group. The better survival of T1 and T2 showed that moderate dietary inclusion of insect by-products had beneficial effects on the health of fish and their resistance to stress. Nevertheless, more addition of inclusion up to 15% (T3) did not add extra survival advantages and was statistically equal to the control group. In general, the findings indicate that the 10% dietary inclusion did not only improve the growth performance but also increased the survival in the current experimental conditions.

Table 5. Survival (%) of *P. hypophthalmus* fingerlings fed experimental diets containing graded levels of black soldier fly meal (BSF meal) for 60 days (Mean ± SD).

Treatment	Survival (%)
Control	88.3 ± 2.1^{b}
T1 (5%)	92.4 ± 1.8^{a}
T2 (10%)	95.6 ± 1.5^{a}
T3 (15%)	90.2 ± 2.0^{b}

Different superscripts indicate significant differences ($P < 0.05$).

Proximate Body Composition

The effects of dietary supplementation of black soldier fly meal (BSF meal) on the whole-body proximate composition of *P. hypophthalmus* fingerlings are presented in Table 6. Dietary inclusion of BSF meal significantly influenced whole-body crude protein, lipid, and ash contents among the experimental groups ($P < 0.05$). Whole-body crude protein content showed a significant increase with increasing BSF meal inclusion. The highest crude protein deposition was observed in fish fed the T2 diet (10% BSF meal) (60.4 ± 1.5%), followed by T1 (5% BSF meal) (57.8 ± 1.3%), while the lowest values were recorded in the control and T3 groups (55.2 ± 1.1% and 56.3 ± 1.2%, respectively). The T1 and T2 groups showed significantly higher crude protein levels compared with the control and T3 treatments ($P < 0.05$). Whole-body lipid content showed an opposite trend, with significantly lower lipid deposition observed in BSF meal-supplemented groups compared with the control. The lowest lipid content was recorded in T2 (10.6 ± 0.4%), whereas the highest value was observed in the control group (12.4 ± 0.6%). Significant differences were observed among all dietary treatments ($P < 0.05$). Ash content was significantly affected by BSF meal inclusion. The highest ash content was recorded in fish fed the T2 diet (16.4 ± 0.5%), followed by T1 (15.2 ± 0.6%), while the control group showed the lowest value (14.1 ± 0.4%). The T1 and T2 groups exhibited significantly higher ash deposition compared with the control and T3 groups ($P < 0.05$). Overall, moderate inclusion of BSF meal (10%; T2) resulted in improved nutrient deposition, reflected by higher whole-body protein and ash contents and reduced lipid accumulation compared with the control and higher BSF inclusion level (Table 6)

Table 6. Whole-body proximate composition of *P. hypophthalmus* fingerlings fed experimental diets containing graded levels of black soldier fly meal (BSF meal) for 60 days (expressed as % dry matter; Mean ± SD).

Parameter	Control	T1	T2	T3

Crude Protein	55.2 ± 1.1[b]	57.8 ± 1.3[a]	60.4 ± 1.5[a]	56.3 ± 1.2[b]
Lipid	12.4 ± 0.6[a]	11.8 ± 0.5[b]	10.6 ± 0.4[c]	11.5 ± 0.5[b]
Ash	14.1 ± 0.4[b]	15.2 ± 0.6[a]	16.4 ± 0.5[a]	14.8 ± 0.4[b]

Different superscripts within rows indicate significant differences ($P < 0.05$).

Apparent Digestibility Coefficient (ADC%)

Protein ADC was found to vary significantly across treatments ($P < 0.05$). T2 (85.9 ± 1.3%), and T1 (82.6 ± 1.5%), were found to be the highest and second-highest protein digester respectively. The least digestible (78.4 + 1.2%) was the control group. Figure 3 presents the growing trends in the inclusion rate up to 10% to digestibility. These results show an increased level of protein consumption at 10% insect inclusion rate. The same was also the case with Mineral ADC. T2 had the largest mineral digestibility (74.3 ± 1.5%), which was significantly high compared to control (65.2 ± 1.6) as shown in table 7. T3 had moderate improvement but was poor compared to T2. T2 has a higher mineral digestibility which can be associated with the elevated ash content in the whole-body composition (Table 7)

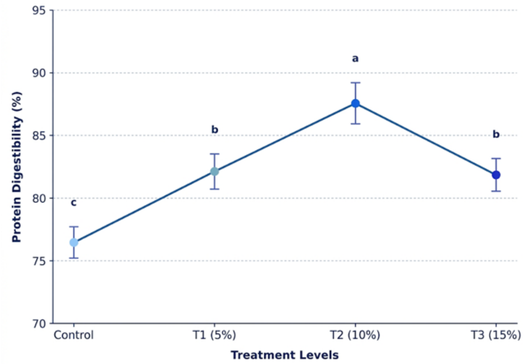

Figure 3. Effect of graded dietary inclusion levels of black soldier fly meal (BSF meal) on protein digestibility of P. hypophthalmus fingerlings after 60 days of feeding trial.

