



## RESEARCH PAPER

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## Effect of black soldier fly (*Hermetia illucens*) larvae meal as a fishmeal protein replacement on the growth, feed utilization, survival and whole-body composition of Black molly (*Poecilia sphenops*)

Mousumi Das\*

Department of Zoology, Vidyasagar College, Block CL, Sector II, Salt Lake, Kolkata, India

**Key words:** Black molly, Black soldier fly larvae meal, Feed utilization, Fishmeal, Growth performance, Survival percentage

<http://dx.doi.org/10.12692/ijb/22.5.168-181>

Article published on May 19, 2023

### Abstract

In search for a potential fishmeal alternative, six-months feeding trial was conducted to examine the effects of replacing fishmeal (FM) with black soldier fly larvae meal (BSFLM) in the diets of black molly, *Poecilia sphenops* on growth performance, feed utilization, survival and whole-body composition. Five iso-nitrogenous diets (40%) were formulated containing 0%, 11.49%, 22.98%, 34.47% and 45.96% BSFLM, replacing 0%, 25%, 50%, 75% and 100% FM protein and named as D0, D25, D50, D75 and D100 respectively. Uniform sized ( $0.025 \pm 0.001$ g) 30 black molly juveniles were randomly assigned to one of fifteen aquariums, having five dietary treatments with three replicates. At the end of feeding trial, significantly higher final weight ( $2.41 \pm 0.03$ g), final length ( $4.75 \pm 0.03$ cm) and specific growth rate in percent (SGR%) ( $2.32 \pm 0.04$ ) were found in the D50 diet fed set, followed by the D25 set and significantly lower final weight ( $0.91 \pm 0.08$ g), length ( $3.43 \pm 0.03$ cm) and SGR% ( $1.97 \pm 0.04$ ) were obtained from the D100 set. Food consumption was observed significantly higher in the D50 set ( $2.79 \pm 0.01$ g) and a lower in the D100 set ( $2.65 \pm 0.05$ g). The value of FCR was significantly higher in the D100 set ( $3.03 \pm 0.25$ ), whereas the best value of was noted in the D50 set ( $1.29 \pm 0.01$ ). Survival percentage was ranged from 88.89% to 98.89% and had no significant effect of dietary treatment. No effects of different dietary treatment on whole-body composition were observed. Overall, BSFLM could be incorporated in black molly diet up to a 75% FM replacement rate with no adverse effect and the optimum FM substitution level is 50% with 22.98g/100 gm BSFLM.

\* Corresponding Author: Dr. Mousumi Das ✉ [mousumidasghosh23@gmail.com](mailto:mousumidasghosh23@gmail.com)

## Introduction

Ornamental fish production and their trade is becoming a profitable alternative aquaculture industry in the aquaculture sector (Pour *et al.*, 2014). *Poecilia sphenops* commonly known as black molly, belongs to the family Poeciliidae, is a shiny black-coloured, omnivorous, tropical, steady and highly adaptable ornamental fish species (Patel *et al.*, 2017). It is one of the high-demanded and most traded aquarium fish in ornamental aquaculture (Uribe *et al.*, 2018; Oliveira *et al.*, 2014).

Suitable fish feed formulation with novel low-cost readily available primary protein source and also with proper proportion of ingredients, are most important criteria in aquaculture as aqua-feed cost is the major expenses (Haghighyan and Shamsaie Mehrgan, 2015). Fishmeal (FM) is the conventionally used primary protein source in fish feed and also in poultry and other livestock feed as it is highly palatable and with good nutritional and amino-acid profile (Gatlin *et al.*, 2007; Ding *et al.*, 2015; Haghighyan and Mehrgan, 2015). High demand of FM due to the ever-increasing development of aquaculture and livestock industries leads to its scarcity and high-cost in commercial markets. Consequently, over exploitation of fish to meet its demand in global market, caused increased pressure in aquatic ecosystems (Haghighyan and Shamsaie, 2015). For sustainable development of aquaculture industry, there is a need of an alternative cost-effective primary protein source for aqua-feed.

