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Effect of four insect meals on the growth performance, survival, total carotenoid content and body composition of an ornamental fish *Xiphophorus helleri* (Poeciliidae)

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Abstract
In order to find alternative sustainable protein sources for ornamental fish culture, a six-months feeding trial was conducted to evaluate the effect of four insect meals (grasshopper, cricket, mealworm and black soldier fly larvae) and commercial meal (control) on the growth performance, food utilization, survival, total carotenoid deposition in skin and muscle tissue, and whole-body composition of swordtail, *Xiphophorus helleri*. A total number of 450 juveniles of uniform size (0.04±0.001 gm) were divided 15 glass aquariums having five experimental dietary sets with three replicates and each aquarium was stocked with 30 juveniles of swordtail. The result of proximate composition of diets revealed that protein content in percent was significantly higher in black soldier fly larvae meal (BSFLM) (52.80±0.50%), followed by mealworm meal (MWM) (45.06±0.53%) and lower in cricket meal (35.67±0.88%). Final weight and length were found significantly higher in MWM (3.45±0.13 gm and 6.62±0.09 cm), followed by BSFLM (2.84±0.12 gm and 6.08±0.06 cm), whereas lower in cricket meal (1.07±0.03 gm and 4.40±0.08 cm). The maximum value of specific growth rate (SGR) in percent and weight gain were obtained from MWM fed set (2.48±0.01% and 3.41±0.13gm) and a lower value was noted in cricket meal fed set (1.84±0.02% and 1.03±0.03gm). Feed conversion ratio (FCR) of the control fish (commercial meal) was 2.31±0.06 whereas the best FCR was found in fishes of MWM fed set (3.01±0.08) and the poorest in cricket meal fed set (2.87±0.03). Survival percentage was ranged from 98.33% to 70% and a higher mean value was observed in MWM fed set and significantly lower in cricket meal fed set. Total carotenoid content in fish skin and muscle tissue was significantly higher (10.53±0.26μg/g) in MWM set and lower in commercial meal fed set (0.54±0.03), whereas a good value was noted in the BSFLM (8.41±0.21μg/g) set. No effect of different dietary treatments on whole-body composition were found. Present study revealed that insect meals were better than commercial meal and among the four insect meals, MWM could significantly enhance growth, feed utilization, survival and skin pigmentation of swordtail.

Keywords: black soldier fly larvae meal, cricket meal, growth performance, grasshopper, mealworm meal, survival, *Xiphophorus helleri*

Introduction

In aquaculture industry, ornamental fish culture is becoming an economic and most profitable sector in Asian countries (Absali and Mohamad, 2010). Swordtail, *Xiphophorus helleri* is one of the popular, high-demanded aquarium fish in ornamental fisheries due to their variety of colours, fin patterns and their sturdiness, stability in environmental and water quality variations, and so relatively easy maintenance (Ghosh *et al*., 2008) [25].

Attractive colouration of ornamental fishes comes from their skin pigmentation. Carotenoids, natural lipid soluble pigments which are not synthesized by fishes de novo (Higuera-Clapara *et al*., 2006) [28], absorb their requirement from their dietary sources (Duru, 2014) [21] like phytoplankton and zooplankton from their habitat, are responsible for fish skin pigmentation. Hence, to increase the commercial value of ornamental fishes, it is required to incorporate carotenoid through dietary supplementation (Bell *et al*., 2019) [7] as carotenoid-deficient diets showed faded colouration in ornamental fishes (Sinha and Asimi, 2007) [49]. Commonly used synthetic carotenoids such as astaxanthin (red) and canthaxanthin (orange-red) (Dananjaya *et al*., 2019) [13] are expensive and their inclusion in fish diets accelerate the feed cost to 10% to 20% (De la Mora *et al*., 2006) [20]. Exploring natural carotenoid sources for the ornamental fish feed producers, is increasingly urgent (Gumus *et al*., 2022) [26]. Though, several prior studies on the natural cost-effective plant-based carotenoid sources (Kaur and Shah, 2017) [31] were conducted, but there is a lacunae of research article on animal-based carotenoid sources. Some arthropods like krill, shrimps, artemia, lobster, copepods, water fleas, are able to deposit excellent amount of carotenoid in their body (Roncarati *et al*., 2016; Pattanaik *et al*., 2021; Seidgar *et al*., 2019; Bell *et al*., 2016; Duru, 2014) [38, 42, 46, 21].

