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Effect of Fishmeal Replacement with Mealworm (*Tenebrio molitor*) Meal on Growth Performance and Survival of the Black Molly (*Poecilia sphenops*)

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ABSTRACT

In order to search of viable alternative protein source for aquaculture, a feeding trial of six-months was carried out to test the effect of mealworm (*Tenebrio molitor*) meal relacing fishmeal protein at 0% (control), 25%, 50%, 75% and 100% in practical diets (named D0, D25, D50, D75 and D100 respectively) on the growth performance, feed utilization and survival of black molly (*Poecilia sphenops*). At the end of six-months, significantly higher final weight and length were observed in D75 diet fed set followed by D50 set and then in D0 set, whereas lowest value was recorded in D100. The specific growth rate (SGR) in percent of control fishes (D0) was 2.25±0.02, whereas the result of SGR (%) was noted best in D75 set (2.44±0.02) and poorest in the D100 (1.97±0.02). Although D75 diet fed set showed lower feed conversion ratio (FCR), black molly fed with D25 and D50 diets still utilized the diets as well as control (D0) as their values did not vary significantly. Significantly higher survival percentage was 96.67%, observed in D75 set, in contrast lowest found in D100 set (64.44%). Hence, mealworm could be successfully incorporated by replacing fishmeal from 50% to 75% in the diet of black molly with optimal growth, feed utilization and better survival.

Keywords Black molly, Fishmeal, Growth efficiency, Mealworm, Survival rate.

INTRODUCTION

Ornamental fish keeping as home decor is becoming the world's second most well-liked and stress relieving hobby in recent times (NFDB 2015). This is the prime cause of their continuous ever-increasing demand in the global ornamental fish trade. *Poecilia sphenops* is an omnivorus freshwater ornamental fish, commonly known as black molly. It is one of the popular aquarium ornamental fishes because of their attractive shiny black color, easy maintenance, ability to tolerate noticeable environmental variations and moreover, of their sturdiness and stability (Pai *et al.* 2016).

Export of ornamental fish played an influential role in a country's economy (Ahilan *et al.* 2010). India exports 0.3% of global ornamental fish (Sumithra *et al.* 2014). About 90% of export from India are caught from diverse natural water bodies (Silas *et al.* 2011). For long term sustainability of export industry of ornamental fishes, it is important to culture them in fish farm. The main obstacle in the industrial development of an ornamental fish culture is the lack of cost-effective protein rich sustainable fish feed. Now,

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aquaculture species production demands the use of fishmeal alternative due to their high cost and scarcity (Sumithra *et al.* 2014).

Therefore, it is necessary to concentrate on finding a potentially sustainable, protein rich, low-cost alternative for fishmeal. Over the last ten years, an interest of incorporating insects as protein source in fish feed production is arising (FAO 2014). Insects seem to be a rich and promising fishmeal substitute for fish feed due to their shorter production time, outstanding production efficiency, rich in nutrients (Khan *et al.* 2018, Van-Huis and Oonincx 2017) and their use has a negligible ecological impact. The numbers of edible insects are approximately 1500 across 113 countries (Ravzanaadii *et al.* 2012). Recent scientific studies have revealed the feasibility and efficacy of incorporating insect meal in fish feed, specifically total or partial replacement of fishmeal protein, for many fish species (Sánchez-muros *et al.* 2014, Mastoraki *et al.* 2020, Barroso *et al.* 2014, Henry *et al.* 2015). In spite of a good number of research papers, there is a need for further research to evaluate the optimum level of fishmeal substitution with insect meal, without hampering their growth performance, feed utilization and survival rate, which is species specific and dependent on fish age and their nutritional requirement and also on insects' nutritional value.