Table 7. Apparent digestibility coefficients (ADC, %) of nutrients in *P. hypophthalmus* fingerlings fed experimental diets containing graded levels of black soldier fly meal (BSF meal) for 60 days (Mean ± SD).

Parameter	**Control**	**T1**	**T2**	**T3**
Protein ADC	78.4 ± 1.2[b]	82.6 ± 1.5[a]	85.9 ± 1.3[a]	80.2 ± 1.4[b]
Mineral ADC	65.2 ± 1.6[b]	70.8 ± 1.7[a]	74.3 ± 1.5[a]	68.9 ± 1.8[b]

Different superscripts indicate significant differences ($P < 0.05$).

Intestinal Morphology

The effects of dietary black soldier fly meal supplementation on intestinal morphology of *P. hypophthalmus* fingerlings are presented in Table 8. Dietary inclusion of BSF meal significantly influenced intestinal morphometric parameters, including villus height and goblet cell density ($P < 0.05$). The highest villus height was observed in fish fed the T2 diet (10% BSF meal), reaching 502 ± 28 µm, followed by T1 (5% BSF meal) with 468 ± 24 µm. The control group had the lowest villus height (412 ± 22 µm) and the T3 group had an intermediate villus height (435 ± 20 µm). There was a significant increase in the height of the villi in the fish fed T1 and T2 diets than in those fed the control and T3 diets ($P < 0.05$). Likewise, BSF meal supplementation had a significant impact on the density of goblet cells. The T2 group (18 ± 2 cells/field) had the highest number of goblet cells, followed by T1 (16 ± 3 cells/field) and the lowest number of goblet cells was observed in the control group (12 ± 2 cells/field). The T3 treatment resulted in 14 ± 2 cells/field, significantly less than the T1 and T2 treatments, but more than the control treatment. In general, BSF meal 10% (T2) showed the effects of improved intestinal structural development in terms of increased villus height and abundance of goblet cells, indicating better intestinal absorptive capacity and intestinal health in *P. hypophthalmus* fingerlings (Figure 4).

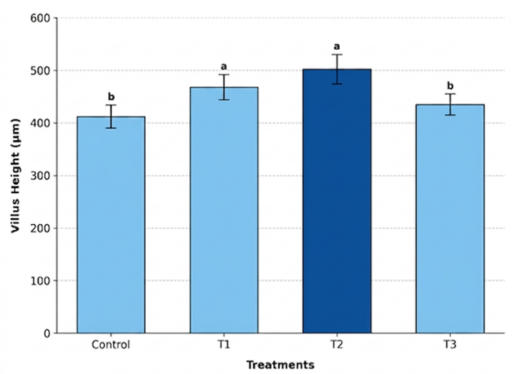

Figure 4. Effect of graded dietary inclusion levels of black soldier fly meal (BSF meal) on intestinal villus height of P. hypophthalmus fingerlings after 60 days of feeding trial.

Table 8. Intestinal morphometric measurements of *P. hypophthalmus* fingerlings fed experimental diets containing graded levels of black soldier fly meal (BSF meal) for 60 days (Mean ± SD).

Parameter	Control	T1	T2	T3
Villus Height (µm)	412 ± 22^{b}	468 ± 24^{a}	502 ± 28^{a}	435 ± 20^{b}
Goblet Cells (No./field)	12 ± 2^{b}	16 ± 3^{a}	18 ± 2^{a}	14 ± 2^{b}

Different superscripts indicate significant differences ($P < 0.05$).