In ornamental fish culture, mostly commercial feed and live feed such as Zooplankton and phytoplankton are used as feed (Anjur, 2017) [3]. In most times, live feed is the cause of spreading harmful protozoan diseases in ornamental fishes (Debnath *et al*., 2022) that lead to health hazard issues and affect their growth, survival and colouration (Shu-chien *et al*., 2004) [47]. Now, commercial ornamental fish producers are relying on commercial feed which is highly expensive due to high-demanded high-cost fishmeal, which is the primary protein source of fish feed. Fish protein is a human consumable protein, when fed to ornamental fishes reflect that it is being converted to non-consumable luxury items (Tlusty, 2002). Overexploitation of fish harms fish population and also aquatic ecosystem (Kowalska *et al*., 2021) [32]. Therefore, in fish feed, fishmeal usage is no longer be sustainable as its production is decreased day-by-day.

In this scenario, sustainable, alternative, novel, cost-effective, available and carotenoid-rich animal protein sources should be explored. One such potential alternatives are insect meals as they contain good quality protein with other nutrients (Nogales-Merida *et al*., 2019) [37]. Moreover, the amino-acid profile of insect meals is comparable with fishmeal (Sanchez-Muros et a., 2014). In addition, insects can be mass reared sustainably and economically (Wang *et al*., 2012; Das *et al*., 2012a) [53, 15] in a small space (Das *et al*., 2009) [17] on organic waste (Kim *et al*., 2021) or fed with grasses (Das *et al*., 2012b) [16]. A great deal of attention has recently been paid to some most common insect species (Gasco *et al*., 2019) [23] such as grasshopper (Das and Mandal, 2014) [18], cricket (Perera and Bhujel, 2022; Das, 2023) [14], mealworm (Das, 2023) [14], superworm (Rumbos *et al*., 2021) [43], waxworms (Finke, 2015) [22], silkworm pupae meal (Raja *et al*., 2019b) [41], black soldier fly larvae (Stejskal *et al*., 2022), blowfly maggot meal (Sing *et al*., 2014) [48] and housefly larvae (Chemello *et al*., 2020) [9] as their nutritional estimations proved that these insects could be an efficient and high-quality alternative protein for the carnivorous and omnivorous fishes (Alfiko *et al*., 2022) [2] as these fishes naturally consume aquatic arthropods (e.g. crustaceans like krill, shrimps, artemia, lobster, copepods, water fleas) from their habitat (Roncarati *et al*., 2016; Pattanaik *et al*., 2021; Seidgar *et al*., 2019; Bell *et al*., 2016; Hekimoglu, 2005; Duru, 2014) [38, 42, 46, 27, 21].

In earlier studies, several insects were successfully included partially or completely in fish diets to investigate their potential as fishmeal alternative without compromising their growth, survival and skin pigmentation (Das, 2023; Kamalii *et al*., 2023) [14]. Most of the studies were conducted on food fishes [e.g. for rainbow trout (Chemello *et al*., 2020) [9], Atlantic salmon (Belghit *et al*., 2019), minor carp (Xu *et al*., 2020), common carp (Azizah *et al*., 2019) [6], Grass carp (Li *et al*., 2022) [33], Nile tilapia (Perez-Pacheco *et al*., 2022) [40], pikeperch (Stejskal *et al*., 2022), climbing perch (Mapanao *et al*., 2021) [36], Common catfish (Roncarati *et al*., 2015), and African catfish (Gebremichael *et al*., 2023) [24]. A limited studies were carried out on the influence of insect meals on the ornamental fishes [e.g. for Goldfish (Kamalii *et al*., 2023) and Platy fish (Das, 2023) [14]] and no reports have yet been published on the impact of insect meal on growth, survival and skin colouration in the swordtail, *X. helleri*.