In the ornamental fish culture, mealworm, larval stages of darkling beetles (*Tenebrio molitor*), belongs to Tenebrionidae family, could be used as an alternative non-conventional unexplored protein source since they are easy to mass rear (Liu *et al.* 2020), nutrient rich (Khan 2018) and have commercial value (Grau *et al.* 2017). Mealworm have the potential of replacing conventional fishmeal as it has high protein content with an adequate amino acid profile (Hussain *et al.* 2017, De-Marco *et al.* 2015), unsaturated fatty acids (linoleic and linolenic), vitamins and minerals (Finke and Oonincx 2017, Makkar *et al.* 2014, Nowak *et al.* 2016, Smetana *et al.* 2016). So, mealworm can satisfyingly serve as a complete or partial substitute for high-priced fishmeal in fish feed. That will lower the production cost of fish in the aqua-industry. Moreover, it is reported that in a formulated diet when mealworm is added in a proper amount, it would able to promote fish growth performance and also boost their immunity by making them resistance to invading pathogens (Su *et al.* 2017, Sankian *et al.* 2018, Ido *et al.* 2019). Previous investigation revealed that mealworms could be added effectively as a partial fishmeal substitute in fish feed for fresh water and marine fishes (Makkar *et al.* 2014). Mealworm was also used in practical diet for European sea bass (Gasco *et al.* 2016, Henry *et al.* 2018), red sea bass (Ido *et al.* 2019), rainbow trout (Gasco *et al.* 2014, Jeong *et al.* 2020) and common catfish (Ng *et al.* 2001, Roncarati *et al.* 2015).

Therefore, the current work aimed to formulate a sustainable feed containing suitable portion of mealworm meal replacing fishmeal protein for black molly. With these, a feeding trial was conducted which specifically focused to investigate the effects of dietary replacements of fishmeal protein with mealworm meal in the diet of black molly, on their growth performance, feed utilization and survival rate.

MATERIALS AND METHODS

Experimental location and experimental fish

A feeding trial was conducted and maintained in the laboratory of Department of Zoology, Vidyasagar College, West Bengal, located in India. For the study 500 juveniles of black molly of uniform size with average weight 0.0123±0.002 gm and average length 1.3±0.022 cm, were purchased from a commercial local ornamental fish market during the month of April, 2023. Then fishes were kept in a glass aquarium (36"×12"×12") for 15 days in the laboratory condition for acclimatization. For the experiment of six months, fishes were randomly chosen and five experimental sets in glass aquariums (18"×12"×12") were made ready with three replications following a Completely Randomized Design (CRD). Each set was with 30 fishes. Diets were given twice a day in the morning and evening, to the fishes, at the amount of 5% of body weight per day.

To remove the extra feed particles and metabolic wastes from the water of aquariums, the water was siphoned off and was again kept in the respective aquariums, done twice in a day. Total water exchange was done twice a week.

Experimental diet

Dried mealworm larvae used to prepare diets, was purchased from nearby local pet market (Kolkata, India) and oven-dried at 40^{O}C for 72 hrs in the laboratory, and then ground into powder form using an electric grinder. It was kept in airtight zipper plastic bags until further use in preparing experimental diets for the feeding trial and analysis.

Five experimental diets were formulated with 40% protein to meet the nutritional requirements of black molly. A control (D0) without mealworm, and four diets in which fishmeal protein was replaced with mealworm meal at 25% (D25), 50% (D50), 75% (D75) and 100% (D100) were formulated. All the ingredients were finely ground into powder form and then well mixed, and pelleted by pelletizer through 1.5 mm die. The pellets are oven-dried at 40^{O}C for 24 hrs and kept at -20^{O}C in airtight bags until used.

Proximate composition analysis

Proximate composition of mealworms and the prepared experimental diets were carried out to assess the crude protein, crude lipid, crude fiber, moisture, ash contents of the samples as per the stipulated methods (AOAC 2006).

Growth efficiency

At the end of the six-months experimental period, final weight and length of fishes were recorded and the growth efficiency indices like weight gain, specific growth rate (SGR), total food consumption and food conversion ratio (FCR) were calculated.

Water quality analysis

Physico-chemical parameters of water such as temperature, dissolved oxygen, pH, free carbon dioxide, hardness, alkalinity and total dissolved solids (TDS) of experimental aquariums were analyzed and noted during the experimental period using standard methods (APHA 1989).

Statistical analysis

Data were presented in tables and figures as mean ± SE of three replications. One way analysis of variance (ANOVA) was done for all the data and then Duncan's multiple range tests (DMRT) were carried out for each case to separate the mean values according to significance.