DISCUSSION

The present study demonstrated that moderate dietary inclusion of BSF meal improved growth performance and feed utilization in *Pangasianodon hypophthalmus*, with the most favorable response observed at the 10% inclusion level. The improved performance at this inclusion level suggests that BSF meal can provide a suitable nutritional balance when incorporated at moderate levels. Insect meals are recognized as promising alternative protein ingredients because they provide highly digestible protein, essential amino acids, lipids, minerals, and bioactive compounds that may support growth and physiological functions in fish. The improved response at 10% BSF meal inclusion may be related to better synchronization between nutrient availability and fish nutritional requirements. Moderate inclusion likely provided additional high-quality nutrients without substantially increasing dietary components that may limit nutrient utilization. Similar improvements in growth performance have been reported in several aquaculture species fed moderate levels of BSF meal, suggesting that partial replacement of conventional protein sources can maintain or enhance growth when dietary formulation is balanced. In contrast, the reduced performance observed at the highest inclusion level indicates that increasing insect meal inclusion does not always result in proportional benefits. Higher inclusion levels may influence nutrient utilization due to changes in dietary composition, including increased structural components such as chitin, variations in amino acid balance, or reduced availability of certain nutrients. However, because chitin content and amino acid composition of the BSF meal were not measured in the present study, these factors should be considered possible explanations rather than confirmed mechanisms. Similar observations have been reported in other fish species where moderate inclusion of BSF meal supported growth, whereas higher inclusion levels resulted in reduced performance. These findings emphasize that the optimal inclusion level of BSF meal is species-specific and depends on dietary formulation, processing method, fish developmental stage, and nutrient requirements.

In several previous studies moderate inclusion of insect meal resulted in similar improvements in fish growth. In aquaculture, feed plays a very important role not only in fish growth but also in environmental sustainability. Fishmeal which has always been a traditional food is costing more and is linked to overexploitation of fish populations. Thus, the necessity to investigate alternative feeds that are nutritionally efficient and environmentally friendly exists. The optimal feed product must be rich in protein and essential amino acids, omega-3 fatty acids, and good digestibility with minimal amounts of fiber and detrimental compounds (Egessa, 2025). The insects have become a potential substitute in this respect. Past research has pointed out that insect feeds are sustainable and cost-effective (Sogari et al., 2019). Moreover, insect foods have a high content of polyunsaturated fatty acids, which play a role in enhancing fish health (Egessa, 2025). The black soldier fly among other insect sources has been identified to be more viable compared to plant-based substitutes, especially with regard to gut health. Mealworms also start to attract interest as the production systems are turning more efficient and commercially feasible (Usman & Yusuf, 2021).

Prior to the analysis of the biological responses, it is significant to ensure that there was no change in the environmental conditions throughout the experiment. There were no significant differences in the temperature, pH, and dissolved oxygen among the treatments in the current study. This means that all the groups were kept in an equal condition and the differences in performance which were observed can be mainly due to diet treatments and not due to environmental factors Henry et al. (2015) and Hassan et al. (2009) report similar findings. In such controlled conditions, the growth performance of fish fed on 10% insect inclusion (T2) was better as evidenced by an increase in final weight, weight gain, and specific growth rate. These findings align with other researchers that indicated increased growth at moderate insect meal inclusion levels (Belforti et al., 2015 and Nogales-Mérida et al., 2019). The results were similar to those reported by Ismat et al. (2025) in *Labeo rohita* and the same responses were obtained in *Pangasianodon hypophthalmus* in the present study which suggest that the effect of insect meal will be the same in different fish species. The same pattern was witnessed in the survival rate. Fish that received moderate amounts of insect meal had better survival rates than the control group, which showed that they were healthier and more adaptable. Other past research also indicated improved immune response and survival of fish kept on insect-based diet (Gasco et al., 2018). Nevertheless, less survival at high levels of inclusion indicates that over inclusion is not likely to be of more benefit. Food also had an effect in the composition of the body of fish.

The growth-promoting effect of moderate BSF meal inclusion observed *in Pangasianodon hypophthalmus* is consistent with findings reported in other warm-water omnivorous and catfish species. Unlike salmonids, which are strict carnivorous species with high dietary protein requirements, *P. hypophthalmus* has an omnivorous feeding habit and greater adaptability toward alternative plant- and insect-derived protein sources. Studies in African catfish (*Clarias gariepinus*) have demonstrated that partial replacement of fishmeal with black soldier fly larvae meal can maintain or improve growth performance when diets are appropriately balanced, indicating efficient utilization of insect-derived nutrients by tropical catfish species. Similarly, research on channel catfish *(Ictalurus punctatus*) has shown that insect-based ingredients can serve as sustainable protein alternatives without negatively affecting growth when included at suitable levels. These findings support the response observed in the present study, where moderate BSF meal inclusion resulted in improved growth performance in *P. hypophthalmus*. The similarities among catfish species may be related to their comparable feeding strategies, digestive adaptability, and ability to utilize diverse protein sources. However, differences in species-specific nutrient requirements, dietary formulation, processing methods, and BSF meal characteristics can influence the optimal inclusion level. Therefore, results obtained from salmonid species should be interpreted cautiously when applied to tropical omnivorous fish such as *P. hypophthalmus.*