Therefore, the present study aimed to evaluate the effect of four insect meals and commercial feed on the growth performance, feed utilization, survival, whole-body composition, carotenoid concentration in the skin and muscle tissue of swordtail, *X. helleri.*

Material and Methods

Experimental site and experimental fish

The feeding trial was conducted using 450 juveniles of swordtail with an average initial weight of 0.04±0.001 gm. Fishes were purchased from a local ornamental fish market. Prior to start the experiment, fishes were acclimatized to the laboratory facilities and conditions in a glass aquarium (36"×12"×12") with continuous aeration, for two weeks, fed on a commercial diet. After the acclimatization period, juveniles were randomly captured and distributed in 15 experimental glass aquariums (18"×12"×12") at the rate of 30 fishes per aquarium. The 15 aquariums were grouped into five sets with three aquariums in each set. The fishes were fed twice daily (09:00 and 18:00), at the amount of 3% of body weight per day. According to the weight of the fishes, the diets were adjusted at 15 days intervals.

The extra uneaten feed particles were collected, dried and weighted to find out the amount of diet consumed daily. Aquariums were cleaned properly by scrubber and the total water of the aquarium was exchanged twice a week.

Experimental diet

Commercial meal and dried grasshoppers, crickets, mealworm and black soldier fly larvae were purchased from local pet shop. These insects were oven-dried at 40^{O}C for 48 hours. The dried specimens were manually crumbled by mortar and pestle and kept in airtight glass containers until further use in feeding trial and analysis.

Proximate Composition Analysis of experimental meals and whole-body of fish

Proximate analysis of experimental meals and fish tissue from the different experimental sets were carried out to assess the crude protein, crude lipid, fibre, ash and moisture content of the samples as per the stipulated methods (AOAC, 2006) [4].

Growth study

At the end of the six-months feeding trial, the fishes were counted in each aquarium and starved for 24 hours. Then final weight and final length of the fishes were noted and the growth performance parameters such as weight gain, specific growth rate (SGR%), and food utilization parameters in terms of total food consumption and feed conversion ratio (FCR) were calculated.

Estimation of carotenoid

Carotenoid content of experimental feed samples were estimated by Cyanotech (2002) [12] method. At the end of the feeding trial, coloured region of skin and muscle tissue of fishes from five experimental sets were collected to measure the total carotenoid content following the method of Tiewsoh *et al*. (2019) [51].

Water Quality Analysis

Water quality parameters from all the experimental aquariums were analysed twice a week as per standard methods (APHA, 1989) [5].

Statistical Analysis

All data were presented in the text as mean ± standard error (SE). Data were subjected to one way analysis of variance (ANOVA) followed by Duncan's multiple range tests (DMRT) for each case to separate the mean values according to significance. Significant was considered at $P < 0.05$.

Results and Discussion

Water quality parameters

In water quality parameters, the range of water temperature was 26^{O}C to 39^{O}C, pH of water was 7.4 to 7.8, dissolved oxygen was 7.8ppm to 8.2ppm, free carbon dioxide was 1.28ppm to 2.34ppm, total hardness was 194ppm to 212ppm and TDS was 148ppm to 172ppm.

Proximate Composition of Experimental Meals

The proximate nutritional composition of commercial meal, cricket meal, grasshopper meal, MWM and BSFLM diets were listed in Table 1. Crude protein percentage was significantly higher in BSFLM, followed by MWM, and then in grasshopper meal. Its value was significantly lower in cricket meal, whereas crude protein content of commercial meal was found better than that of cricket meal. Crude fat content was noted significantly higher in BSFLM, whereas lower in commercial meal. A higher value of crude fibre content was observed in grasshopper meal and a lower value in cricket meal. Moisture content of commercial meal was significantly higher and it was lower in MWM.

Table 1: Proximate nutritional composition of commercial meal, cricket meal, grasshopper meal, MWM and BSFLM.

Nutritional composition (%)	Commercial meal	Cricket meal	Grasshopper meal	Mealworm meal	BSFLM
Crude Protein	37.83±0.44b	35.67±0.88a	42.29±0.13c	45.06±0.53d	52.80±0.50e
Crude fat	3.60±0.26a	4.63±0.19b	5.82±0.11c	15.30±0.06d	17.57±0.25e
Crude fibre	4.00±0.10b	2.90±0.12a	11.18±0.47e	6.52±0.15c	8.95±0.19d
Ash content	8.70±0.15d	5.95±0.13b	6.34±0.08c	4.64±0.08a	13.60±0.14e
Moisture	12.43±0.38e	6.87±0.22b	10.98±0.42d	5.63±0.20a	7.68±0.30c

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

Growth Performance

The growth performance parameters of the swordtail at the end of the six-months experimental period are presented in table 2. At the start of feeding trial, the initial length and the initial weight varied from 1.30 cm to 1.33 cm and 0.039 gm to 0.041 gm respectively. At the end of the experimental period, the significantly highest values of final length, final weight and weight gain were noted in the MWM fed sets followed by the BSFLM fed sets and the lowest in the cricket meal fed sets, whereas the results of the parameters of control (commercial meal) were higher than the cricket meal set. The data of SGR followed a similar trend of results, where a higher value was found in BSFLM set and a lower value in cricket meal set.

