

RESEARCH PAPER

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On-farm validation of black soldier fly larvae meal as a sustainable replacement for shrimp meal in rainbow trout diets in the mid hills of Nepal

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Key words: Sustainable aquaculture, BSFL, Trout farming, Partial replacement

DOI: <https://dx.doi.org/10.12692/ijb/27.6.189-200>

Published: December 22, 2025

ABSTRACT

Rainbow trout (*Oncorhynchus mykiss*) aquaculture in Nepal faces constraints regarding high feed costs and the sustainability of marine-derived proteins. This study validated the on-farm partial replacement of shrimp meal with Black Soldier Fly Larvae Meal (BSFLM). Juvenile trout in Rasuwa, Nepal, were fed four isonitrogenous, isolipidic diets for 90 days: Control (100% shrimp meal), BSF25 (25% replacement), BSF50 (50%), and BSF75 (75%). Results indicated that the BSF25 treatment achieved the highest final weight (23.13 ± 0.13 g) and Specific Growth Rate (SGR: 1.34 ± 0.01 %/day), significantly outperforming the control (14.39 g; 0.81 %/day). Feed efficiency was optimal in BSF25, showing the lowest Feed Conversion Ratio (FCR: 1.49 ± 0.02) and the highest Protein Efficiency Ratio (PER: 1.50 ± 0.02). Water quality monitoring showed stable temperature and pH, and significantly higher dissolved oxygen in BSFLM groups, correlating positively with growth. Survival rates remained high in Control and BSF25 (84%) but dropped significantly at BSF75 (48.27%). In conclusion, replacing shrimp meal with 25% BSFLM optimizes growth, feed utilization, water quality conditions, and survival. Higher inclusion levels negatively affected performance. These findings support BSFLM as a sustainable, cost-effective protein alternative for Nepalese trout aquaculture.

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INTRODUCTION

Rainbow trout (*Oncorhynchus mykiss*) aquaculture represents a significant advancement in the diversification of Nepal's aquaculture sector, particularly in the mid-hill regions where cold-water resources remain underutilized. First introduced to Nepal in the late 1960s and early 1970s from the UK, Japan, and India, the species could not survive due to lack of technical expertise (Gurung, 2008). Following its successful re-introduction from Japan in 1988 and subsequent breeding in 1990, the Nepal Agricultural Research Council (NARC) developed comprehensive breeding and culture technology for this carnivorous species.

Rainbow trout culture was initiated experimentally in 1993, and commercial production began in the mid-1990s, establishing it as a viable aquaculture enterprise in the temperate regions of Nepal (Gurung, 2008).

The mid-hill districts of Nepal, characterized by pristine mountainous rivers fed by glacier and snow melt, provide ideal environmental conditions for rainbow trout farming. With water temperatures ranging from 10-20°C, optimal for trout growth and abundant cold-water resources flowing through elevations between 1,500 to 3,000 meters above sea level, these regions possess considerable potential for commercial trout production (Khanal and Gautam, 2008). The Fisheries Research Station, Trishuli (NARC) in Nuwakot district, established in 1971 (2027 BS), has been instrumental in conducting research on rainbow trout breeding, rearing, and germplasm maintenance, utilizing the cold water of the Trishuli River (Gurung *et al.*, 2014). Rainbow trout production has expanded from merely 10 metric tons annually from two government stations in the early 2000s to approximately 1244 metric tons currently, with farming activities now spanning 8.4 hectares of water surface area (CFPCC, 2025) across 39 districts including Nuwakot, Solukhumbu, Sindhupalchok, Kaski, Rauwa, Kaski, Gulmi, Jumla, and Doti (FRST, 2025).