On the whole, this research has indicated that intermediate inclusion of insect feed, especially at 10 percent, has the best benefits regarding growth performance, nutrient use, and gut health. Besides these biological benefits, insect-based feeds have environmental benefits such as less reliance on fishmeal and sustainable use of aquaculture methods. Van der Fels-Klerx et al. (2018) also reported similar results claiming that insect based feed ingredients do not negatively impact aquaculture water quality when fed at moderate levels. Similarly, the feeding trials conducted by Egessa (2025) with different types of insect feed ingredients showed that they did not create any negative impacts on the environment during the feeding trials, showing that insect feed ingredients are environmentally safe in an aquaculture system. The results obtained for the fish body weight, weight gain, specific growth rate (SGR) and feed conversion ratio (FCR) showed significant improvement when fed the 10% insect inclusion diet in comparison to other diets, which matched the findings of previous studies. Moderate levels of insect meal feeding improved growth performance and feed efficiency because the protein quality of insect meal based feeds was good and the balanced amino acid profile of insect meal was observed in the study done by Divakaran and Obaldo, (2002). Likewise, Belforti et al. (2015) showed that the inclusion of IM at moderate levels (20–40%) in the diet of rainbow trout resulted in better growth and nutrient use efficiency compared to fishmeal.

In the present study, the above-mentioned better growth performance of T2 could therefore be explained as due to better digestibility of nutrients and better utilization of protein. The reduced growth performance observed at the 15% BSF meal inclusion level may be related to limitations associated with higher insect meal incorporation. Although chitin has been reported as one of the factors that can influence nutrient digestibility in insect-based diets, chitin content was not quantified in the present BSF meal. Therefore, the contribution of chitin to the observed reduction in performance cannot be confirmed. Other factors, including differences in nutrient availability, dietary amino acid balance, fibre-associated effects, or processing characteristics of insect meal, may also contribute to the response observed at higher inclusion levels. Future studies should include detailed characterization of BSF meal, including chitin concentration, amino acid profile, and potential anti-nutritional components, to better explain the physiological responses of fish. The same findings were reported by Sánchez-Muros et al. (2014) and Ismat et al. (2025), who reported that inclusion rates between 10 and 20% contributed to fish growth while over 20% negatively affected fish performance. Insect by-products seemed to have a positive effect on fish health and stress tolerance as seen from the higher survival rate recorded in T1 and T2. Józefiak et al. (2016) described that the insect food source is rich in antimicrobial peptides, lauric acid and chitin derivatives which can strengthen immunity and disease resistance in aquatic animals. Similarly, Usman and Yusuf (2021) emphasized the nutrition system of animals in relation to edible insects and their nutritional and health benefits. The whole body proximate analysis revealed that the 10% diet inclusion level of insects had significantly more crude protein and ash and less lipid deposition when compared to the other diet inclusion levels. Gasco et al. (2018) presented the same results, with the protein retention and mineral deposition in fish fed with insect protein sources being higher. This translated to a lower amount of lipids being accumulated in T2, which means that nutrient partitioning was more efficient toward the development of muscle mass and not fat.

The apparent digestibility coefficient (ADC%) of protein and minerals were significantly higher in T2 indicating better digestibility and utilization of minerals and protein. Moderate supplementation of insect meal was also found to favor the development of the gut and enhance its functionality in aquaculture species by Antonopoulou et al. (2019); Fielder and Allan, (2003) inclusion of insects' protein led to better growth and protein efficiency ratio of African catfish. The higher growth rate in T2 could likely be explained by the balanced amino acid composition, digestibility and palatability of the nutrients from insects. Feed efficiency decreased with the amounts of insect by-product included and was significantly reduced with greater amounts, up to 10%, suggesting better feed efficiency. The FCR value for T2 group of fish (1.48 ± 0.03) was comparatively the lowest which indicates that feed was utilized more efficiently in production of body mass compared to the control group. The results are similar to those of Gasco et al. (2018) who reported positive feed efficiency in the case of rainbow trout fed insect diet. Likewise, Ido et al. (2015) found that the FCR of species raised in aquaculture also significantly reduced when they substituted fish meal with insect meal by 50%. Bioactive compounds in the insect meals might contribute to improvements in FCR due to enhanced digestibility of nutrients and/or greater absorption in the gut. Survival rate has been regarded as an important criterion of fish health and tolerance to stress. The maximum number of survivors (T2, 95.6 ± 1.5%) were obtained in T2 and T1 and minimum number of survivors (88.3 ± 2.1%) was obtained in control group. The outcomes show that moderate inclusion of insect might enhance immunity and health of fish. The same results were reported by Józefiak et al. (2016) who noticed that fish fed with insect protein diets exhibited higher disease resistance and survival rate. This better survival rate may relate to the antimicrobial peptides, chitin, and lauric acid present in the ingredients from insects, and the latter might have a positive effect on immune response and gut microbiota. As indicated in the whole-body composition analysis, fish fed insect-based diets had a significant increase in crude protein and ash content particularly for the 10% inclusion rate. The highest crude protein content (60.4 ± 1.5%) was shown for T2 while lipid deposition was significantly lower than the control. These results show that protein deposition and lean tissue growth was efficient in the moderate insect supplementation fish.