The results of feed utilization depicted that there had been a positive effect by all the insect meals except the cricket meal one on the experimental swordtail. In the case of food consumption, the highest amount of food consumed by the fishes of MWM sets, followed by the fishes of BSFLM sets then of grasshopper meal sets, and the least amount of food was eaten by the swordtail of cricket meal set. Though the best FCR was obtained from the BSFLM sets, the significantly highest value of FCR was found in the cricket meal set.

Table 2: Growth parameters of swordtail, *X. helleri* fed with five experimental diets in six months feeding trial

Growth parameters	Commercial meal	Cricket meal	Grasshopper meal	MWM	BSFLM
Initial weight (gm)	0.041±0.001a	0.039±0.001a	0.04±0.001a	0.04±0.002a	0.041±0.001a
Initial length (cm)	1.30±0.03a	1.32±0.02a	1.30±0.00a	1.32±0.04a	1.33±0.06a
Final weight (gm)	1.53±0.07b	1.07±0.03a	2.06±0.04c	3.45±0.13e	2.84±0.12d
Final length (cm)	5.12±0.07b	4.40±0.08a	5.68±0.04c	6.62±0.09e	6.08±0.06d
Weight gain (gm)	1.49±0.07b	1.03±0.03a	2.02±0.04c	3.41±0.13e	2.80±0.12d
SGR (%)	2.01±0.02b	1.84±0.02a	2.18±0.02c	2.48±0.01e	2.36±0.01d
Total food consumed (gm)	3.43±0.07b	2.97±0.06a	3.85±0.05c	4.43±0.05e	4.17±0.05d
FCR	2.31±0.06d	2.87±0.03e	1.91±0.01c	1.30±0.03a	1.49±0.05b

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

Survival percentage

Survival percentage was found better in all the insect meal fed sets except the cricket meal set when compared with the control. The value of survival percentage was varied from 98.33% to 70% (Figure 1). Swordtail fed with the MWM showed a significantly higher survival percentage followed by the grasshopper meal set (86.67%) and was lower in the cricket meal fed sets (Figure 1). Survival percentage of the BSFLM fed sets and the commercial meal fed sets (control) did not vary significantly.

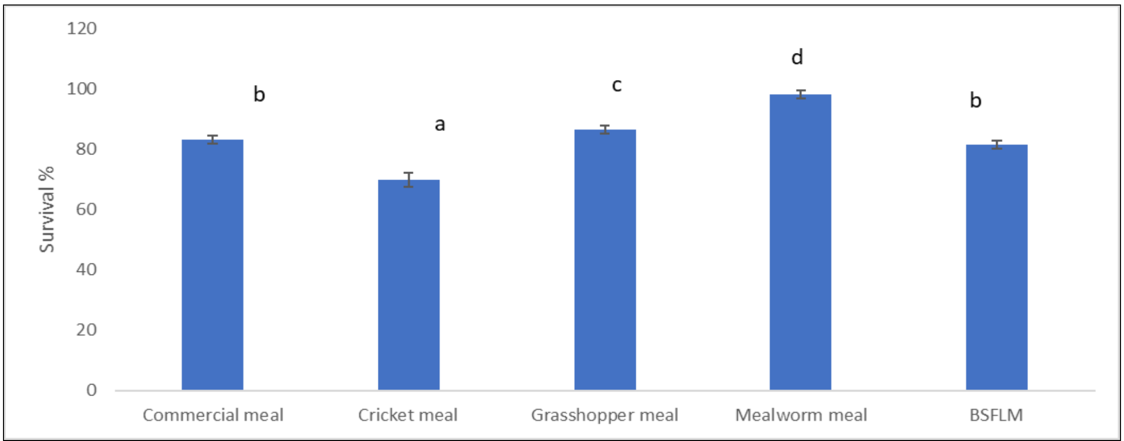

Fig 1: Survival percent of swordtail, *X. helleri* fed with five experimental diets.
Values are mean ± SE. Bars with different letters are significantly different (P<0.05) using DMRT after one way ANOVA.