Many scientific research studies have proposed that plant-based protein sources might be alternatives for FM (Aas *et al.*, 2019), but plant proteins are associated with some complications such as possessing imbalanced amino-acid profiles (Kamalii *et al.*, 2022) and, having low palatability, digestibility (NRC, 2011) and also containing anti-nutritional factors, tannin and oxalate (Mastoraki *et al.*, 2020), consequently having negative impact on growth performance and fish health (Gai *et al.*, 2012; Oliveira-Teles *et al.*, 2015). Moreover, crops as FM alternative could cause efficient conflict with human nutrition (Kroeckel *et al.*, 2012). Therefore, the search for alternative economic, readily available, nutrient rich,

animal-based protein source which is not competed by human is continued throughout the world. In recent times, insect has gained a significant attention (Gasco *et al.*, 2019; Barragan-Fonseca *et al.*, 2017) as a potential high-quality fishmeal protein alternative for poultry (Neumann *et al.*, 2018; Secci *et al.*, 2018; Gariglio *et al.*, 2019; Pieterse *et al.*, 2019; Yoo *et al.*, 2019), swine (Biasato *et al.*, 2019; Chia *et al.*, 2019), carnivorous fishes such as rainbow trout (Chemello *et al.*, 2020), European sea bass (Gasco *et al.*, 2016), Atlantic salmon (Belghit *et al.*, 2019) and gilthead seabream (Piccolo *et al.*, 2017), and for omnivorous fishes such as common carp (Li *et al.*, 2017), Nile tilapia (Devic *et al.*, 2018) and goldfish (Kamalii *et al.*, 2022). In most cases the acceptable results without any adverse effect on growth and survival, were observed in the partial replacements of FM with insect meal. Insects, such as grasshoppers, crickets, termites, silkworm, Asiatic rhinoceros beetles, housefly larvae, yellow mealworm larvae, mosquito larvae, black soldier fly larvae, have high protein content (Henry *et al.*, 2015) and have similar amino acid profiles that of fishmeal (Barroso *et al.*, 2014). These insects can easily be grown and mass reared on organic waste or they can be harvested, for their continuous supply to the aqua-feed industry. Few insects are able to transform organic biowaste into good proteins with high conversion efficiency (Makkar *et al.*, 2014). Some species of insects are regarded as potential sustainable FM alternatives for fish feed (Ido *et al.*, 2019; Li *et al.*, 2019; Taufek *et al.*, 2016; Wang *et al.*, 2019; Chemello *et al.*, 2020).

Among insects, one candidate species, the larvae of *Hermetia illucens*, black soldier fly larvae (BSFL) being considered most suitable due to its superior feed conversion rate (Diener *et al.*, 2009), do not spread disease (Wang and Shelomi, 2017) and have potential to convert organic waste to protein (Zheng *et al.*, 2012; Zhou *et al.*, 2013). Moreover, the protein content of BSFL ranged from 35% to 59.8% on dry weight basis (Smets *et al.*, 2020; Cammack and Tomberlin, 2017; Meneguz *et al.*, 2018) and its amino-acid and fatty-acid profile make it suitable FM substitute in animal feed (Nogales-Merida *et al.*, 2019; Smets *et al.*, 2020; Makkar *et al.*, 2014).

Earlier studies showed that BSFLM could be substitute for FM in aqua-feed without any adverse effect on *Cyprinus carpio* (Zhou *et al.* 2018), *Salmo salar* (Belghit *et al.* 2019; Lock *et al.*, 2016), *Dicentrarchus labrax* (Magalhães *et al.* 2017), *Pelteobagrus fulvidraco* (Xiao *et al.* 2018), *Lates calcarifer* (Katya *et al.* 2017) and some warm water fish species (Sudha *et al.*, 2022; Sealey *et al.*, 2011). But there are insufficient records of feeding trials on ornamental fish species fed with diets replacing FM with BSFLM (Kowalska *et al.*, 2021).

Therefore, the present study aimed to evaluate the effects of different dietary inclusion levels of BSFLM in replacement of FM protein on the growth performance, feed utilization, survival and whole body composition of resulting fishes, black molly (*Poecilia sphenops*).

## Materials and methods

### *Experimental fish*

A total number of 500 black molly juveniles were procured from local ornamental fish market, Galiff Street, Kolkata, India in March 2022 and apparently, healthy ones were acclimatized for two weeks in a glass aquarium (36"×12"×12") in the laboratory facilities and conditions, by feeding them with a commercial diet containing 40gm/100gm of protein. Before starting the six-month feeding trial, the fishes of average weight of  $0.025 \pm 0.001$  gm were selected and stocked at a density of 30 fishes per aquarium.