RESULTS AND DISCUSSION

Water quality parameters

Table 1 presented the data-range of water physico-chemical parameters of all the experimental aquariums. The high value of water hardness and TDS of ground water of experimental area used in the aquariums of the experiment, did not exhibit any adverse effect on growth efficiency parameters and survival percentage of black molly during the acclimatization period. This finding was affirmed by the previous investigation, where it was observed that adaptability of ornamental fishes was very high and they can survive in water with a wide range of hardness and TDS (Roncarati *et al.* 2015).

Nutrient composition of primary protein sources of experimental diets

Proximate compositions of mealworm meal and fishmeal were listed in Table 1. Crude protein percentage of mealworm meal was comparable to the fishmeal protein percentage, though significantly varies. Crude lipid and crude fiber content of mealworm meal was slightly higher than that of the fishmeal.

The proximate compositions of all the experimental diets of black molly were similar and the protein content ranged from 39.98% to 40.27%. (Table 2). The values of crude lipid, ash and crude fiber

Table 1. Physico-chemical water quality parameters of experimental aquarium sets during the experiment.

Parameter	Minimum	Maximum
Temperature (^{O}C)	26	33
Dissolved oxygen (ppm)	8.1	8.4
pH	7.2	7.4
Free carbondioxide (ppm)	1.45	2.16
Hardness (ppm)	590	660
Alkalinity (ppm)	320	336
TDS (ppm)	920	1085

Table 2. Proximate nutritional composition of mealworms meal and fishmeal (%, dry weight basis). *Data are presented as mean ± SE, values with different letters are significantly different ($p<0.05$) using DMRT.

Nutritional composition (%)	Meal worm meal	Fishmeal
Crude protein	49.00±0.75[a]	52.00±0.95[b]
Crude lipid	17.73±0.79[b]	13.10±1.55[a]
Crude fiber	7.22±0.33[b]	5.06±0.55[a]
Ash	4.64±0.07[a]	13.01±1.20[b]
Moisture	5.90±0.06[a]	19.10±1.12[b]
NFE	21.40±0.89[b]	16.84±2.30[a]

content of analytic composition of the experimental diets, as presented in Table 3, were as expected as the level of replacement of fishmeal with meal worm meal in the formulated diets.

Growth performance

The growth efficiency parameters of the black molly at the end of the experiment are shown in Table 4. At the start of feeding trial, initial length and initial weight varied from 1.25 cm to 1.35 cm and 0.0118 gm to 0.0127 gm respectively. At the end of the 6-months experimental period, the significantly highest values of final length and final weight and weight gain were noted in the D75 set, followed by the D50 set and the lowest in the D100 set, whereas the results of both the parameters of control (D0) were higher than the D25 set. The data of SGR (%) showed that the fishes provided with Diet D75 had a significantly higher value and D100 had a lower value, but D50 had a better value when comparing with control. The result of feed utilization showed that there had been a positive impact by all the diets on the experimental diets. In the case of food consumption, the highest amount of food eaten by fishes was in the D75 set followed by the D50 and then in the D0 set. Though the best FCR was noted in the D75 diet containing mealworm at 75% replacement of fishmeal, the significantly highest value of FCR was found in the D100 set and the data of the D25, D50 and control (D0) did not vary significantly.

In this study, molly fishes from the D75 set showed a higher value for weight gain, total food consumption and SGR (%) followed by the D50, D0, D25, D100 sets (Table 4). It clearly indicated that the values of growth efficiency parameters increased with the increased level of food consumption. The results supported the observation of Johnston *et al.* (2003) and Sapkale *et al.* (2017) where it was found that higher growth was obtained with increased food consumption by *X. maculatus*. Higher consumption of diets corresponded with higher weight gain and higher growth of selected fish (Rad *et al.* 2003).

Table 3. Ingredients, their proportion and proximate composition of experimental diets. *MOC- mustard oil cake, *Vitamin-Mineral Premix (mg/kg diet): Vitamin A 1000 µg, Vitamin B1 1.8 mg, Vitamin B2 2.5 mg, Vitamin B3 18 mg, Vitamin B5 3 mg, Vitamin B6 2.4 mg, Vitamin B12. 2 µg, Vitamin C 80 mg, Vitamin D3 5 µg, Vitamin E 10 mg, Biotin 30 µg, Zinc 17 mg, Magnesium 250 µg, Manganese 3 mg, Iodine 140 µg, Copper 30 µg, Selenium 40 µg, Chromium 50 µg, Folic acid 150 µg.

