Original Research Article

Studies on Effect of Insect Meals on the Growth Performance, Survival and Total Carotenoid Content of an Ornamental fish - Platy (*Xiphophorus maculatus*, Gunther, 1866)

ABSTRACT

Aims: To evaluate the effects of three non-conventional insects (cricket, grasshopper and mealworm) and commercial diet (control) on growth, survival and total carotenoid content of ornamental fish, platy (*Xiphophorus maculatus*).

Study design: For feeding trial of six months, 250 juveniles of platy fishes were used to prepare four experimental sets in glass aquariums (18"×12"×12") with three replications each set 20 fishes.

Place and Duration of Study: Zoology laboratory, Vidyasagar College, West Bengal, India, between November 2021 and May 2022.

Methodology: Proximate analysis of oven-dried powdered insects were carried out (AOAC). Weight and length of fishes were recorded at the start of the experiment and in 15 days intervals for the evaluation of growth performances of platy fish in terms of weight gain percentage, specific growth rate (SGR), Feed conversion ratio (FCR).

Results: Protein content in percent is significantly higher in mealworm (46±0.58) and then in grasshopper (42.50±0.29). Up to 4th month, values of fortnightly taken growth performance parameters were found better in mealworm fed fishes, followed by grasshopper fed fishes and lowest in commercial meal fed fishes. Whereas 4th month onwards cricket meal fed sets showed superior results. Final weight of fishes was significantly higher in mealworm fed sets (2.134gm±0.008) and lower in commercial meal fed sets (0.827gm±0.003). The poorest food conversion ratio was found in commercial meal (0.458±0.002) and best in cricket meal (0.280±0.001). Higher values of specific growth rate percent were obtained in mealworm fed sets (2.209±0.002) and lower in commercial meal fed sets (1.683±0.002). Mean survival percentage was ranging from 55% to 90%, where significantly higher value was obtained in mealworm fed sets. Carotenoid deposition in fish body was noticed significantly higher in mealworm fed sets (9.23µg/g±0.071) while lowest was observed in commercial meal fed sets (0.47µg/g±0.001).

Conclusion: Present study revealed that the chosen insects were preferable than the commercial meal for platy. Among the three insects, up to 4th month the mealworm and then cricket meal was more effective in improving the growth, but for maximum survival and enhancement of colouration, mealworm was recommendable for platy fish, *X. maculatus.*

Keywords: carotenoid, cricket, grasshopper, growth, mealworm.

1. INTRODUCTION

In recent times, aquarium fish culture is a household small-scale fish farming with low investment and high profit yield due to their always ever-increasing demand in the worldwide export trade. In India there is a sharp rise in international trade of ornamental fish with 6-billion-dollar turnover and 8 percent annual growth rate (1). To meet their high requirement and to get the profit, ornamental fish farmers' constant effort is to enhance the fish skin colouration as it determines their market value and their commercial acceptability. Platy, *Xiphophorus maculatus* is a freshwater brilliantly coloured ornamental fish. Their attractive colour is due to chromatophores, colour pigment containing cells which are found in skin of platy (2). Different shades of red and orange (carotenoid), yellow (xanthophylls), and brown and black (melanin) of platy are the results from the utilization of carotenoids by these pigments of chromatophores (3). Therefore, the chief agent of fish skin pigmentation are the carotenoids (4) that could not be synthesized by fish *de novo* (5) and are taken through diet, then absorbed and sometimes convert to other carotenoids, and later taken in into different tissues and are deposited there and in their skin. So, carotenoid pigmentation of their body muscle and skin comes from their diet, containing pigments (6). Some crustaceans and insects consumed natural carotenoid through their diets and deposited it in their tissue and skin. In nature fish obtain their required carotenoid by consuming carotenoid rich aquatic plants, weeds, algae and some arthropods (7, 8, 9, 10, 11, 12, 13). Several works have been documented that fish pigmentation can be enhanced by adding different processing wastes and plant sources (14, 15). Hence, colour supplementing diets should include additional natural or synthetic carotenoids to enrich the colour of ornamental fish. In that, required natural carotenoid sources and such sources should be easily available and have low cost or nearly costless.
Therefore, there is an urgent need of investigation for exploring a low-cost, easily available, alternative non-conventional protein source with natural carotenoid for the ornamental fishes. In this scenario, insects are an alternative protein source that are easily available, could be reared or harvested. Insects have high nutritional value and consumed as food in the different parts of the world. But insects as food is not practiced in many developing counties where this huge insect protein biomass are wasted. Aquatic insects are natural diets for omnivorous and carnivorous fishes (16, 17). Hence, the insect biomass could serve as an alternative feed for ornamental fish culture and a cost effective non-conventional feed for fish. Very recently, insects have started to play a key role in fish culture as alternative protein sources (18). Among insects, grasshoppers, crickets and mealworms are easy to breed and rear (19, 20, 21), and have a valuable protein profile (22; 23 24). Since, there is no works on ornamental fish using insects as feed to find whether insects have any role in enhancing their growth and skin pigmentation.

