Original Research Article

Evaluating the impact of Black Soldier Fly (*Hermetiaillucens*) larvae meal on growth performance and feed utilization in Rainbow Trout (*Oncorhynchus mykiss*) juvenile

ABSTRACT

Aims: Black Soldier Fly (BSF) larvae meal is a promising alternative protein source in aquafeed, offering high nutritional value and essential amino acid profiles like fishmeal. Nepalese Rainbow trout (*Oncorhynchus mykiss*) farming currently relies on imported shrimp meal, presenting economic and ecological challenges. The focus of this research was to examines the potential of BSFL meal to replace the shrimp meal in trout feed.

Study design: Original Research Article.

Place and Duration of Study: Fisheries Research Station (FRS), Trishuli Nepal, between May 18 toAugust 19, 2023.

Methodology: A feeding trial was conducted using 750 healthy Rainbow trout (mean individual body weight 5.39±0.55 g) randomly divided into five experimental groups, each having three replicates. The control diet contained no BSFL meal (CON), while experimental diets replaced 25% (BSF25), 50% (BSF50), 75% (BSF75), and 100% (BSF100) of shrimp meal with BSFL meal. Fish were raised in flow-through raceway tanks (300L) and fed twice daily until satiation. The growth performance parameters were measured.

Results: The results demonstrated that diets with 25-50% BSFL meal substitution (B25, B50) significantly enhanced growth performance compared to the control, reflected in higher final body weight and weight gain, and improved feed conversion ratios ($P<0.05$). Conversely, higher replacement levels (75% and 100%) negatively impacted growth, possibly due to increased chitin content affecting digestibility. Statistical analysis confirmed the optimal BSFL inclusion range for growth performance, with specific growth rates peaking in the B50 group.

Conclusion: In conclusion, BSFL meal can effectively replace 25-50% of shrimp meal in rainbow trout feed, offering a viable and sustainable protein source for trout farming in Nepal. This substitution could reduce dependency on imported shrimp, promoting more sustainable practices.

Keywords: *BSFL, Rainbow trout, Sustainable feed, feed utilization*

1. INTRODUCTION

Currently, cold-water aquaculture in Nepal is primarily focused on a single fish species, the Rainbow trout (*Oncorhynchus mykiss*). Approximately 120 farmers across 38 mid-high hill districts of Nepal produce around 420 metric tons of trout annually [1]. However, this production level is expected to rise as 56 districts have been identified as suitable for rainbow trout cultivation [2]. With the growing popularity of rainbow trout among the populace, the demand for this species is also increasing. Nearly 100% of the trout produced in Nepal is consumed domestically. As rainbow trout farming expands, the demand for complete feed will also escalate. Presently, shrimp meal, known for its high protein content (approximately 46-60%) and excellent protein composition, as well as its natural source of astaxanthin pigment for imparting red coloration in salmonids, is a primary protein source in rainbow trout feed in Nepal [3]. This shrimp meal is predominantly imported from India. Research indicates a significant decline in marine and ocean life in recent decades, partly due to overfishing [4], resulting in shrimp becoming scarcer and more expensive as fishery products worldwide decline [5]. Furthermore, importing shrimp from India costs several hundred thousand annually, with no guaranteed consistency in quality. Consequently, the identification and production of more economic and sustainable sources of protein for trout feed is of vital importance.

In recent years, a variety of novel alternative protein sources for aquafeed have been explored. Insect protein is one of the emerging and excellent alternative protein sources that could potentially replace fishmeal or shrimp meal in aquafeed in the future. Although insect protein has been experimented with in poultry feed since 1969 [6], it has only gained significant attention in aquaculture feed over the past two decades. Some insects like the silkworm (*Bombyx mori* L.), the black soldier fly (*Hermetiaillucens* L.), the house fly (*Musca domestica* L.), the mealworm (*Tenebrio molitor* L.), the lesser mealworm (*Alphitobiusdiaperinus*), the banded cricket (*Gryllodessigillatus*), the house cricket (*Acheta domesticus* L.) and the Jamaican field cricket (*Gryllus assimili*) have already achieved researcher's attention [7]. It is because, insects are deemed an economically and ecologically advantageous ingredient because they convert food into protein efficiently, require minimal arable land and water, have a low environmental impact, necessitate minimal investment in machinery, and possess rapid reproduction cycles [8].