Despite the technical feasibility and growing popularity of rainbow trout farming, the sector faces significant economic and sustainability challenges, primarily

related to feed costs (Khanal and Gautam, 2008; Mahato *et al.*, 2024). As a carnivorous species, rainbow trout requires high-protein feed containing no less than 20-30% animal protein for optimal growth (Cho *et al.*, 1976). Currently, shrimp meal serves as the predominant animal protein source in commercial trout feeds in Nepal, constituting 43.2-48.0% of formulated diets. However, shrimp meal is costly, with prices reaching NPR 550 per kilogram, making feed costs account for 40-60% of total production expenses in intensive trout farming systems (Dheke and Gubhaju, 2013). Moreover, the dependence on marine-derived protein sources such as shrimp meal and fishmeal raises critical sustainability concerns. The global aquaculture industry's reliance on fishmeal has contributed to overfishing and depletion of marine fish stocks, with approximately 38% (by number) and 23% (by volume), the world's marine fish populations are unsustainably exploited or overfished (FAO, 2024). This unsustainable extraction of marine resources not only threatens ocean ecosystem health but also compromises food security in coastal communities where small pelagic fish serve as important dietary staples (Biasato *et al.*, 2022).

The search for sustainable, locally available, and cost-effective alternative protein sources to replace conventional marine-based ingredients has become imperative for the long-term viability of aquaculture (Rosle *et al.*, 2024). Among various alternatives explored globally, black soldier fly larvae (*Hermetia illucens*) meal (BSFLM) has emerged as one of the most promising sustainable protein sources for aquaculture feeds. BSFL are highly efficient bioconverters, capable of transforming low-grade organic waste and agricultural by-products into high-quality protein and lipid-rich biomass. The nutritional composition of BSFL is particularly suitable for fish feed, containing 40-63% crude protein (dry matter basis), essential amino acids with favorable profiles comparable to fishmeal, beneficial lipids including medium-chain fatty acids such as lauric acid with antimicrobial properties, and important minerals including calcium, phosphorus, iron, and zinc (Rampure *et al.*, 2025).

Research on insects as a fishmeal or shrimp meal replacement in rainbow trout diets has demonstrated encouraging results (Ma *et al.*, 2025; Terova *et al.*, 2021). Studies have shown that BSFL meal can replace up to 50% of fishmeal in rainbow trout feeds without negative effects on weight gain, feed conversion ratio, or survival. The inclusion of BSFL in rainbow trout diets has been associated with improved intestinal microbiota diversity, enhanced immune function through upregulation of immune-related genes, and potential functional benefits including antioxidant properties (Sayramoğlu *et al.*, 2023). However, higher inclusion levels (>50%) have sometimes resulted in reduced growth performance and nutrient digestibility, potentially due to the presence of chitin in the insect exoskeleton, which may act as an anti-nutritional factor by reducing protein and lipid bioaccessibility (Eggink *et al.*, 2022). Despite this limitation, research indicates that both Nile tilapia and rainbow trout possess endogenous chitinolytic enzymes, and chitin digestibility can be enhanced through adaptive enzyme responses, suggesting that moderate dietary inclusion levels can be well-tolerated (Eggink *et al.*, 2022).

In the Nepalese context, preliminary research has demonstrated the feasibility of BSFL meal as a partial replacement for shrimp meal in rainbow trout feeds. A recent feeding trial conducted at the Fisheries Research Station, Trishuli, Nepal, evaluated BSFL meal as a replacement for shrimp meal at inclusion levels of 25%, 50%, 75%, and 100%. Results indicated that BSFL meal could effectively replace 25-50% of shrimp meal in rainbow trout feed, with these treatment groups demonstrating significantly enhanced growth efficiency, improved feed conversion ratios, and comparable or superior performance to the control diet.

However, higher replacement levels (75% and 100%) negatively impacted growth, likely due to increased chitin content affecting digestibility (Mahato *et al.*, 2024). These findings suggest that BSFL meal represents a viable and sustainable protein source for

rainbow trout farming in Nepal, with the potential to reduce dependency on imported shrimp meal and promote more sustainable aquaculture practices.

Technology verification and outreach research programs play a crucial role in bridging the gap between research station findings and practical application in farmers' fields. Such participatory approaches enable the validation of research outcomes under real-world production conditions, facilitate technology adoption by farmers, and ensure that developed technologies are economically viable and socially acceptable (Bhattarai, 2022). The Nepal Agricultural Research Council has established outreach research as a key mechanism for testing and disseminating aquaculture technologies, with research stations identifying outreach sites and implementing participatory research programs with active farmer involvement (Bhattarai, 2022).