Diet ingredients (gm/100 gm)	D0	D25	D50	D75	D100
Fishmeal	48	36	24	12	0
Mealworm	0	12.73	25.47	38.2	50.94
Soybean	21	21	21	21	21
Corn	7	7	7	7	7
MOC	14.5	14.5	14.5	14.5	14.5
Rice bran	3.23	2.79	2.25	0.87	3.74
Wheat	4.27	3.98	3.78	4.43	0.82
Vitamin-mineral premix	2	2	2	2	2
Total	100	100	100	100	100
Crude protein	40.27	40.19	40.11	40.02	39.98
Crude lipid	14.1	14.2	14.5	14.8	15.2
Ash	12.25	11.3	10.25	9.3	8.1
Crude fiber	9.25	10.75	11.4	13.5	13.7
NFE	24.13	23.56	23.74	22.38	23.02

Table 4. Growth parameters of black molly, *Poecilia sphenops* fed with different mealworm diets in six months feeding trial. *Values are mean ± SE, values with different letters are significantly different (p<0.05) using DMRT.

Growth parameters	Diets				
	D0	D25	D50	D75	D100
Initial length (cm)	1.300±0.029[a]	1.267±0.017[a]	1.317±0.017[a]	1.300±0.029[a]	1.317±0.017[a]
Final length (cm)	4.183±0.044[c]	3.867±0.033[b]	4.383±0.034[d]	4.750±0.029[e]	3.450±0.029[a]
Initial weight (gm)	0.0123±0.0002[a]	0.0123±0.0003[a]	0.0123±0.0002[a]	0.0123±0.0003[a]	0.0123±0.0002[a]
Final weight (gm)	0.702±0.029[c]	0.575±0.022[b]	0.825±0.021[d]	0.995±0.006[e]	0.427±0.022[a]
Weight gain (gm)	0.690±0.029[c]	0.562±0.022[b]	0.813±0.021[d]	0.983±0.007[e]	0.414±0.022[a]
SGR (%)	2.25±0.02[c]	2.14±0.01[b]	2.34±0.01[d]	2.44±0.02[e]	1.97±0.02[a]
Total food consumption (gm)	0.818±0.040[c]	0.653±0.015[b]	0.948±0.036[d]	1.007±0.016[e]	0.602±0.013[a]
FCR	1.19±0.02[b]	1.16±0.03[b]	1.17±0.02[b]	1.02±0.01[a]	1.46±0.11[c]

In case of the amount of fishmeal replacement with mealworm meal in the fish diet, the present study confirmed the results of the previous study of Su *et al.* (2017) who reported that up to 75% inclusion of mealworm meal in the yellow catfish diet, showed better results in growth performance in terms of food intake, SGR (%) and FCR. Fishmeal replacement at the level of 80% with mealworm in Catfish (*Clarias gariepinus*) diets, displayed better growth and feed utilization (Ng *et al.* 2001). In the current study, 100% replacement of fishmeal with mealworm meal showed significantly poorest results for weight gain, food intake, SGR (%) and FCR. A similar result was found by Mazlum *et al.* (2021) in narrow-clawed crayfish juveniles and Redman *et al.* (2019) in black sea bass where in 100% fishmeal replacement, fishes showed disinterest in feeding and consumed significantly less amounts of feed that led to significantly poorest result for growth performance.

However, in the case of rainbow trout, when fed diet with 100% replacement of fishmeal with mealworm meal, showed significantly best results were shown in final body weight, food intake, SGR (%) and FCR (Saravanan *et al.* 2015). A similar result was noted in the case of Red Seabream (*Pargus major*) by Ido *et al.* (2019). In the present study, 100% replacement showed significantly decreased growth performance. Jeong *et al.* (2022) reported that in black porgi (*Acanthopagrus schlegelii*), fishmeal could be replaced with meal worm meal up to 60%, which was supported by Harsij *et al.* (2019), Antonopoulou *et al.* and Basto *et al.* (2019) in case of rainbow trout, gilthead sea bream (*Sparus aurata*), European sea bass (*Dicentrarchus labrax*) respectively. Few studies depicted that meal worm meal could replace up to 50% of fishmeal in fish feed for rainbow trout (Belforti *et al.* 2015), black spot seabream (Iaconisi *et al.* 2017), Asian seabass (Alghada *et al.* 2022). Though, in the earlier reports the level of fishmeal substitution by mealworm meal for better food utilization of fishes, varied greatly depending on the chosen fish species, in this study significantly highest FCR of black molly was noted in 75% replacement of fishmeal which was also found by Valipour *et al.* (2019).