Carotenoid content in the experimental diets and in the fish skin and tissue

The spectrophotometric analysis of pigment content of insect meals and commercial meal were estimated and a significantly higher value was observed in BSFLM, followed by MWM, and then in grasshopper meal and the lowest value was found in commercial meal (Figure 2).

Among the five experimental meal sets, a maximum value of the concentration of carotenoid deposition in the body of swordtail was observed in the fishes of BSFLM fed sets, whereas a lower value was reported in commercial meal fed fish sets (Figure 3). When the value of carotenoid deposition in the fish body was compared with the control, it was noted that all the four insect meals fed sets showed a better result.

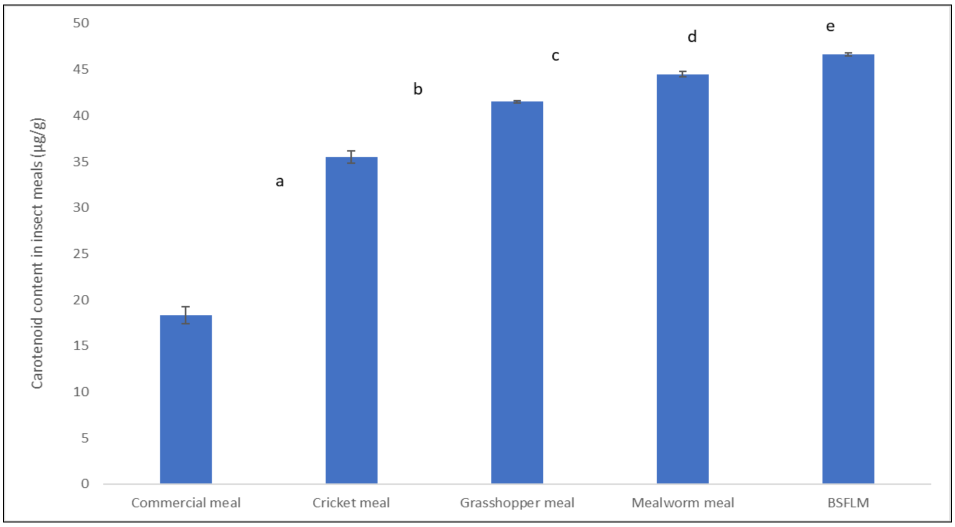

Fig 2: Carotenoid content (µg/g) in five experimental meals. Values are mean ± SE. Values are mean ± SE. Bars with different letters are significantly different (P<0.05) using DMRT after one way ANOVA.