Building upon the successful research conducted at the Fisheries Research Station, Trishuli, the present study was undertaken as part of an outreach and technology verification project to evaluate the effect of BSFL meal as a replacement for shrimp meal in rainbow trout under actual farming conditions. The research was conducted in farmers' raceways at Sole, Dhunche, Rasuwa district, the same region where the rainbow trout research stations are located and where commercial trout farming has been established. This on-farm validation is essential to confirm whether the promising results obtained under controlled research station conditions can be replicated in commercial production systems, thereby providing practical evidence for technology dissemination and adoption by the trout farming community in mid-hill regions of Nepal.

MATERIALS AND METHODS

Experimental site and duration

The trial was conducted at the commercial rainbow trout farm, Himalayan Rainbow Trout PVT. LTD located at Sole, Ward 6 of Gosaikunda Rural Municipality of Rasuwa district, Nepal (28.08998° N, 85.274889°E) (Fig. 1), owned by Mr. Shanta Kumar

Shrestha, as part of the outreach and technology verification program led by the Fishery Research Station (FRS), Trishuli during the fiscal year 2082-83 (2025-26). The experiment ran for 90 days during the main trout growing season, under ambient mid-hill environmental conditions similar to those practiced commercially in Nepal's trout culture sector.



Fig. 1. Himalayan Rainbow Trout Pvt. Ltd. in Rasuwa district (Map: National Statistics Office 2078)

Experimental fish and rearing conditions

Juvenile rainbow trout (*Oncorhynchus mykiss*) with uniform size and age were sourced from the FRS hatchery and acclimatized for 7 days using standard local trout feed containing 45% crude protein. After acclimatization, healthy fish with a mean initial body weight comparable to previous FRS trial (6.91-6.97 g) were randomly allocated to flow-through raceway tanks.

Each tank measured approximately 300 L in volume, supplied continuously with clean, spring water at 6–8 L/min flow, maintaining water temperature ($16.9 \pm 0.2^{\circ}\text{C}$) and dissolved oxygen (7-10 mg/L) and pH (7.5 ± 0.1) near optimal levels for rainbow trout culture.

For the present study, 115 fish were stocked per tank. Treatments included the control (CON, conventional shrimp meal-based feed), and three diets with black soldier fly larvae meal partially replacing shrimp meal: BSF25, BSF50, and BSF75, corresponding to 25%, 50%, and 75% replacement levels. Each treatment was conducted in duplicate, resulting in a total of eight tanks (2 tanks per treatment \times 4 dietary groups).

Diet formulation and preparation

Experimental diets were formulated to be isonitrogenous ($\approx 45\%$ crude protein) and isolipidic ($\approx 15\%$ ether extract) to meet the nutritional requirements of rainbow trout (Kamalam *et al.*, 2020). The control diet (CON) used shrimp meal as the primary animal protein source. For the other treatments, partially defatted black soldier fly (*Hermetia illucens*) larvae meal (BSFL) replaced 25% (BSF25), 50% (BSF50), or 75% (BSF75) of the shrimp meal's protein. Diets were formulated using premium feed-grade ingredients: BSFL meal and shrimp meal (imported), full-fat soybean, wheat gluten, corn meal, wheat flour, soybean oil, vitamin premix, and trace mineral premix in proportions given in Table 1.

Table 1. The detail of feed composition and proximate analysis of experimental diets.