The increased protein retention observed by Belghit et al. (2018) and reduced lipid accumulation when Atlantic salmon were fed black soldier fly meal are also similar trends. Also, Sealey et al. (2011) reported that diets containing insects resulted in better protein content of trout carcasses. This decrease in lipid content during the present study could be an indication of a more efficient partitioning of nutrients to muscle development rather than fat deposition. Ash was also significantly higher in T2 indicating better mineral retention and bioavailability. Insect meals are known to contain essential minerals, including calcium, phosphorus, iron and zinc, which play a role in the development of the skeleton, as well as in metabolism. The results are similar to those found by Kroeckel et al. (2012) who found that mineral deposition improved in rainbow trout on insect protein feeds. Higher ash accumulation in the present study also may be due to improved digestibility of minerals. The apparent digestibility coefficient (ADC%) of protein and minerals significantly increased in the fish fed with insect by-products especially at 10% inclusion. The digestibility of protein was 85.9 ± 1.3% in T2 and 74.3 ± 1.5% for minerals in T2. The findings showed that there was efficient utilization of insect derived nutrient by Pangasius fingerlings. Similarly Piccolo et al. (2017 and Ismat et al. (2025) reported that protein digestibility was improved by the inclusion of moderate quantities of insect meal because of its high levels of digestible amino acids and functional nutrients.

The digestibility, however, was slightly reduced at the 15% inclusion rate, which may be related to the increase in chitin content, as it may decrease nutrient absorption at high levels. The positive effects of insect by products on digestive health was confirmed by intestinal morphology analysis. The villi of fish fed with T2 were found to be significantly taller than the villi of the control and the number density of goblet cells was significantly more than the control. More villi means more surface area for absorption and more goblet cells means more protection for the gut and more mucus production. The same results were reported by Bruni et al. (2018) as they noted better intestinal morphology in European sea bass fed with diets containing insect meal. The improved nutrient digestibility and growth performance in the current experiment could be attributed to the improved gut morphology. The positive effects can be attributed to bioactive compounds like chitin and antimicrobial peptides, which have a positive impact on the intestinal health and the intestinal flora. All of the above results suggest that up to 10% of dietary inclusion of insect by-products can be used to enhance the growth performance, feed utilization, nutrient digestibility, body composition, survival rate and intestinal morphology of *P. hypophthalmus* fingerlings without adversely affecting water quality.

Results showed that performance was slightly decreased with a higher level of inclusion (15%), which may be due to an increase in chitin levels or the imbalances in nutrient levels with such a high level of incorporation. In general, the results of the present study are in agreement with the results of some previously published works on the use of insect by-products in aquaculture, which have shown that these products can be successfully added at moderate levels to diets and that they can positively affect the growth performance, feed utilization, nutrient digestibility, body composition, survival and intestinal health of aquaculture species. According to the overall study, insect by-products can be a potential alternative protein source in aquaculture feed which is sustainable and economical. Given the growing demand for the ingredients of aqua feeds to be sustainable and the growing price of fish meal, the insect products have potential in the area of environmentally friendly fish production. Future studies with long-term feeding trials, immune response studies and economic viability could yield further insights to potentials for commercial application of insect diets in aquaculture systems. This experiment showed that 10% inclusion rate was the best diet for the present experimental conditions of all the dietary treatments tested.

Although periodic body weight measurements were recorded during the feeding trial, the present study focused on endpoint growth performance parameters because treatment comparisons were based on final weight gain, specific growth rate, and feed conversion ratio. Future studies may include detailed growth trajectory analysis to evaluate the timing of treatment-related differences. The histological evaluation in the present study focused on villus height and goblet cell density as indicators of intestinal structural development and mucosal function. However, additional morphometric parameters such as villus width, crypt depth, and muscularis thickness were not evaluated. Furthermore, detailed information regarding intestinal segment selection and some histological processing parameters was not included in the original manuscript. These limitations have now been acknowledged, and future studies should incorporate comprehensive intestinal histomorphometric analysis, including multiple intestinal regions and additional structural parameters, to provide a more complete understanding of dietary effects.