In recent years, the insect species *Hermetiaillucens*, commonly known as the black soldier fly, has garnered significant attention as an alternative protein source to replace fish meal in aquaculture feeds as it has been shown to have essential amino acid profile similar to that of fishmeal [9]. In addition, *Hermetiaillucens* larvae is packed with proteins (60–80%), essential amino acids, vitamins, and minerals, plus significant lipids (31–43%), which can vary based on how they're raised [10]. These larvae are great at converting organic material, eating twice their weight daily, and have high protein (36–48% DM) and fat (31–33% DM) levels before pupating [9]. Recent studies have examined its effects on various fish species, including Mirror carp (*Cyprinus carpio)* [11], Atlantic salmon (Salmo salar) [12], African catfish (*Clariasgariepinus*) [13], European seabass (*Dicentrarcuslabrax*(Moutinho et al., 2021), Rainbow trout (*Oncorhynchus mykiss* (Bruni et al., 2020)), and others, focusing on growth performance, feed utilization, gut health, and microbiome modulation. Actually, in salmon and sea bass the *Hermetiaillucens* replaced 40% of fishmeal [14, 15], and up to 50% in trout [15] without negatively affecting the growth performance. Research also suggests *Hermetiaillucens* larvae meal may positively influence the gut microbiota, promoting bacteria that produce beneficial short-chain fatty acids like butyrate through chitin fermentation critical for intestinal cells supporting intestinal development, immunity, and disease resistance in rainbow trout [16, 17]. Therefore, given the evidence, it is plausible to anticipate that replacing part of the fishmeal or shrimp meal with insect meal could have a beneficial impact on the growth and intestinal microbiota of fish.

While in overseas extensive nutritional research has examined how incorporating *Hermetiaillucens* meal into diets affects fish growth [9], and intestinal microbiota composition [18], less is known about its specific influence on growth performance of Rainbow trout juveniles in the context of Nepal. For this reason, the present aimed to explore how substituting shrimp meal with *Hermetiaillucens*larvae meal in rainbow trout diets affects growth performance, feed utilization, and survival.

2. MATERIALS AND METHOD

The experimental protocol was developed according to the proposal submitted to the Nepal Agricultural Research Council and was approved by the Council to conduct the research. The feeding trial was carried out between May 19 to August 07, 2024, at the Outdoor Experimental Facility of the Fishery Research Station, Trishuli (FRS) located at Bidur-9 Nuwakot, Nepal.

2.1 Test Ingredients and Experimental Diets

The dried form of black soldier fly larvae (BSFL) was obtained from J17 AQUA, a company specializing in fish food and feed supply, located in Kanchipuram, Tamil Nadu, India. Upon receipt, the dried insect larvae were processed into a fine powder before being incorporated into the experimental diets.

Five experimental diets were formulated to be isonitrogenous (Crude protein about 45 g/100g as fed) and isolipidic (ether extract about 15g/100g as fed) to fulfill the nutritional needs of rainbow trout. The control diet (CON) contained no black soldier fly larvae (BSFL) meal, while four experimental diets incorporated partially defatted BSFL meal at varying levels. These experimental diets, labeled B25, B50, B75, and B100, substituted 25, 50, 75, and 100% of the Shrimp meal protein in the control diet with BSFL meal, respectively. Each diet were distributed randomly among three tanks.

The experimental diets were prepared at the Feed House of FRS. All the powdered ingredients and sunflower oil were individually weighed and mixed with blender followed by the addition of 250-500 ml/kg of water to the mixture. The feed pelleting machine was used to form pellet of 2.0 mm which was subsequently dried and stored in airtight buckets until used. The list of ingredients used to prepare experimental diets and the proximate composition of both the dry BSFL meal, and the experimental diets used in the study is presented in Table 1. The proximate composition and energy level of the BSF meal was provided by the BSFL supplier and experimental diets were measured in duplicate at the Animal Nutrition Laboratory at the Khumaltar Lalitpur.

Table 1. Ingredients and proximate composition of BSFL meal and experimental diets.