Ingredients	BSFL	CON	BSF25	BSF50	BSF75
Shrimp meal		500	375	225	125
BSFL meal		0	125	225	375
Soybean full fat		355	355	355	355
Wheat gluten		30	35	80	60
Corn meal		20	20	5	5
Wheat flour		50	50	80	65
Soybean oil		40	35	25	10
Vitamin premix		3	3	3	3
Trace mineral premix		2	2	2	2
Total		1000	1000	1000	1000
Proximate composition					
Dry matter (%)	91.67	93.81	93.39	93	92.66
Crude protein (%)	51.56	45.71	45.21	45.23	45.4
Lipid (%)	15.39	15.25	15.78	15.26	15.31
Digestible energy (MJ/kg)	26.46	18.75	19.9	20.89	21.92
Reference	J17 Aqua	NANRC	NANRC	NANRC	NANRC

All dry ingredients, including BSFL and shrimp meals, were finely ground and thoroughly mixed in a blender. Soybean oil and water (250–500 mL/kg) were added during mixing to achieve adequate dough consistency for pelleting. The feed was extruded through a pelleting machine into about 2.0 mm diameter pellets (Kamalam *et al.*, 2020), dried, and stored in airtight containers until use. The chemical composition and proximate analysis of all diets were confirmed in duplicate at the National Animal Nutrition Research Centre, Khumaltar, Lalitpur (NANRC) and by supplier certificates for BSFL (J17 AQUA, Kancheepuram, Tamil Nadu, India). The detail of feed composition and proximate analysis is presented in Table 1.

Feeding and management

Fish were hand-fed twice daily (09:00 and 16:00) to apparent satiation (confirmed visually by cessation of feeding). Uneaten feed and feces were siphoned daily, and dead fish were removed and recorded. Bulk weighing of fish and cleaning of tanks were performed every 15 days to monitor growth parameters and maintain tank sanitation respectively.

Water quality monitoring

Water quality parameters such as Temperature, dissolved oxygen (DO), and pH were monitored using portable water quality meter from ERMA Instruments (pH and Temperature) and Lutron PDO-519 (DO) were recorded every 2–3 days after calibration to ensure optimal rearing conditions throughout the trial period.

Growth performance and data collection

Every 15 days, and at the end of the 90-day trial, all fish were fasted for 24 hours before sampling. Fish from each tank were netted, counted, and individually weighed to determine final body weight and total length. The following growth and feed utilization indices were calculated for each replication according to (Wani *et al.*, 2025):

Survival rate (%) = (Number of fish survived/Initial number of fish) × 100

Individual weight gain (iWG, g) = Final mean individual body weight – Initial mean individual body weight.

Specific growth rate (SGR, %/day) = $[\ln(\text{final body weight}) - \ln(\text{initial body weight})] / \text{number of days} \times 100$

Feed conversion ratio (FCR) = Total feed intake (dry matter) / Total weight gain

Protein efficiency ratio (PER) = Total weight gain / Total protein fed (dry matter).

Statistical analysis

All data were analyzed using IBM SPSS Statistics (version 25). Treatment effects were tested by one-way ANOVA ($\alpha = 0.05$). Prior to ANOVA, data were evaluated for normality and homogeneity (Kolmogorov-Smirnov and Levene's tests, respectively). The Brown-Forsythe test was used when variances were not equal. Multiple comparisons were performed using Tukey's (equal variance) or Tamhane's T2 (unequal variance) post-hoc tests. Results are presented as means ± pooled standard error (SEM).

Ethical considerations

All experimental procedures adhered to scientific best practices for animal welfare, minimizing harm and stress to fish throughout the study.

RESULTS

Growth performance

Growth performance indicators including weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), and protein efficiency ratio (PER) were measured in Rainbow trout fed experimental diets containing varying levels of black soldier fly meal (BSFM) as a substitute for shrimp meal (SM). The effects of the different dietary treatments (CON: 100% SM and 0% BSFM, BSF25: 75% SM and 25% BSFM, BSF50: 50% SM and 50% BSFM, BSF75: 25% SM and 75% BSFM) on fish growth and survival were assessed using statistical analysis to determine

significant differences among groups and results are presented as mean and standard error of mean (Mean \pm SEM) in Table 2.

Initial weights ranged from 6.92 ± 0.02 g to 6.97 ± 0.01 g across all four groups and did not differ significantly ($p = 0.058$), confirming homogeneous starting conditions.