Based on these aspects, the present study was an attempt to determine the potentiality of grasshoppers, crickets and mealworms as fish feed and to evaluate their suitability based on growth, survival and colouration of *X. maculatus*.

2. MATERIAL AND METHODS

2.1 Experimental site and experimental fish

The experiment was conducted and maintained in the laboratory of Department of Zoology, Vidyasagar College, West Bengal, India. For the experiment 250 juveniles of platy fish of uniform size group (average weight 0.04 gm and average length 0.6 cm) were purchased from a commercial local ornamental fish market during the month of November, 2021. After purchasing, fishes were acclimatized to a glass aquarium (36"×12"×12") for 10 days in a laboratory condition. In this period, crushed commercial diets were offered to the fishes. For the experiment of six months, fishes were randomly collected and four experimental sets in glass aquariums (18"×12"×12") were prepared with three replications following a completely randomized design (CRD). In each set 20 fishes were stocked. The fishes were fed twice daily in the morning and evening at the rate of 5% of body weight per day. The weight and length of fishes were recorded in 15 days intervals.

Twice in a day, the water of the experimental glass aquariums was siphoned off to remove the extra feed particles and metabolic wastes from the water and the same water was stored in the respective aquariums. Water exchange was done twice a week.

2.2 Experimental diet

Grasshoppers and crickets were collected from nearby grassland field of Vidyasagar college, Kolkata, India, by sweeping method using insect net. Mealworms were purchased from local market. These insects were freeze killed and then they were oven-dried at 60^{O}C for 72 hours. The dried specimens were manually ground into small flakes and powder form, and kept in airtight zipper plastic bags until further use in feeding trial and analysis.

2.2.1 Proximate Composition Ansalysis

Proximate analysis of experimental meals was carried out to assess the crude protein, crude fat, crude fibre and moisture contents of the samples as per the stipulated method of AOAC (2006)(25).

2.3 Growth study

The weight and length of fishes were recorded at the start of the experiment and in 15 days intervals of the 6 months experimental period. The growth performances of platy fish were evaluated in terms of weight gain percentage, specific growth rate (SGR), Feed conversion ratio (FCR) by using following formulae:

Weight gain (%) = (final weight – initial weight) / initial weight × 100;

Length gain (%) = (final length – initial length)/ initial length × 100;

Condition Factor (CF %) = (wet weight of fish / (length of fish)^3) × 100.

SGR = 100 × $\log_e$ average final – lag_e average initial weight)/number of rearing days;

FCR = Total feed given (dry weight in g) / weight gain (wet weight in g)

2.4 Estimation of carotenoid

Carotenoid content of experimental feed samples were estimated by Cyanotech (2002) (26) method. Olson (1979) (27) method was followed to measure the total carotenoid content of coloured part of fish skin.

2.5 Water Quality Analysis

Physico-chemical parameters of water like temperature, dissolved oxygen, pH, free carbon dioxide, hardness, alkalinity and TDS of experimental aquariums were analysed and noted during the experimental period using standard methods (28).

2.6 Statistical Analysis

Data were presented as mean ± SE. One way analysis of variance (ANOVA) was done for the data of proximate composition, carotenoid content and survival rate of sample diets and samples of fish skin. Data of growth parameters were statistically processed by two-way analysis of variance (ANOVA) and then Duncan's multiple range tests (DMRT) were carried out for each case to separate the mean values according to significance.

3. RESULTS AND DISCUSSION

On 15 days intervals samplings were done of feeding trial for the assessment of growth in length, weight, specific growth rate and FCR.

3.1 Water quality parameters

Physico-chemical water quality parameters of different experimental aquarium sets during the experimental period is represented in Table 1. In all the experimental aquarium sets, the temperature of water fluctuated from 26^{O}C to 33^{O}C. The pH was noted in the range of 7.2 to 7.4. The dissolved oxygen and free carbon dioxide level varied in the range of 8.1 ppm to 8.4 ppm and 1.45 ppm to 2.16 ppm. The TDS of different experimental sets was recorded in the range of 920 ppm to 1085 ppm. Hardness of water of all the aquarium sets varied from 39 ppm to 78 ppm. Alkalinity of water of all the meal sets was found in the range from 320 ppm to 336 ppm. All the estimated physico-chemical water quality parameters of experimental aquariums were within the optimal range known for the fishes.

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

Parameter	Minimum	Maximum
Temperature	26^{O}C	33^{O}C
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

3.2 Nutrient Composition of experimental meals

The proximate nutritional composition of commercial feed, grasshopper meal, cricket meal and mealworms meal were listed in table 1.