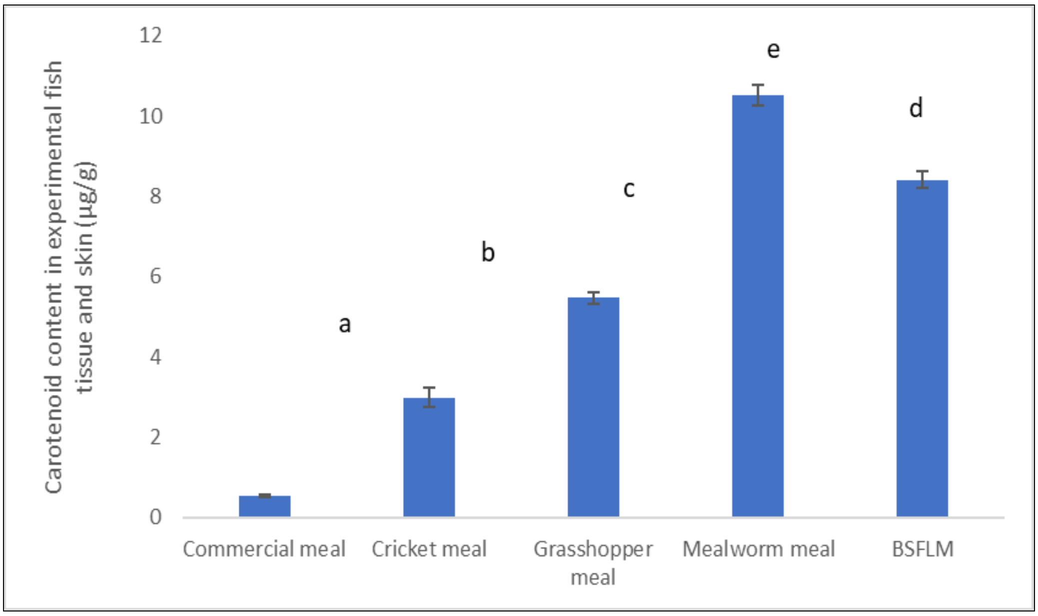

Fig 3: Total carotenoid content (µg/g) in the skin and muscle tissue from the experimental fishes. Values are mean ± SE. Bars with different letters are significantly different (P<0.05) using DMRT after one way ANOVA.

Whole Body Composition

The whole-body composition of swordtail from all the four insect meals and commercial meal fed set is shown in Table 3. The data of proximate compositions of the whole body of fishes from all the insect meals and commercial meal fed sets, did not show any significant differences.

Table 3: Proximate composition (%) of the whole body of Swordtail, *X. helleri* fed with five experimental meals in six-months feeding trial.

	Commercial meal	Cricket meal	Grasshopper meal	Mealworm meal	BSFL
Crude Protein	14.17±0.58a	14.38±0.39a	14.67±0.45a	14.74±0.37a	14.58±0.43a
Crude fat	3.39±0.32a	3.45±0.41a	3.48±0.42a	3.50±0.51a	3.50±0.39a
Crude fibre	3.90±0.23a	3.91±0.24a	3.80±0.45a	3.90±0.15a	3.99±0.39a
Ash content	4.49±0.15a	4.51±0.11a	4.47±0.09a	4.52±0.06a	4.48±0.09a
Moisture	66.69±0.67a	66.97±0.87a	66.90±0.65a	66.99±0.99a	66.69±0.69a

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

The overall results of growth performance and survival of swordtail confirmed that insect meals could be an alternative of commercial meal. In the present study, except cricket meal, all the insect meals had a positive effect on the growth performance parameters of swordtail. Growth performance of swordtail fed with cricket meal was observed poor than the control. But, in previous study on guppy it was noted that formulated diet with cricket meal as primary protein source showed a growth comparable to the control (Perera and Bhujel, 2022) which indicated that cricket meal should have some deficient in required nutrients for fish. Amino acid composition of cricket meal revealed that when compared with fishmeal, the house cricket meal had a very little deficient in leucine, lysine, methionine and valine, and the field cricket meal was only deficient in methionine. Therefore, for obtaining favourable growth of fish fed with cricket meal it is suggested that formulated diets with cricket meal is better than the solely cricket-included diet.

In the current study, weight gain and specific growth rate were found highest in the set of mealworm meal which contain 45% protein followed by the set of BSFLM containing 52.80% of protein and then in grasshopper meal set with 42.29% protein and lowest in cricket meal set as it had least percentage of protein (35.67%). This finding demonstrated that the protein content in diets had an influential role in the growth of swordtail as with the increased protein percentage in diet, the growth of fish accelerated accordingly. An acceptable growth was obtained in the fishes fed with mealworm meal, BSFLM and grasshopper meal, where crude protein percentage ranged from 42% to 53%. In this aspect, Chong *et al*. (2004) [10] demonstrated that dietary protein level had a crucial role in SGR of swordtail and reported that diet containing 20%, 30% and 40% protein showed 0.94%, 1.16 % and 2.78% of SGR, whereas diets containing 40% to 60% protein showed about 3% of SGR. In another feeding trial on *Xiphophorus maculates* with commercial meal (33.83% protein), cricket meal (37.67% protein), grasshopper meal (42.50% protein) and mealworm meal (46.00% protein), mentioned that a highest SGR was found in mealworm fed fishes (2.21%), followed by grasshopper meal fed fishes (1.19%) and then in cricket meal fed set (1.95%) and lower in commercial meal fed set (1.68%). The present study confirmed that growth of swordtail is positively affected by the protein content of diet except the result of BSFLM fed fishes, where a lower value of SGR was noted when compared with MWM fed sets, though containing higher percentage of protein in BSFLM (52.80%). It is clear that the growth of fish is also affected by some other factors such as protein quality, level of lipid and other nutrients in the diets (Chong *et al*., 2004) [10]. Here, growth performance of swordtail varied significantly among the dietary sets, the primary cause of this variations might be due to the dissimilar nutrient compositions of their diets, unequal amount of food consumption, and the variation in food energy utilization. Now, the swordtail from MWM fed set showed a maximum value for weight gain and SGR, where highest food consumption was noted and lower values for growth parameters were found in cricket meal fed set, where the least consumption was recorded. Here, a higher consumption of diets by swordtail reflected higher weight gain and higher growth of selected fish. The conclusion was asserted by Johnston *et al*. (2003) [29] on juvenile clown fish, Sapkale *et al*. (2017) on *X. maculatus* and Das (2023) [14] on *X. maculatus.* A significant higher value of weight gain and SGR with concurrent decreased value of FCR was found in the MWM fed fishes, followed by BSFLM fed set, and then in grasshopper meal fed fishes which showed better than that of commercial meals. In earlier investigation it was noted that the higher value of weight gain and SGR, and the best value of FCR is an indicator of improved utilization of food nutrients (Weisburger and Chung, 2002) [54].