At the end of the experiment, the final weight was found to be significantly affected by the dietary treatments ($p = 0.000$). Fish fed BSF25 had the highest final weight (23.13 ± 0.13 g), significantly greater than all other groups. BSF75 (16.5 ± 0.28 g) and BSF50 (18.08 ± 0.5 g) were intermediate, while the control group (CON: 14.39 ± 0.86 g) had the lowest final weight.

Table 2. Growth parameters of growing rainbow trout fish fed on experimental diets

Parameters	CON	BSF25	BSF50	BSF75	p-value
Initial weight (IW, g)	6.95 ± 0.01	6.96 ± 0.02	6.92 ± 0.02	6.97 ± 0.01	0.058
Final weight (FW, g)	14.39 ± 0.86^c	23.13 ± 0.13^a	18.08 ± 0.5^b	16.5 ± 0.28^{bc}	0.000
Weight gain (WG, g)	7.44 ± 0.87^c	16.18 ± 0.15^a	11.17 ± 0.48^b	9.53 ± 0.27^{bc}	0.000
Specific Growth Rate (SGR, %/day)	0.81 ± 0.07^c	1.34 ± 0.01^a	1.07 ± 0.03^{ab}	0.96 ± 0.02^{bc}	0.000
Survival rate (SR, %)	84.35 ± 0.00^a	84.79 ± 2.26^a	77.83 ± 8.79^a	48.27 ± 2.77^b	0.002
Feed conversion ratio (FCR)	3.06 ± 0.41^c	1.49 ± 0.02^a	2.04 ± 0.16^{ab}	2.56 ± 0.12^{bc}	0.006
Protein efficiency ratio (PER)	0.76 ± 0.11^c	1.50 ± 0.02^a	1.11 ± 0.09^b	0.87 ± 0.04^{bc}	0.000

CON (100% SM, 0% BSFM), BSF25 (75% SM, 25%BSFM), BSF50 (50% SM, 50%BSFM), BSF745 (25% SM, 75%BSFM). Values were expressed as mean \pm SEM (n=2).

Similarly, weight gain mirrored the final weight results, with BSF25 showing a significantly higher gain (16.18 ± 0.15 g) than BSF50 (11.17 ± 0.48 g), BSF75 (9.53 ± 0.27 g), and CON (7.44 ± 0.87 g) ($p = 0.000$).

Similarly, specific growth rate (SGR) was highest in BSF25 (1.34 ± 0.01 %/day), significantly exceeding all other groups ($p = 0.000$). SGR for BSF50 (1.07 ± 0.03) and BSF75 (0.96 ± 0.02) were intermediate, with the lowest rate in the control group (0.81 ± 0.07).

Survival rate was also significantly affected ($p = 0.002$). Both CON ($84.35 \pm 0.00\%$) and BSF25 (84.79 ± 2.26 %) exhibited the highest survival, whereas BSF50 had a moderate rate ($77.83 \pm 8.79\%$), and BSF75 had the lowest ($48.27 \pm 2.77\%$), indicating reduced survival at higher BSFM inclusion.

Feed conversion ratio (FCR) was lowest and most efficient in BSF25 (1.49 ± 0.02), followed by BSF50 (2.04 ± 0.16), BSF75 (2.56 ± 0.12), and highest in CON (3.06 ± 0.41), demonstrating that inclusion of BSFM up to 25% improved feed efficiency ($p = 0.006$).

Protein efficiency ratio (PER) results were similar to growth parameters, with BSF25 showing the highest PER (1.50 ± 0.02), significantly greater than BSF50 (1.11 ± 0.09), BSF75 (0.87 ± 0.04), and CON (0.76 ± 0.11) ($p = 0.000$).

Water quality parameters

The results of water quality parameters measured across the four treatment groups, CON, BSF25, BSF50, and BSF75, included temperature, dissolved oxygen, and pH for are represented in Fig. 2.

The mean temperature values were $13.84 \pm 0.01^\circ\text{C}$ for CON, $13.86 \pm 0.00^\circ\text{C}$ for BSF25, $13.85 \pm 0.00^\circ\text{C}$ for BSF50, and $13.85 \pm 0.04^\circ\text{C}$ for BSF75. The Kruskal–Wallis test showed no significant difference in temperature among treatments ($p = 0.421$), indicating that temperature remained stable across all groups during the study period.