Table 2. Proximate nutritional composition of commercial feed, cricket meal, grasshopper meal and mealworms meal.

Nutritional composition (%)	Commercial meal	Cricket meal	Grasshopper meal	Mealworm meal
Crude Protein	33.83±0.44a	37.67±0.88b	42.50±0.29c	46±0.58d
Crude fat	3.60±0.31	4.67±0.17b	4.57±0.30b	18.97±0.28c
Crude fibre	3.87±0.07b	2.90±0.60a	11.83±0.17d	6.47±0.15c
Moisture	11.33±0.67c	9.07±0.12a	11.33±0.20c	9.63±0.20b

Data are presented as mean ± SE. Within a row a, b, c, d indicates significant differences between mean values. (P= .001, DMRT)

3.3 Growth performance

Initial weight and length of the fishes was almost the same (0.04 gm and 0.6 cm). Fishes of all the four dietary sets preferred and accepted all the selected diets and consumed these experimental diets actively. Rejection of diets were not observed until the end of the experiment. Similar acceptability for all the diets were observed.

The data of length gain in percent in 15 days intervals of *X. maculatus* were summarized in Figure 1. When data of length gain in percent were compared between time periods it was noticed that significantly highest values were obtained in 15th day for all the meals and significantly lowest value for commercial meal found on 6th month and for mealworm meal on 5th month. Lower values for cricket meal and grasshopper meal were found in last four time period where data did not vary significantly.

When data were compared within time period and between the experimental meals, on 15th day significantly lower value and higher value were noticed in commercial meal fed sets and mealworm meal fed sets respectively. On first month data of length gain in percent did not vary significantly (DMRT). On 45th day and 75th day significantly lower values were found in commercial meal fed sets and higher value on 45th day in grasshopper and mealworm meal fed sets and on 75th day in grasshopper meal fed sets. On 3rd month and 105th day significantly higher values were obtained in commercial meal fed sets. In the last remaining time periods data did not show any significant differences.

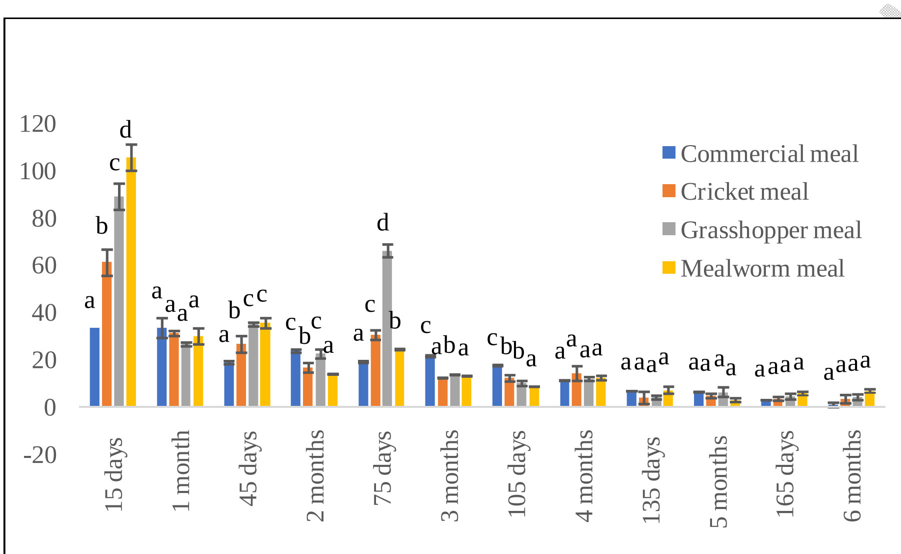

Fig. 1. Length gain in percent of *X. maculatus* fed with four experimental meals on 15 days interval time periods for 6 months

Values are mean ± SE. Bars with different letters within growth stage are significantly different (P=.001) using DMRT

When data were compared between time period intervals, on 2nd month significantly higher values for weight gain in percent of *X. maculatus* were noticed for all the four experimental meals fed sets. Whereas significantly lower data were found on 6th month for all the experimental sets. Within time period a similar trend of results was observed up to 105th day i.e. mealworm meal fed sets attained significantly higher weight gain in percent, then in grasshopper meal fed sets and then in cricket meal fed sets. Whereas significantly lower values were obtained in commercial meal fed sets up to 4th month. On 4th month the values of weight gain in percent did not vary significantly between mealworm and grasshopper meal fed sets. On 135th day the value was significantly higher in grasshopper meal fed set and on 5th month onward highest value was noted in cricket meal fed sets. On 135th day onwards mealworm meal fed sets showed significantly lower values for weight gain in percent.