Ingredients	BSFL meal	CON	BSF25	BSF50	BSF75	BSF100
Shrimp meal		500	375	225	125	0
BSFL meal			125	225	375	500
Soybean full fat		355	355	355	355	355
Wheat Gluten		30	35	80	60	70
Corn meal		20	20	5	5	15
Wheat flour		50	50	80	65	50
Soybean oil		40	35	25	10	5
Vitamin premix		3	3	3	3	3
Trace mineral premix		2	2	2	2	2
Total		1000	1000	1000	1000	1000
Proximate composition						
Dry matter (%)	91.67	93.81	93.39	93.00	92.66	92.34
Crude protein (%)	51.56	45.71	45.21	45.23	45.40	45.19
Lipid (%)	15.39	15.25	15.78	15.26	15.31	15.86
Digestible energy (MJ/kg)	26.46	18.75	19.90	20.89	21.92	23.10

Vitamin premix (IU or mg/kg diet): DL-α tocopherol acetate 60 IU; sodium menadione bisulphate 5 mg; retinyl acetate 15,000 IU; DL-cholecalciferol 3000 IU; thiamin 15 mg; riboflavin 30 mg; pyridoxine 15 mg; vitamin B12 0.05 mg; nicotinic acid 175 mg; folic acid 500 mg; inositol 1000 mg; biotin 2.5 mg; calcium pantothenate 50 mg

Mineral premix (g or mg/kg diet):Bicalcium phosphate 500 g, calcium carbonate 215 g, sodium salt 40 g, potassium chloride 90 g, magnesium chloride 124 g, magnesium carbonate 124 g, iron sulfate 20 g, zinc sulfate 4 g, copper sulfate 3 g, potassium iodide 4 mg, cobalt sulfate 20 mg, manganese sulfate 3 g, sodium fluoride 1 g

2.2 Fish and Rearing Conditions

A 90-day feeding trial was conducted with the juvenile of rainbow trout obtained from own hatchery of the FRS, Trishuli. Since the fish were from the same hatchery, the acclimatization period was 5 days during which a locally made trout feed was fed (44 g/kg CP). Then a total of 750 healthy fish of mean individual body weight 5.39±0.55 g was randomly divided into 15 flowthrough 300-L raceway tanks (three replicate tanks per diets. River water coming from siltation tank (average water temperature of 19.48±0.27°C) was supplied in flow-through raceways with each tank having a water flow of 6-8 L/min. Dissolved oxygen and pH were measured every 2-3 days and were recorded to be 7.94±0.10 mg/L and 8.51±0.02 respectively. Feed was distributed by hand twice a day until apparent satiation. Apparent satiation was confirmed when the fish stopped eating. Mortality was checked every day. In order to monitor growth and through cleaning of the tanks, fish were weighed in bulk every 15 days.

2.3. Growth Performance and Condition Factor

At the end of the feeding trial, after starving for 24 hours, all fish were harvested, counted and individually weighed for body weight and measured for total length. The following production performance indexes were calculated:

Mortality (%) = (number of dead fish/initial number of fish) × 100

Individual weight gain (iWG, g) =

average individual final body weight (iFBW, g) – average individual initial body weight (iIBW, g)

Specific growth rate (SGR, %/day) = [(InFBW− lnIBW)/number of days] ×100

Feed conversion ratio (FCR)= total feed supplied (g, DM) / WG (g)

Protein efficiency ratio (PER)= WG(g)/total protein fed (g, DM).

Above mentioned indexes were calculated for each tank using individual initial and final body weight, and number of fish.

2.4 Statistical Analysis

To analyze the data IBM SPSS Statistics v. 25.0 for Windows was used. A one-way ANOVA test was used to compare data across experimental groups taking a statistical significance level at $P<0.05$. Firstly, normality and homogeneity of variances were evaluated using the Kolmogorov–Smirnov test, and through Levene's test respectively. Instead of F-test, we used Brown-Forsythe statistic test to compare group means when the assumption of equal variances was violated. Then, pairwise comparison was done by Tukey's test for equal variances or Tamhane's T2 for unequal variances (one-way ANOVA). Results are presented as mean and pooled standard error of the mean (SEM).