The mean dissolved oxygen (DO) levels were 9.47 ± 0.01 mg/L for CON, 9.70 ± 0.00 mg/L for BSF25, 9.66 ± 0.05 mg/L for BSF50, and 9.64 ± 0.00 mg/L for BSF75. The Kruskal–Wallis test revealed a significant difference in dissolved oxygen among treatments ($p = 0.046$). This indicates that the

inclusion of black soldier fly meal in the diets influenced the dissolved oxygen concentration, likely due to differences in fish activity, feed utilization, or microbial activity within the culture system (He *et al.*, 2022).

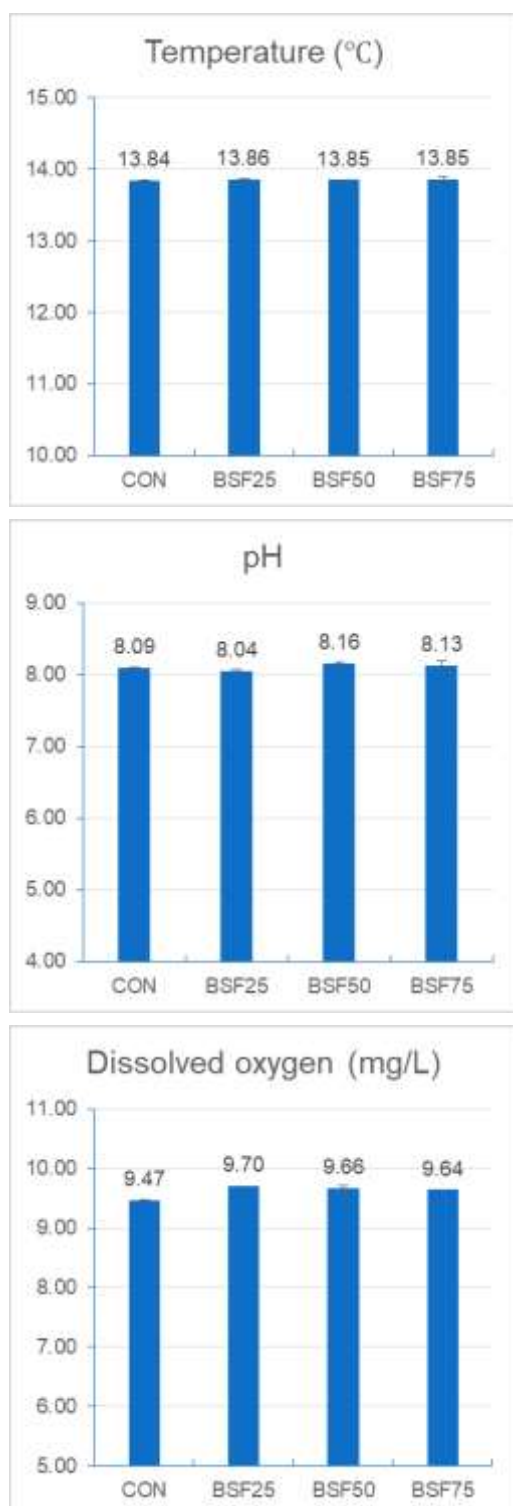


Fig. 2. Water quality parameters recorded across different treatments

The mean pH values were 8.09 ± 0.01 for CON, 8.04 ± 0.03 for BSF25, 8.16 ± 0.03 for BSF50, and 8.13 ± 0.07 for BSF75. The pH did not differ significantly among treatments ($p = 0.227$), showing that all groups maintained similar water pH levels.

Since dissolved oxygen (DO) levels showed significant variation among treatments, a correlation analysis was conducted to understand its relationship with fish growth performance and feed utilization parameters. The Pearson correlation results indicated that DO had a strong positive correlation with final weight ($r = 0.691$, $p = 0.013$), weight gain ($r = 0.693$, $p = 0.012$), and specific growth rate ($r = 0.726$, $p = 0.008$). This shows that higher DO levels were associated with better fish growth. Adequate dissolved oxygen likely supported efficient metabolism and feed utilization, leading to improved growth performance.