A total of 450 fishes were distributed into 15 indoor glass aquariums (18"×12"×12") having five dietary treatment sets with three replications. Diets were given twice a day (09:00 and 18:00 H) to the fishes, at the amount of satiation level and feed consumption was noted daily. Water exchange was done twice a week at 25% level in each aquarium. For continuous aeration throughout the feeding trial mini air blower pumps were used.

### *Experimental diet*

Dried black soldier fly larvae were purchased from nearby local pet shop (Kolkata, India) and then oven-dried at 40°C for 72 hours in the hot-air-oven and

then ground into powder form. It was stored in airtight plastic container until further use for the preparation of experimental diets and for proximate analysis. Five different practical BSFLM and FM based iso-proteic experimental diets were formulated with 40% of crude protein (Table 3) to meet the nutritional requirement of black molly. A practical FM-based diet was used as control (D0), and the four other diets were prepared containing 0%, 11.49%, 22.98%, 34.47% and 45.96% BSFLM, replacing 25%, 50%, 75% and 100% of fishmeal protein and named as D25, D50, D75 and D100 diets respectively. For the preparation of each experimental diet, all the oven-dried dietary ingredients were finely ground, well mixed and thoroughly blended with double-distilled water into dough, and pelleted by pelletizer through 1.5mm die. The obtained moist stands were oven dried at 40°C for 48 hours and then crushed into desirable particle size and kept in airtight container at room temperature until used. Dietary ingredients proportion and proximate composition of all the experimental diets were shown in Table 3.

### *Growth efficiency and survival percentage*

After the six-months feeding trial, fishes were counted in each aquarium to determine their survival percentage. Then final weight and length of fishes were taken in order to estimate their growth efficiency parameters like weight gain, specific growth rate (SGR%) and food utilization parameters in terms of feed conversion ratio (FCR).

### *Proximate Composition Analysis*

Fishes from experimental dietary treatment sets were collected at the end of feeding trial to estimate whole-body composition. The proximate composition such as crude protein, crude lipid, ash and moisture contents of BSFLM, experimental diets and whole body was measured following the guideline of Association of Official Analytical Chemists methods (AOAC, 2006).

### *Water Quality Analysis*

Physicochemical parameters of water such as temperature, dissolved oxygen, pH, free carbon dioxide, hardness and total dissolved solids (TDS) of experimental aquariums were analysed and recorded

weekly, throughout the experimental period using standard methods (APHA., 1989).

#### Statistical Analysis

The experimental data were presented as mean  $\pm$  standard error (SE) of the mean of three replications. One-way analysis of variance (ANOVA), followed by Duncan's multiple range tests (DMRT) for multiple comparisons at the significance level of 0.05 was used to compare the differences among the five dietary treatments.

## Results

#### Water quality

The range of values of water quality parameters recorded was given in Table 1. Temperature, dissolved oxygen, pH, free carbon dioxide, hardness and TDS were within the optimum range for aquarium fish rearing.

**Table 1.** Water quality parameters of experimental aquarium sets during the experiment.

Parameter	Minimum	Maximum
Temperature (°C)	27	39
Dissolved Oxygen (ppm)	8.2	8.4
pH	7.3	7.5
Free Carbon dioxide (ppm)	1.36	2.32
Hardness (ppm)	195	238
TDS (ppm)	148	172

#### Proximate composition of primary protein sources of experimental diets

Proximate compositions of BSFLM and FM were listed in table 2. Crude protein percentage of BSFLM were comparable to the FM protein percentage, though significantly varies. Crude lipid and crude fibre and ash content of BSFLM were slightly higher than FM.

**Table 2.** Proximate nutritional composition of BSFLM and FM (% dry weight basis).

Nutritional composition (%)	BSFLM	FM
Crude Protein	47.00 $\pm$ 0.20a	52.00 $\pm$ 0.95b
Crude Lipid	16.50 $\pm$ 0.20b	11.43 $\pm$ 1.25a
Crude Fibre	9.10 $\pm$ 0.15b	5.06 $\pm$ 0.55a
Ash	13.57 $\pm$ 0.14b	10.01 $\pm$ 0.58a
Moisture	5.58 $\pm$ 0.06a	9.77 $\pm$ 1.58b
NFE	13.83 $\pm$ 0.66a	21.50 $\pm$ 1.39b

Data are presented as mean  $\pm$  SE. Different letters (a,b) in a row denote significance difference ( $P < 0.05$ ) indicated by one-way ANOVA followed by DMRT.