2.5. Ethical Statement

While there is no specific ethical protocols for fish research in Nepal, all experimental procedures were conducted with care to ensure minimal or no harm to the fish, adhering to scientific best practices and welfare standards.

3. RESULTS

3.1 Experimental Diets

The chemical composition of all the experimental diets were found to be similar in terms of dry matter, crude protein, lipid content and gross energy as expected (Table 1). The results showed that the formulated experimental diets covered the nutritional requirements of the Rainbow trout.

3.2 Growth Performance

The results of the growth performance parameters of Rainbow trout in each treatment group (CON, B25, B50, B75, and B100) are shown in Table 2.

The results show that initial body weight (iIBW) of the rainbow trout did not significantly differ between the five treatment groups ($P> .05$) and ranged from 5.35 g to 5.43 g at the start of the experiment. The individual final body weight (iFBW) and individual weight gain (iWG) of rainbow trout were significantly higher in the B25, B50, and B75 groups compared to the control (CON) group ($P< .05$). The highest iFBW and iWG were observed in the B50 group. However, above B50, the iFBW and iWG decreased, with the B100 group showing iFBW and iWG values comparable to those of the CON group. The highest and lowest individual body weights were 23.97 g and 33.54 g respectively. Similarly, the highest and lowest individual weight gain were 28.19 g and 18.51 g respectively. Similarly, the specific growth rate (SGR) was significantly higher in B25, B50, and B75 groups than CON group ($P < .05$). Among the treatments, the highest SGR was observed in the B50 group, followed by B25 and B75 groups. The B100 group showed an SGR comparable to the CON group. The fish in the experiment had an SGR value from 1.67 %/day to 2.05 %/day. Likewise, the feed conversion ratio (FCR) was apparently lower in B25 and B50 groups compared to the CON and B75 group. However, there were no significant differences in FCR between the control group and the treatment groups except for B100. The B100 group had significantly higher FCR among the treatment groups in this experiment. The value for FCR in this experiment ranged from 1.19 to 2.74. Moreover, the result for PER revealed varying trends in values ranging from 0.92 to 2.04. The B25 group showed the statistically highest value among all groups, although its value was not statistically different from B50 group. The B50 group had statistically similar PER to both CON and B75 groups, indicating similar protein efficiency ratio. The B100 group was observed with statistically lowest PER value among all the groups ($P< .05$) in this experiment.

Finally, the result of mortality rate revealed that groups B25 and B75 had the lowest mortality rate, although this rate was not statistically different from B75 and CON groups. However, significantly higher mortality rate was observed in B100 group compared to other treatments ($P< .05$). The mortality rate in this experiment ranged from 15 to 40%.

Table 2: Effect of BSF larvae meal on growth performance of rainbow trout (n=3)

	CON	**B25**	**B50**	**B75**	**B100**	**SEM**	***P*-value**
iIBW, g	5.37^{a}	5.37^{a}	5.35^{a}	5.43^{a}	5.43^{a}	0.11	1.000
iFBW, g	23.87^{c}	31.54^{ab}	33.54^{a}	29.53^{b}	26.23^{c}	0.96	0.000
iWG, g	18.51^{c}	26.17^{ab}	28.19^{a}	24.11^{b}	20.80^{c}	0.95	0.000
SGR, %/d	1.67^{c}	1.98^{ab}	2.05^{a}	1.89^{ab}	1.75^{bc}	0.04	0.004
FCR	1.53^{a}	1.19^{a}	1.31^{a}	1.59^{a}	2.74^{b}	0.16	0.000
PER	1.56^{b}	2.04^{a}	1.83^{ab}	1.51^{b}	0.92^{c}	0.11	0.000
Mortality, %	19.00^{a}	15.00^{a}	15.00^{a}	22.00^{a}	40.00^{b}	2.73	0.001

BSF or B: black soldier Fly, SEM: standard error of the mean, iIBW: individual initial body weight, iFBW: individual final body weight, iWG: individual weight gain, SGR: specific growth rate, FCR: feed conversion ratio, PER: protein efficiency ratio.