Although the present study provides evidence that BSF meal can be used as an alternative protein ingredient in diets for *Pangasianodon hypophthalmus* fingerlings, several limitations should be considered. First, the experiment was conducted using three replicate tanks per dietary treatment, with ten fish maintained per tank. Although this experimental design allowed detection of significant differences among treatments, the relatively limited number of experimental units may reduce statistical power for identifying moderate treatment effects. Future studies involving larger numbers of replicate tanks would provide stronger statistical confidence. Second, the present study evaluated four predefined dietary inclusion levels (0, 5, 10, and 15% BSF meal) and identified 10% inclusion as the best-performing treatment among the tested diets. However, no dose–response modelling or regression analysis was performed to estimate the mathematically optimal inclusion level. Future research should evaluate a wider range of inclusion levels and apply dose–response approaches to determine the precise dietary optimum. Third, the BSF meal used in this study was characterized based on proximate composition; however, detailed nutritional characterization, particularly amino acid profiling, was not performed. Therefore, the contribution of specific amino acid availability to the observed growth response could not be confirmed. Future studies should include amino acid composition, digestibility of individual amino acids, and evaluation of other nutritional components such as chitin content to better explain the mechanisms underlying fish responses. Finally, the study was conducted using a single batch of BSF meal under specific experimental conditions. Since the nutritional composition of BSF meal can vary depending on larval substrate, production system, developmental stage, and processing method, the findings may not represent all BSF meal products. Multi-source and multi-batch evaluations are therefore required to confirm the broader applicability of BSF meal in commercial aquafeed formulations.

CONCLUSIONS

The present study demonstrates that black soldier fly (*Hermetia illucens*) meal can be effectively incorporated into diets of *Pangasianodon hypophthalmus* fingerlings as a partial alternative protein ingredient. Among the tested dietary treatments, 10% BSF meal inclusion resulted in the best overall growth performance, nutrient utilization, digestibility, survival, and intestinal health responses. The 10% BSF meal diet reduced fishmeal inclusion from 30% to 20%, representing approximately a one-third reduction in dietary fishmeal incorporation while maintaining or improving fish performance under the experimental conditions. These findings suggest that moderate inclusion of BSF meal has potential to reduce dependence on conventional fishmeal in *P. hypophthalmus* aquafeeds. However, the present study evaluated only a limited range of inclusion levels and used a single BSF meal source; therefore, further investigations involving broader dose-response trials, economic evaluation, and characterization of different BSF meal products are required before commercial-scale recommendations can be established. The partial replacement of fishmeal with BSF meal may provide economic and sustainability benefits by reducing dependence on expensive marine-derived protein sources. However, the actual reduction in feed cost will depend on the production cost, availability, and market price of BSF meal compared with fishmeal. A detailed economic analysis should therefore be included in future studies.

REFERENCES

Acutis, M., Scaglia, B., & Confalonieri, R. (2012). Perfunctory analysis of variance in agronomy, and its consequences in experimental results interpretation. *European Journal of Agronomy, 43*, 129–135. https://doi.org/10.1016/j.eja.2012.06.006

Antonopoulou, E., Nikouli, E., Piccolo, G., Gasco, L., Gai, F., Chatzifotis, S., & Kormas, K. A. (2019). Reshaping gut bacterial communities after dietary *Tenebrio molitor* larvae meal supplementation in three fish species. *Aquaculture, 503*, 628–635. https://doi.org/10.1016/j.aquaculture.2018.12.013

Belforti, M., Gai, F., Lussiana, C., Renna, M., Malfatto, V., Rotolo, L., De Marco, M., Dabbou, S., Schiavone, A., & Zoccarato, I. (2015). *Tenebrio molitor* meal in rainbow trout (*Oncorhynchus mykiss*) diets: Effects on animal performance, nutrient digestibility and chemical composition of fillets. *Italian Journal of Animal Science, 14*(4), 4170. https://doi.org/10.4081/ijas.2015.4170

Belghit, I., Liland, N. S., Waagbø, R., Biancarosa, I., Pelusio, N., Li, Y., Krogdahl, A., & Lock, E. J. (2018). Potential of insect-based diets for Atlantic salmon (*Salmo salar*). *Aquaculture, 491*, 72–81. https://doi.org/10.1016/j.aquaculture.2018.03.016

Bruni, L., Randazzo, B., Cardinaletti, G., Zarantoniello, M., Mina, F., Secci, G., Tulli, F., Olivotto, I., & Parisi, G. (2018). Dietary inclusion of insect meal affects intestinal morphology and gut health in European sea bass. *Aquaculture, 484*, 42–50.