#### Proximate composition of the experimental diets

Proximate compositions of all the practical diets for black molly were shown in Table 3. Crude protein content in all the experimental diets were ranged from 39.77% to 40.40% and did not vary significantly ( $P < 0.05$ ). The values of crude lipid content in the diets tended to increase with the increased level of BSFLM inclusion.

On the contrary, the obtained values of crude fibre and ash content of the experimental diets were decreased with the increased level of BSFLM inclusion.

#### Growth performance

The results of the growth performance of black molly in each set of dietary treatment at the end of the feeding trial were shown in Table 4.

At the start of experiment, initial length and initial weight varied from 1.00cm to 1.50cm and 0.023gm to 0.027gm respectively. At the end of six-months experimental period, significantly highest value of final weight was found in the D50 diet fed set followed by the D75 diet fed set and the lowest was observed in the D100 set.

A similar result was noted in case of the final length where significantly higher value was found in the D50 set and a lower value in the D100 set.

In case of SGR in percent, a maximum (2.52 $\pm$ 0.02) value was obtained from the D50 diet fed set, whereas a minimum value was noticed in the D100 (1.97 $\pm$ 0.04) diet fed set.

Total food consumption was significantly higher in the D50 diet fed set, followed by the D25, and then in the control (Do), whereas a lower mean value was obtained from the D100 set and the values of the D75 and the D100 did not vary significantly.

A higher value of food conversion ratio (FCR) was noted in the D100 set, followed by the D75 and then in the control (Do). The best value of FCR was observed in the D50 diet fed set and the value of the D25 was good when compared with the control (Do).

**Table 3.** Ingredients, their proportion and proximate composition of experimental diets.

Diet ingredients (gm/100gm)	Do	D25	D50	D75	D100
Fishmeal	40	30	20	10	0
BSFLM	0	11.49	22.98	34.47	45.96
Soybean	26.50	26.50	26.50	26.50	26.50
Corn	6.50	6.50	6.50	6.50	6.50
MOC*	18.20	18.20	18.20	18.20	18.20
Rice bran	3.11	2.36	1.49	1.36	0.43
Wheat	3.69	2.95	2.33	0.97	0.41
Vitamin-Mineral Premix*	2	2	2	2	2
Total	100	100	100	100	100
Crude Protein	40.40	40.24	40.08	39.93	39.77
Crude lipid	12.10	12.26	12.58	12.83	13.28
Ash	12.06	11.83	11.48	11.05	10.72
Crude Fibre	15.25	14.3	13.25	12.3	11.1
NFE	21.53	22.15	22.61	23.11	23.79

\*MOC- mustard oil cake

\*Vitamin-Mineral Premix (mg/kg diet): retinol-18,000 IU, Cholecalciferol-2000 IU, thiamine-15, menadione sodium bisulphate-10, riboflavin-25, pyridoxine-5,  $\alpha$ -tocopherol-35, nicotinic acid-200, Ca-pantothenate-50, biotin-1.5, folic acid-10, cyanocobalamin-0.03, ascorbyl monophosphate-50, inositol-400, copper sulphate-20.2, dibasic calcium phosphate-5.9, sodium fluoride-2.21, potassium iodide-0.78, zinc oxide-37.5, ironsulphate-200, magnesium oxide-840, manganese oxide-26, cobalt sulphate-1.85, sodium selenite-0.65, potassium chloride-1.17, sodium chloride-0.45.