Survival percentage

There was a positive effect of mealworm meal on survival percentage which ranged from 64.44% to 96.67%. Black molly fed with the D75 diet showed a significantly higher survival percentage followed by the D50 set (91.11%) and was lower in the D100 (Fig. 1). The survival percentage of the D25 diet fed fishes was higher than control (D0).

In the study, 100% fishmeal replacement with mealworm meal showed the lowest survival per-

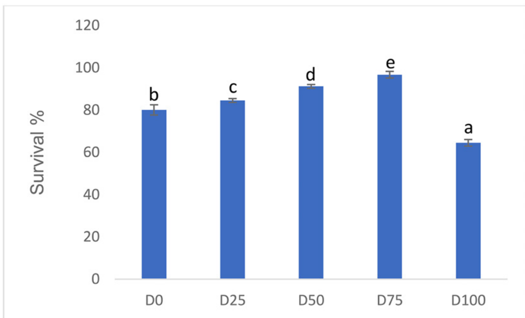

Fig. 1. Survival percent of black molly, *Poecilia sphenops* fed with five experimental diets. Values are mean ± SE, Bars with different letters are significantly different (p<0.05) using DMRT.

centage. This result confirmed the results of Ido *et al.* (2019) in red seabream, but opposed the results of Mazlum *et al.* (2021) in narrow-clawed crayfish juveniles, where survival percentage was significantly higher in 100% fishmeal replacement set. In case of rainbow trout and European seabass, higher survival percentages were obtained from 50% fishmeal replacement with mealworm meal (Ennayer *et al.* 2022, Belforti *et al.* 2015), whereas in African catfish the best value of survival percentage was found in 60% fishmeal replacement set.

In the past decades, several related studies carried out in different fishes to shed light on the feasibility and efficiency of mealworm dietary meal in aquaculture, revealed that dietary mealworm meal dose does not affect growth performance negatively (Mastoraki *et al.* 2020, Sankian *et al*. 2018, Belforti *et al.* 2015, Iaconisi *et al*. 2017, Choi *et al.* 2018, Feng *et al.* 2018, Panini *et al.* 2017, Song *et al.* 2018, Tubin *et al.* 2020 Piccolo *et al.* 2014, Rema *et al.* 2019). Several feeding trials with different fishes provide conflicting reports about the proper fishmeal replacement level of mealworm meal that supports best growth performance. Few studies recommends that the highest level of replacement of fishmeal with mealworm meal could not be more than 25–30% (El-Ouny *et al.* 2023), whereas other noticed excellent results at more than 80% replacement (Ido *et al.* 2019). The suitable level of fishmeal replacement with meal worm meal might be species specific (Osimani *et al.* 2016, Ng *et al.* 2001).

CONCLUSION

Overall, this current study suggests that black molly has excellent ability to efficiently utilize mealworm meal at 75% fishmeal replacement, with significantly improved growth efficiency with higher survival percentage. Hence it can be concluded that fishmeal could be replaced up to 75% with the mealworm meal without any adverse effect on growth performance and survival rate of black molly. Even 50% fishmeal replacement showed better results when compared with the control.

Therefore, the present feeding trial with black molly, elucidate that mealworm larvae are not only a promising alternative animal protein source for aquaculture, but also have good potential in replacing fishmeal as functional ingredient in fish feed. Moreover, mealworm is easy to mass rear on agricultural byproducts and will be a low-cost fishmeal substitute, it has a nutrient profile comparable to fishmeal, could be recommended as a candidate ingredient for aquafeed production.

However, earlier reports on fishmeal replacement with meal worm meal for fish feed varied greatly, therefore the observations in this study might be species-specific. More research and a detailed study, especially long-term feeding trials, on growth performance on other aquarium ornamental fishes are still needed to find the optimum fishmeal substitution levels for specific species which will be suitable for their maximum growth performance and survival rate.

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