A significant negative correlation was observed between DO and feed conversion ratio ($r = -0.660$, $p = 0.019$), indicating that fish reared in water with higher oxygen levels required less feed to gain the same amount of weight. This suggests better feed efficiency under higher oxygen conditions. Additionally, DO showed a positive correlation with protein efficiency ratio ($r = 0.618$, $p = 0.032$), meaning that oxygen availability enhanced protein utilization efficiency in fish. However, the correlation between DO and survival rate ($r = -0.286$, $p = 0.367$) was weak and not significant, suggesting that variations in DO within the observed range did not affect fish survival.

Overall, the correlation analysis highlights that dissolved oxygen played a crucial role in influencing fish growth and feed efficiency. Higher DO concentrations were linked with improved growth rate, weight gain, and protein utilization, while reducing feed conversion ratio. This indicates that lower BSFL level helps to maintain optimal dissolved oxygen levels which essentially enhanced growth performance and feed efficiency in BSFL supplemented groups.

DISCUSSION

The use of black soldier fly larvae (BSFL) meal has been gaining momentum as a promising and sustainable protein alternative for aquaculture feeds. Its appeal lies in BSFL's ability to convert organic waste into a high-quality protein ingredient, thereby supporting a circular economy and reducing the pressure on marine resources traditionally used for fish and shrimp meals (Dîrvariu *et al.*, 2025). Given these advantages and the urgent need for sustainable aquafeed ingredients, our first study was designed to investigate the effects of partially replacing shrimp meals with BSFL meal in the diets of rainbow trout at the Fishery Research Station, Trishuli. After achieving a significant results of BSFL inclusion in the rainbow trout diets in controlled setting at Fishery Research Station, Trishuli in previous year, current study was designed to verify that achievement.

The present study was an on-farm experiment to verify the outcomes of previously achieved results in our controlled laboratory trial at the Fishery Research Station, Trishuli (Mahato *et al.*, 2024). Current study have added a depth to our understanding of black soldier fly larvae meal (BSFLM) as a sustainable replacement for shrimp meal in rainbow trout diets. Notably, our earlier laboratory study demonstrated that a partial replacement of shrimp meal (25–50%) with BSFLM significantly enhanced growth efficiency, feed conversion, and protein efficiency, without compromising survival (Survival rate, 85%) (Mahato *et al.*, 2024). The best results, as measured by weight gain (26.17–28.19 g), SGR (1.97–2.05%/day), FCR(1.19–1.31), and PER (2.02–1.83), were observed at the lower inclusion levels, while higher levels (75–100%) resulted in diminished growth, feed efficiency, and survivability, likely due to increased chitin content affecting digestibility (Mahato *et al.*, 2024).

These outcomes are echoed robustly in the current farm-based validation. Results under practical rearing conditions again confirmed that 25% replacement of shrimp meal by BSFLM generated superior growth performance with marked improvements in final weight (23.13–18.08 g), SGR (1.34–1.07 %/day), FCR

(1.49–2.04), and PER (1.50–1.11), and a consistently high survival rate (84.79–77.83%). Importantly, similar declines in performance at higher BSFL inclusions were observed, providing continuity between lab and field evidence, and validating the practical applicability of the previous laboratory findings.

However, slightly reduced performance at the field level might be due to the lower average temperatures at 16.9°C at the Sole, Rasuwa (Farmer's condition) throughout the duration of the experiment compared to 19.48°C in previous experiment at Fishery Research Station, Trishuli.

The consistency of these findings across research settings is supported by multiple academic studies globally. (Biasato *et al.*, 2022) found that up to 50% fishmeal replacement by BSFLM in rainbow trout diets maintains or improves weight gain and feed utilization efficiency.