**Table 4.** Growth parameters of black molly, *P. sphenops* fed with different BSFLM diets in six months feeding trial.

Growth parameters	Diets				
	Do	D25	D50	D75	D100
Initial length (cm)	1.27±0.15a	1.23±0.07a	1.25±0.058a	1.23±0.09a	1.22±0.08a
Final length (cm)	4.17±0.04c	4.48±0.04d	4.75±0.03e	3.88±0.04b	3.43±0.03a
Initial weight (gm)	0.024±0.001a	0.027±0.001a	0.026±0.001a	0.025±0.001a	0.026±0.001a
Final weight (gm)	1.53±0.05c	1.96±0.07d	2.41±0.03e	1.33±0.05b	0.91±0.08a
Weight gain (gm)	1.51±0.05c	1.94±0.07d	2.38±0.03e	1.30±0.05b	0.89±0.08a
SGR (%)	2.32±0.04c	2.38±0.03c	2.52±0.02d	2.21±0.01b	1.97±0.04a
Food consumption (gm)	2.79±0.01b	2.95±0.04c	3.07±0.03d	2.70±0.05a	2.65±0.05a
FCR	1.85±0.07c	1.52±0.04b	1.29±0.01a	2.08±0.09d	3.03±0.25e

Values are mean ± SE. Values with different letters are significantly different ( $P < 0.05$ ) using DMRT after one way ANOVA.

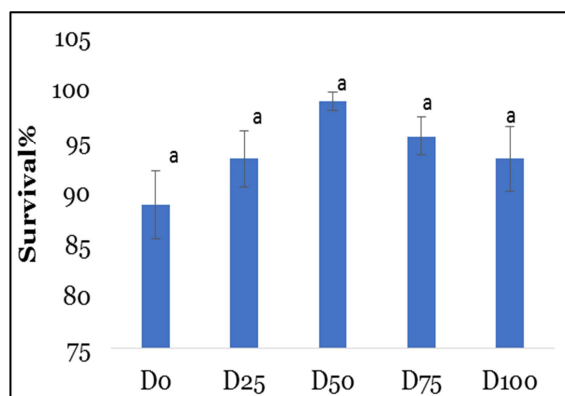
**Table 5.** Proximate composition (%) of the whole body of black molly, *P. sphenops* fed with different BSFLM diets in six months feeding trial.

Diets	Crude Protein	Crude lipid	Moisture	Ash
Do	14.02±0.34ns	6.30±0.12ns	74.27±0.05 ns	3.41±0.02 ns
D25	14.29±0.21 ns	6.30±0.17 ns	74.33±0.03 ns	3.42±0.02 ns
D50	14.27±0.21 ns	6.43±0.21 ns	74.25±0.05 ns	3.43±0.01 ns
D75	14.53±0.24 ns	6.34±0.18 ns	74.24±0.07 ns	3.43±0.02 ns
D100	14.26±0.28 ns	6.42±0.22 ns	74.26±0.07 ns	3.44±0.03 ns

Values are mean ± SE. Values with ns letters are not significantly different ( $P > 0.05$ ) in DMRT after one way ANOVA.

### Whole Body Composition

The whole-body composition of black molly from different dietary treatments of BSFL and control is presented in Table 5. Dietary replacements of FM protein with BSFLM did not show any significant differences in whole-body crude protein, crude lipid, ash content and moisture content of the selected fish.



**Fig. 1.** Survival percent of black molly, *P. sphenops* fed with five experimental diets. Values are mean ± SE. Bars with different letters are significantly different ( $P < 0.05$ ) using DMRT after one way ANOVA.

### Survival percentage

Survival percentage was varied from 88.89% to 98.89% (Fig.1.) and had no significant effect of dietary treatment where a higher mean value was recorded in the D50 diet fed set followed by the D75 set (95.56%) and a lower mean value in the Do set.

### Discussion

#### Water quality

Dissolved oxygen level in aquarium during fish rearing is crucial factor for fish health and growth (Patil *et al.*, 2019). For optimum growth of fishes, dissolved oxygen level should be maintained above 5mg/l (Lawrence 2007; Bahnasawy *et al.*, 2009).

In the present study, throughout the feeding trial, its levels were found higher than the maintenance value. Warm water fishes avoid water having free carbon dioxide level above 5mg/l (Boyd and Lichtkoppler, 1979), whereas in current study its values throughout the experimental period were below than that of the value. According to Mukhopadhyay *et al.*, (2003) the safe level of pH of water for freshwater fishes is 7 to 8.5. The pHs of water of experimental aquariums were within that mentioned reference range. Therefore, it could be affirmed that the water physicochemical parameters of the experimental aquariums were ideal for the rearing of black mollies.