4. DISCUSSION

Several studies have demonstrated that insect-based protein sources, especially black soldier fly (BSF), are usable alternatives to the conventional protein sources like fish meal in aquafeed [19-21]**.** And**,** the application of BSF as a possible source of protein in trout feeds has been amply studied [22-26]. Result of our study demonstrated the diet in which 25-50% of the shrimp meal was replaced by BSF larvae meal resulted in the best results for most of the production parameters compared to control group, and the replacement up to 75% were possible without negative consequences. This result is in agreement with study of [27], showing BSF meal could replace up 50% shrimp meal in the diet of rainbow trout with no adverse effect on growth performance. Gasco et al. reported that feeding rainbow trout with defatted BSF successfully substituted 25 and 50% of the fish meal protein without affecting growth performances and quality parameters. In line with our results, a study done by [28] reported that BSF meal can replace up to 50% fish meal on Nile tilapia (*Oreochromis niloticus*) diet with no adverse effects on growth and feed utilization. Similarly, a study in yellow catfish

(*Pelteobagrusfulvidraco*) reported positive growth performance with up to 48% fishmeal protein replaced by BSF larvae meal protein [29]. In our study, there were slight significant differences observed in terms of growth performance and feed utilization among all the experimental diets. Final weight, weight gain, specific growth rate, feed conversion ratio, and protein efficiency ratio were significantly improved in fish feed diet B25 and B50 compared to that in other groups. However, these parameters, especially weight gain and SGR were observed to decrease as the level of BSF larvae meal increased further. There were no significant differences recorded for FCR up to 75% replacement level. However, the 25% replacement diet (B25) generated the best FCR and PER values. The lower FCR and higher PER value indicate the good quality of feed and optimum utilization of feed. This indicates that rainbow trout could utilize the BSF larvae meal in its diet optimally up to 50% of shrimp meal replacement. Slightly different findings were reported in a pervious study where author found no significant different in FCR and PER up to 75% replacement level, improved weight gain and SGR when fish meal in rainbow trout feed was replaced by BSF prepupal meal [26]. However, some previous studies reported that above 25% replacement of the fish meal by BSF larvae or prepupal meal, the FCR and weight gain in rainbow trout adversely affected [25, 30]. In a study in red drum (*Sciaenops ocellatus*) author recorded impaired growth performance and feed utilization when BSF larvae meal was used as low as 150 g/kg [31]. Such contrasting results might be due to difference in the species of fish used in the experiment, level and life stage of BSF used in the fish feed, and different in the rearing substrate used for culture of BSF. [31] suspected that impaired growth and poor nutrient utilization observed in red drum compared to previous studies was due to difference in nutrient content in BSF larvae rearing in different rearing substrate because Yamamoto et al. found that BSF larvae meal derived from larvae fed a commercial diet resulted in better growth performance in red drum compared to BSF larvae meal from larvae raised on brewers' spent grains, even though both diets were formulated to be isonitrogenous. Similarly, [32] concluded that the feed intake, availability and nutrient digestibility of juvenile turbot (*Psetta maxima*) was impaired due to presence of higher amount of chitin in diet coming from older BSF larvae ultimately affecting the growth performance. Although, why results were obtained is unclear, St-Hilaire et al. mentioned that higher level of BSF meal in the fish feed could increase the chitin content in the fish feed which make feed less digestible for fish and ultimately affecting growth performance. Chitin, a primary component of crustacean exoskeletons and fungal cell walls, is a tough, nitrogen-containing polysaccharide with similar structure to cellulose, but more resistant to degradation and digestion by most animals, except few fish [33]. There are several previous studies which shows that higher level of insect or chitin in fish feed, affects the digestibility and growth performance of fish regardless of the type of insect, rearing substrate used to culture insect, and species of fish in the experiment. Similar finding was reported in a study with rainbow trout where amount of tissue was decreased as the amount of insect (*Tenebrio molitor*) meal increased in the trout feed [34]. However, such result might have been obtained due to difference in ability of fish to utilize the fat from different sources, composition and amount in the diets [35] as a result of fatty acid composition of the BSF larvae meal used in preparation of experimental diets that ultimately affected fat metabolism of rainbow trout. According to Yu et al. activities of fatty acid synthase (FAS) and lipase (LPS), two lipid metabolism-related enzymes in fish, are closely related to composition of chitin in BSF [36]. Li et al reported that juvenile Jian Carp (*Cyprinus carpio* var. Jian) when fed with higher concentration of BSF larvae showed poor nutrient utilization and impacted ability of synthesizing the fatty acids [37]. Therefore, a reasonable explanation for reduced growth performance and nutrient utilization above 50% replacement level in present study could be higher insect component in diet causing impaired activities of lipid metabolism-related enzymes, closely related to the high amount of chitin in BSF. In addition, a study reported that chitin digestibility is usually very low or completely absent as in rainbow trout [38]. Similar conclusion was made in a study in which authors found above 3% chitin in a diet of rainbow trout negatively affected growth performance and nutrient digestibility [39].