(Caimi *et al.*, 2021) and others have noted that moderate substitution supports comparable carcass protein retention and SGR to conventional fishmeal-based diets, helped by the well-balanced amino acid profile and high digestibility of insect meal (Kuo *et al.*, 2022). Likewise, (Borland *et al.*, 2024) observed similar trends, reporting that BSFL components (1% oil and 4% chitin) may even enhance hepatic gene expression linked to protein metabolism, but that chitin at excessive amounts can reduce digestibility in trout and salmonids (Eggink *et al.*, 2022).

Survival rates recorded in both our studies are in agreement with previous research, showing no significant compromise in fish health or survival up to 50% BSFLM inclusion in the diet, provided the feed is carefully formulated to the species' nutritional needs. For example, (Kari *et al.*, 2023) and (Mikołajczak *et al.*, 2022) demonstrated that BSFL-based feeds produce survival rates similar to fishmeal-based feeds, reinforcing the safety of moderate BSFLM replacement. The reduced survival seen at higher inclusions may point to digestibility challenges and

chitin-related effects, as highlighted by (Mahato *et al.*, 2024) in the earlier laboratory trial and by several international reports (Eggink *et al.*, 2022; Pascon *et al.*, 2025).

Besides growth performance and survival rate, the current study provides valuable insights into water quality management. Notably, dissolved oxygen (DO) levels reacted positively to BSFL inclusive diets, correlating strongly with growth metrics and feed efficiency. This phenomenon can be attributed to lower organic load and improved nutrient assimilation associated with highly digestible feeds (He *et al.*, 2022), as evidenced by lower FCR and superior PER. Improved nutrient retention, lessening nitrogenous and organic waste emission, supports better DO levels, a pattern also reported in studies investigating the sustainability and water quality benefits of insect meals in aquaculture systems (He *et al.*, 2022). This linkage between efficient feed utilization and favorable water quality represents a significant win for sustainable trout farming in resource-limited environments.

Recent literature also elaborates on the sustainability aspect. Life Cycle Assessment studies emphasize that BSFLM production substantially lowers environmental impacts including greenhouse gas emissions, water use, and land requirements compared to marine derived ingredients (Salahuddin *et al.*, 2024), making it an attractive option for countries such as Nepal with limited feed resource security. Supplementation of BSFLM with select amino acids or fatty acids (such as methionine or omega-3s) can further optimize growth and nutritional outcomes (Lemme and Klüber, 2024).

Complementing these observations, global meta-analyses and industry reviews underline BSFLM's broad biological roles, from serving as a high-quality protein and energy source to modulating immune function and gut health via its natural chitin and peptide content (He *et al.*, 2022; Koutsos *et al.*, 2022; Lee *et al.*, 2018). While moderate levels support these benefits, higher levels may overwhelm digestive capacity, lessen fat and protein retention, and affect biochemical structure and

immunity (Eggink *et al.*, 2022), underlying why careful balancing is paramount.

CONCLUSION

In summary, Rainbow trout fed diets with 25% BSFM (BSF25) consistently exhibited superior final weight, weight gain, SGR, feed conversion, and protein efficiency compared to other diets, with survival rate remaining high. Increasing levels of BSFM beyond 25% resulted in decreased growth and survival, highlighting 25% BSFM substitution as optimal for growth and feed utilization in Rainbow trout. In addition, temperature and pH remained consistent across treatments, while dissolved oxygen showed significant improvement, suggesting that BSFL inclusion in diet may positively influence oxygen dynamics in the fish culture system.

Therefore, our transition from laboratory to farm-based experimentation demonstrates convincingly that partial shrimp meal replacement with BSFLM at 25–50% is both effective and safe for rainbow trout, underpinned by improved growth, feed efficiency, survivability, and water quality. These outcomes, reinforced by multiple academic studies and our own previous controlled finding at Fishery Research Station, point decisively toward the practical adoption of BSFLM in Nepalese aquaculture. BSFLM's sustainability and cost efficiency, corroborated by international literature, further support its wider use.

Future research should focus on refining BSFLM processing technologies to better control chitin levels, exploring supplementation strategies for essential amino acids and fatty acids, and expanding trials to include longer-term effects, larger production scales, and different environmental scenarios. This continuing line of research will deepen our ability to harness insect protein for a resilient, environmentally sound aquaculture sector.

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