Proper growth, health condition, normal behaviour, external appearance, colour of ornamental fishes mainly depends on the amount of protein present in fish diet. Therefore, before designing any feeding trial for searching fishmeal alternative, the dietary protein content should be considered first for getting the optimum fish growth (Guillaumee, 1997). According to Shim and Chua (1986) the diets of molly should have 30 to 40% protein for their suitable growth. Parker (2011) also stated that 35% to 40% dietary protein is necessary for optimum growth of molly. Keeping these opinions in mind, in the current study, crude protein percentage for all the formulated experimental diets were fixed to 40%.

The overall results of growth and survival asserted that insect meal could replace fishmeal efficiently when the suitable species-specific formulations of diets are available.

In the past decades, there is a rapid development of aquaculture practices, both in the food fish culture and ornamental fish culture (Kujawa *et al.*, 2010; Tlustý 2002). Recently, aquaculture researchers turn their efforts on ornamental fish culture and their rearing with suitable diets having novel protein sources to provide sufficient information on low-cost alternative aquarium-fish feed for their sustainable development. However, insects as alternatives for fishmeal in the diet of ornamental fish has been hardly investigated globally, although in case of food fish, sufficient relevant information are available

(Tran *et al.*, 2015; Biancarosa *et al.*, 2019; Motte *et al.*, 2019; Mapanao *et al.*, 2021; Tervora *et al.*, 2021; Yuan *et al.*, 2022).

Previous published research works suggested that 10% to 20% of lipid in fish feeds showed optimum growth performances without producing any adverse effect (Cowey and Sargent, 1979). In this regard, it was clear that the lipid content of the diets of the present feeding trial were within the mentioned range. According to NRC (1993), 8% fibre in fish feed is suitable for most fishes as it is indigestible. But the diets of the present study contain a little higher amount of fibre (near about 11.1% to 15.25%). Here, the diets' fibre content did not adversely affect the food utilization, growth of black molly though it was higher than the NRC (1993) mentioned value. Mollies are live bearer and their zero-day old fries smoothly consume formulated feed (Coad, 2017). Molly feeds on algae, some arthropods such as artemia, copepods, cladocerans, organic detritus and also insect larvae like mosquito larvae chironomus larvae in their natural habitats (Davis and Selvaraju, 2019; Sumithra *et al.*, 2014; Arunkumar *et al.*, 2019; Lawal *et al.*, 2012). Insects are natural diets of carnivorous and omnivorous fishes and are the main food items of their diets. In this context Kowalska *et al.* (2021) investigated that molly has a thrust for insect feed.

Although Black soldier flies are terrestrial insects, molly's natural feeding habit helped spontaneous intake of diets containing BSFLM which indicate that replacement of FM had no negative effect on palatability. Thus, inclusion of insects in fish diets would not affect their voluntary ingestion, on the contrary fishes will consume the insect-based diets as palatable one (Nogales-Merida *et al.*, 2019). Therefore, partial or complete FM replacement could be possible in the fish diet as insect meal were attractive to fishes. Though insect-based meals are attractive to fishes, they are unable to digest the chitin generally (Rust, 2002). But the presence of chitinase enzyme in the gastrointestinal tract of several fishes, enable them to digest insect meal (Rangaswamy, 2006). As the current study showed that statistically superior results of growth performances were

obtained from the diets with different level of BSFLM inclusions, confirmed that black molly can have the enzymes for digesting the insect meal.

For sustainable ornamental fish culture, requires an optimum percentage of FM replacement with BSFLM and this level of replacement depends on various factors like cultured fish species, culture conditions and the black soldier fly larvae rearing conditions and the ingredients used in fish diet preparation, and also their proportions and nutritional quality. But the values of replacement percentages of FM with BSFLM for a specific fish species are not sufficient to compare between the earlier investigations. For better comparison, it is necessary to have the data of inclusion amount of BSFLM along with the value of percentage of FM replacement. In this context, previous investigations provide conflicting reports. Few studies showed highest growth performance on 20% fishmeal replacement with the inclusion of 20gm BSFLM per 100gm of diet in Dusky kob fish (Madibana *et al.*, 2020). Whereas it was 25% with the inclusion of 20.20gm and 14.41gm BSFLM per 100gm of diet in African catfish and Tongue Sole respectively (Azri *et al.*, 2022; Li *et al.*, 2022). Rawski *et al.* (2020) and Aisyah *et al.* (2022) stated that the best result of growth was found in 30% fishmeal substitution with 30gm and 19gm of BSFLM incorporation in 100gm of diet in case of sturgeon and red hybrid tilapia respectively.