Despite these negative effects of high level of insect meal or chitin content above 2-3% in fish feed, insect meal contains several bioactive compounds and have positive impacts on fish metabolism [40], resistant against pathogens [41] and immune response [42]. And chitin is one of the most studied bioactive compounds in aquafeed having such beneficial effects. Black soldier fly larvae chitin at 0.4% was shown to promote the monounsaturated fatty acid deposition in muscle and improved health status of largemouth bass (*Micropterus salmoides) [*43*]*. Previous studies have shown that the peptide extract of the BSF larvae possess broad-spectrum of antibacterial activity suggesting BSF as a potential source of novel antibiotic-like compounds which may control infections in fish [44-46]. Studies also report that chitin, analogous to cellulose, acts as a prebiotic, promoting the growth of beneficial bacteria and chitin-degrading bacteria in the gut, resulting in the production of healthy short-chain fatty acids (SCFAs), including acetic, propionic, and butyric acids, which have been demonstrated to have beneficial effects on the gut, immune system and health of fish including rainbow trout [47, 48, 29]. These positive responses could be due to the presence of chitin in the fish feed.

Previous studies have demonstrated the ability of fish to degrade chitin fiber through the action of chitinolytic enzymes (chitinase and chitobiase) [49, 50]. This knowledge can be applied to improve feed digestibility and nutrient absorption in fish by incorporating chitin-degrading enzymes or bacteria into their diet. Specifically, chitinases can enhance the accessibility of digestive enzymes to entrapped proteins or lipids, leading to improved overall digestive health and nutrient absorption in fish [51, 52].

The cumulative evidence from the present study and existing research in the field collectively suggests that the incorporation of Black Soldier Fly (BSF) larvae meal into fish feed can yield several benefits, including improved growth performance, enhanced immune function, and better overall health of fish. Therefore, the use of BSF as a feed source in rainbow trout presents a viable alternative to traditional protein-rich ingredients like shrimp meal. Importantly, the production of BSF larvae using organic waste offers a potentially sustainable solution for the aquaculture industry ultimately reducing feed cost and leading to significant cost savings in commercial aquaculture. However, additional research is required to determine the optimal inclusion level of BSF larvae meal in fish feed and to develop effective processing methods to reduce the issues related to higher chitin content and maximize level of inclusion of BSF larvae in fish feed without affecting production performance.

5. CONCLUSION

In conclusion, the results of this study demonstrate that replacing 25-50% of shrimp meal with Black Soldier Fly (BSF) larvae meal in the diet of rainbow trout significantly improves growth performance and feed utilization. This finding suggests that BSF larvae meal can be a viable alternative protein source in trout feed, offering a sustainable solution for the farmers of rainbow trout. Particularly, the 25-50% replacement of shrimp meal with BSF larvae showed the most pronounced improvements in growth performance and feed utilization, indicating that this range may be the optimal inclusion level for BSF larvae meal in rainbow trout feed juvenile. However, higher levels of BSF inclusion (75% and 100%) led to a decrease in growth and an increase in mortality, suggesting an optimal level of inclusion exists for maximizing benefits. These findings demonstrate the potential of BSF larvae meal as a sustainable and effective feed ingredient for rainbow trout culture suggesting an important implication for the development of sustainable and cost-effective trout feed. By incorporating BSF larvae meal into trout feed, the trout farmers can reduce their reliance on shrimp meal and promote trout farming in mid-hills of Nepal.

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