Results of survival rates of swordtail asserted that insect meals could be a replacement of commercial artificial meal as survival rate showed a higher value in MWM and grasshopper meal fed set when compared to commercial meal fed set, and survival rate of BSFLM fed set was insignificantly varied (DMRT) with control. No previous research work was conducted on *X. helleri* fed with insect meals. Though, a previous study on *X. maculatus* confirmed the results of present study (Das, 2023) [14]. In contrary, Kowalska *et al*., (2021) [32] reported that solely insect-included meals did not affect (P>0.05) the survival of Guppy.

Whole-body composition of *X. helleri* were similar (P>0.05) when they were fed four different insect meals and commercial meal. Therefore, the current study confirmed that whole-body composition is less likely to be affected by the dietary treatments of swordtail. No research data is available for the whole-body composition of swordtail when insect meals were solely used as diets.

The colour intensity of ornamental fishes can be enhanced by incorporating synthetic or natural carotenoids into the diets (Liu *et al*., 2019) [34] as fishes are unable to synthesize the pigments themselves (Higuera-Clapara *et al*., 2006) [28]. Red-, orange- and yellow-coloured ornamental fishes digest astaxanthin and different carotenoids and then store in their skin to impart pigmentation (Lorenz and Cysewski, 2000) [35]. The results of total carotenoid concentration varied significantly among the dietary treatments. The results of total carotenoid content of the five experimental meals and of the experimental fishes of different sets revealed that the carotenoid content in swordtail was increased with the increased level of carotenoid content in the diets. This finding was supported by the observation of Perera and Bhujel (2022), where also carotenoid deposition in guppy increased when the inclusion level of carotenoid was increased by the increased amount of cricket meal incorporation. Maximum carotenoid accumulation in *X. helleri* was observed in MWM fed fishes, followed by BSFLM fed fishes, then in grasshopper meal fed fishes and cricket meal fed fishes showed higher accumulation than the control. In the earlier research on *X. maculatus* fed with the above-mentioned insect meal solely (Das, 2023) [14] obtained a similar result like the present study. Therefore, insect meals might be used solely as fish diet or might get preference to be included in fish diets as better colouration was noted in insect meal fed fishes than the commercial diet fed fishes. As colour of ornamental fishes determines their market value, expensive synthetic colour enhancers are incorporated in the artificial diets that ultimately rises the production costs of ornamental fishes (Ciani *et al*., 2021). Hence, the present study has an importance as it revealed that insect meals are potential ornamental fish feeds without a dedicated expensive synthetic carotenoid and without compromising the growth and survival of fishes.

Conclusion

Among the four insect meals, MWM, BSFLM and grasshopper meal could be used solely as ornamental fish feed. These three insect meals might be the alternatives of commercial meal as these insect meals fed fishes showed higher growth performance, survival rate and skin pigmentation than that of commercial meal fed fishes. Therefore, concerning the results of the current feeding trial, insect meals could be promoted as a good quality feed for *X. helleri*. However, more researches are required on the other ornamental fishes to established that insects are sustainable alternative novel animal protein source for fishes and also to convinced the reluctant ornamental fish producers those are used to in adding expensive synthetic colour enhancer in the fish diets.

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