In juvenile Tench 45% (34.8gm in 100gm diet) and in yellow catfish 48% (22.3gm in 100gm diet) FM replacement with BSFLM was reported suitable for increased growth efficiency (Carral and Saez-Royuela, 2022; Xiao *et al.*, 2018). Several investigations showed that the suitable percentage of FM replacement is 50%, where the BSFLM inclusion amount is 18gm, 30.8gm, 15gm, 0.53gm, 20.04gm and 21gm in 100gm of diet for pikeperch, Asian sea, rainbow trout, bass, Jian carp, common carp and zebrafish respectively (Stejskal *et al.*, 2023; Katya *et al.* 2017; Caimi *et al.*, 2021; Li *et al.*, 2017; Azizal *et al.*, 2019; Zarantoniello *et al.*, 2018). Fewer studies stated that the optimum FM replacement percentage is 60% (20.10gm/100 gm) for gold fish (Kamalii *et*

*al.*, 2022) and 75% for African catfish, climbing perch (20.63gm and 37.5gm per 100gm of diet respectively) (Maranga *et al.*, 2022; Mapanao *et al.*, 2021). According to Nairuti *et al.* (2021) improved growth performance of Nile Tilapia was observed in complete FM replacement with BSFLM (29.3gm/100gm). In the present study with Black molly, significantly highest values of growth performances were obtained from 50% FM replacement with BSFLM (22.98% inclusion). Unsatisfactory results were obtained from D100 diet fed set might be because of black mollies cannot tolerate a diet with complete replacement of FM with BSFLM.

Along with the estimation of growth performances and food utilization, the evaluation of survival percentage is also crucial for any feeding trial. Survival percentages of black molly were found similar in different dietary treatments in the present study. Hence, the study confirmed that survival seems to be not affected by the different level of BSFLM inclusion in experimental diets. This study result was supported by prior reports on other fishes such as on Nile Tilapia, African catfish, common carp where dietary inclusion of BSFLM had no effect on their survival (Tippayadara *et al.*, 2021; Maranga *et al.*, 2022; Azizah *et al.*, 2019). There is no published data on survival rate of molly when BSFLM were added in their diets. Yet, Adil *et al.* (2014) reported that complete FM replacement with insect meal did not affect the survival of molly. In this regard, Kowalska *et al.* (2021) stated that molly showed a similar survival rate in superworm (*Zophobas morio*) and commercial diet. Several reports on molly demonstrated that molly can be reared in different formulated feeds without having any negative impact on survival (Harpaz *et al.*, 2005; Uribe *et al.*, 2018). Overall, BSFLM has the potential to replace the fishmeal in fish diet at a high level, giving satisfactory results of growth and survival without any adverse effect.

## Conclusion

From the results of the present study, it could be concluded that 50% FM protein replacement corresponding to 22.98gm of BSFLM inclusion per 100 gm of diet is the optimum level of FM

substitution, where significantly better growth performances were observed than the other dietary treatment groups. Moreover, 75% of FM protein could be replaced by 34.47% of BSFLM, without compromising the growth and survival of black molly, when comparing with control. Hence, BSFLM should be considered as a good protein source for black molly from the early stages.

But the insect meals are expensive than the other animal protein sources (Morales-Ramos *et al.*, 2020) as nutritionally rich insects are not mass reared in farms due to these insects are less popular as alternative animal protein sources. Under this consideration, in future, similar researches with various insects on several other ornamental fishes are necessary that will emphasize the importance of insect farms and these farms will continuously supply protein rich insects to the aqua-feed industries for producing insect protein-based diets for ornamental fishes, making the industries viable and sustainable. It would be expected that the overexploitation of fishmeal will be reduced through inclusion of insect meals in aqua-feed and the barrier of high-cost of insect meals will be overcome.

### Recommendation

In future, similar studies on feeding trial with various proportions of BSFLM in diets of several other ornamental fishes are necessary.

### Conflict of interest

The author declares no conflict of interest.

### Acknowledgements

I am sincerely thankful to the Principal of Vidyasagar College, Dr. Gautam Kundu and the Head of the department of Zoology, Dr. Ankur Bhawal for providing necessary laboratory facilities.

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