Divakaran, S., Obaldo, L. G., & Forster, I. P. (2002). Note on the methods for determination of chromic oxide in shrimp feeds. *Journal of Agricultural and Food Chemistry, 50*(3), 464–467.

Egessa, R. (2025). *Exploring the potential benefits of insect oil as alternative lipid source in aquafeeds for catfish* [Doctoral dissertation, Magyar Agrár-és Élettudományi Egyetem]. https://doi.org/10.54598/006470

Erokhin, V., Diao, L., Gao, T., Andrei, J. V., Ivolga, A., & Zong, Y. (2021). The supply of calories, proteins, and fats in low-income countries: A four-decade retrospective study. *International Journal of Environmental Research and Public Health, 18*(14), 7356. https://doi.org/10.3390/ijerph18147356

Fielder, D. S., & Allan, G. L. (2003). *Improving fingerling production and evaluating inland saline water culture of snapper, Pagrus auratus* (Final Report Series No. 43). NSW Fisheries, Port Stephens Fisheries Centre.

Gasco, L., Gai, F., Maricchiolo, G., Genovese, L., Ragonese, S., Bottari, T., & Caruso, G. (2018). Fishmeal alternative protein sources for aquaculture feeds. In *Feeds for the aquaculture sector: Current situation and alternative sources* (pp. 1–28). Springer.

Gutasi, A. (2021). *Benefit and drawbacks of fish meal substitution in aquaculture diets* [Unpublished manuscript]. University of Veterinary Medicine Vienna.

Hassan, A. A., Sani, I., Maiangwa, M. W., & Rahman, S. A. (2009). The effect of replacing graded levels of fishmeal with grasshopper meal in broiler starter diet. *PAT, 5*(1), 30–38.

Henry, M., Gasco, L., Piccolo, G., & Fountoulaki, E. (2015). Review on the use of insects in the diet of farmed fish: Past and future. *Animal Feed Science and Technology, 203*, 1–22. https://doi.org/10.1016/j.anifeedsci.2015.03.001

Hoque, M. S., Haque, M. M., Nielsen, M., Rahman, M. T., Hossain, M. I., Mahmud, S., Mandal, A. K., Frederiksen, M., & Larsen, E. P. (2021). Prospects and challenges of yellow flesh pangasius in international markets: Secondary and primary evidence from Bangladesh. *Heliyon, 7*(9), e08060. https://doi.org/10.1016/j.heliyon.2021.e08060

Hussain, S. M., Afzal, M., Nasir, S., Javid, A., Azmat, H., Mamoona Makhdoom, S., Shah, S. Z. H., Hussain, M., Mustafa, I., & Iqbal, M. (2017). Role of phytase supplementation in improving nutrient digestibility and growth performance for *Labeo rohita* fingerlings fed on canola meal-based diet. *Journal of Applied Animal Research, 45*(1), 15–21. https://doi.org/10.1080/09712119.2015.1091331

Ido, A., Iwai, T., Ito, K., Ohta, T., Mizushige, T., Kishida, T., & Miura, C. (2015). Dietary effects of housefly maggot meal on growth performance of fish. *Fisheries Science, 81*(1), 163–168.

Ismat, N., Hayat, S., & Hussain, M. (2025). Sustainable fisheries and biodiversity conservation: The potential of insect protein to replace fishmeal in aquaculture. *Journal of Wildlife and Biodiversity, 9*(3), 374–390. https://doi.org/10.5281/zenodo.17386965

Ismat, N., Hayat, S., & Hussain, M. (2026). Effect of substituting fishmeal with black soldier fly and mealworm larvae meal on nutrient digestibility, body and mineral composition of *Labeo rohita*. *Open Veterinary Journal, 16*(1), 241–255. https://doi.org/10.5455/OVJ.2026.v16.i1.22

Józefiak, D., Engberg, R. M., & Józefiak, A. (2016). Insects as a novel protein source in aquaculture. *Animal Feed Science and Technology, 215*, 1–12.

Khan, I. (2020). *Growth performance and meat quality of Pangas catfish (Pangasianodon hypophthalmus) fed fish silage and linseed oil-supplemented diets* [Doctoral dissertation, Guru Angad Dev Veterinary and Animal Sciences University].

Kim, C. H., Ryu, J., Lee, J., Ko, K., Lee, J. Y., Park, K. Y., & Chung, H. (2021). Use of black soldier fly larvae for food waste treatment and energy production in Asian countries: A review. *Processes, 9*(1), 161. https://doi.org/10.3390/pr9010161

Kroeckel, S., Harjes, A. G., Roth, I., Katz, H., Wuertz, S., Susenbeth, A., & Schulz, C. (2012). Insect meal as protein source in fish nutrition. *Aquaculture, 364–365*, 345–352.

Lock, E. J., Arsiwalla, T., & Waagbø, R. (2016). Insect meal in fish feed and nutrient digestibility. *Aquaculture Nutrition, 22*(6), 1203–1212.

Makkar, H. P. S., Tran, G., Heuzé, V., & Ankers, P. (2014). State-of-the-art on use of insects as animal feed. *Animal Feed Science and Technology, 197*, 1–33. https://doi.org/10.1016/j.anifeedsci.2014.07.008

Mendes, R., Rema, P., Dias, J., Gonçalves, A. T., Teodósio, R., Engrola, S., Sánchez-Vázquez, F. J., & Conceição, L. E. (2024). Socially acceptable feed formulations may impact the voluntary feed intake and growth, but not robustness of Nile tilapia (*Oreochromis niloticus*). *Fishes, 9*(9), 361. https://doi.org/10.3390/fishes9090361

Mueller-Harvey, I. (2004). *Modern techniques for feed analysis* (FAO Animal Production and Health Paper No. 160, pp. 1–38). Food and Agriculture Organization of the United Nations.

Nogales-Mérida, S., Gobbi, P., Józefiak, D., Mazurkiewicz, J., Dudek, K., Rawski, M., Kierończyk, B., & Józefiak, A. (2019). Insect meals in fish nutrition. *Reviews in Aquaculture, 11*(4), 1080–1103. https://doi.org/10.1111/raq.12281

Piccolo, G., Iaconisi, V., Marono, S., Gasco, L., Loponte, R., Nizza, S., Bovera, F., & Parisi, G. (2017). Effect of *Tenebrio molitor* larvae meal on growth performance, in vivo nutrients digestibility, somatic and marketable indexes of gilthead sea bream (*Sparus aurata*). *Animal Feed Science and Technology, 226*, 12–20. https://doi.org/10.1016/j.anifeedsci.2017.02.007

Pigden, W. J., Balch, C., & Graham, M. (1980). *Standardization of analytical methodology for feeds: Proceedings*. IDRC.

Rahman, F., Khan, Y., & Rahman, R. (2024). The impact of climate change on Pakistan's agricultural economy. *ASSAJ, 2*(4), 1236–1254.

Renna, M., Schiavone, A., Gai, F., Dabbou, S., Lussiana, C., Malfatto, V., & Gasco, L. (2017). Evaluation of the suitability of a partially defatted black soldier fly (*Hermetia illucens* L.) larvae meal as ingredient for rainbow trout (*Oncorhynchus mykiss* Walbaum) diets. *Journal of Animal Science and Biotechnology, 8*(1), 57. https://doi.org/10.1186/s40104-017-0191-3

Sánchez-Muros, M. J., Barroso, F. G., & Manzano-Agugliaro, F. (2014). Insect meal as renewable source of food for animal feeding: A review. *Journal of Cleaner Production, 65*, 16–27. https://doi.org/10.1016/j.jclepro.2013.11.068

Sealey, W. M., Gaylord, T. G., Barrows, F. T., Tomberlin, J. K., McGuire, M. A., Ross, C., & St-Hilaire, S. (2011). Sensory analysis of trout fed insect-based diets. *Aquaculture Research, 42*(5), 699–707. https://doi.org/10.1111/j.1749-7345.2010.00441.x

Sogari, G., Amato, M., Biasato, I., Chiesa, S., & Gasco, L. (2019). The potential role of insects as feed: A multi-perspective review. *Animals, 9*(4), 119. https://doi.org/10.3390/ani9040119

Usman, H. S., & Yusuf, A. A. (2021). Legislation and legal framework for sustainable edible insects use in Nigeria. *International Journal of Tropical Insect Science, 41*(3), 2201–2209. https://doi.org/10.1007/s42690-020-00291-9

Van der Fels-Klerx, H. J., Camenzuli, L., Belluco, S., Meijer, N., & Ricci, A. (2018). Food safety issues related to uses of insects for feeds and foods. *Comprehensive Reviews in Food Science and Food Safety, 17*(5), 1172–1183. https://doi.org/10.1111/1541-4337.12385

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