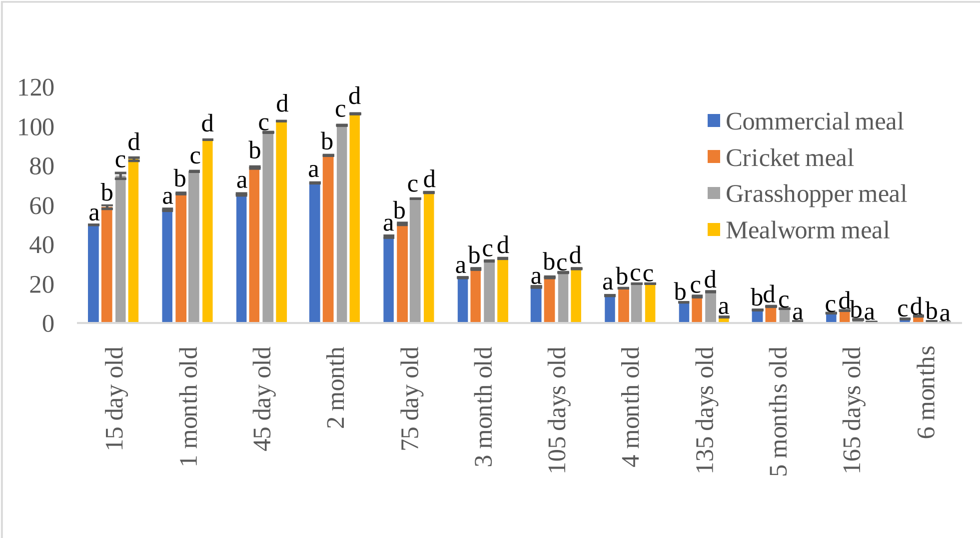

Fig. 2. Weight gain in percent of *X. maculatus* in four experimental meal fed sets on 15 days interval time periods

Values are mean ± SE. Bars with different letters within growth stage are significantly different (P=.001) using DMRT

Figure 3 depicts the food consumption of *X. maculatus* fed with four experimental meals on 15 days interval time periods. Commercial meal consumption recorded significantly higher up to 75th days and then 105th day onward commercial meal sets showed significantly lower values. Among the four meals sets cricket meal consumption were lowest till 3rd month. On 3rd month onwards lowest data obtained from commercial meal fed sets. Among cricket meal, grasshopper meal and mealworm meal, the last one always showed higher consumption.

When data of food consumption were compared between time period intervals within meals, in case of grasshopper and mealworm sets, an increasing trend of data were reported from start to end of the experiment. But cricket and commercial meal fed sets followed this trend till 135th day except on 2nd month in case of commercial meal fed sets where the value of consumption were decreased.

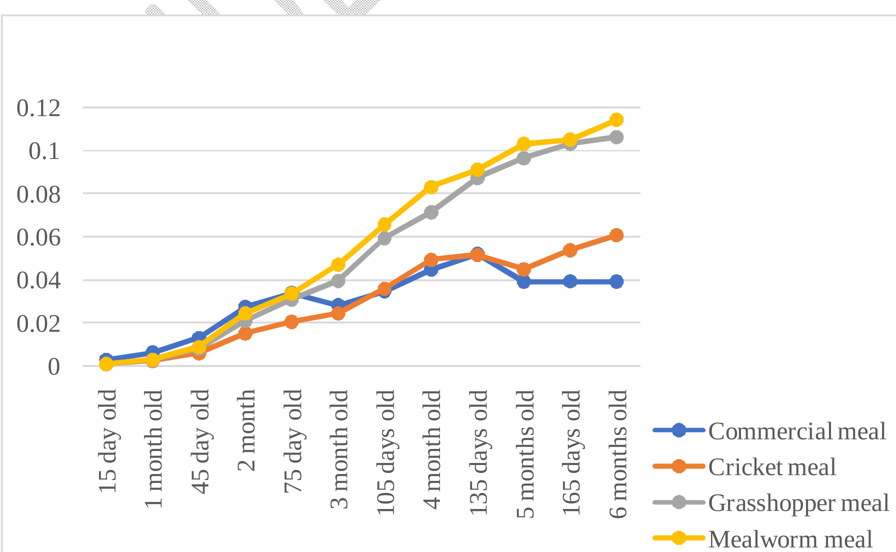

Fig. 3. Food consumption of *X. maculatus* fed with four experimental meals on 15 days interval time periods

Line with marks of food consumption are significantly different (P=.001; DMRT)

Within time periods between different meal fed sets, up to 45 days, a decreasing trend of data of CF i.e. highest value in commercial meal, then in cricket meal, and then in grasshopper meal and lastly in mealworm meal fed sets respectively were found and up to 3rd month significantly higher values were noticed in commercial meal fed sets. On 2nd month lower value of CF were found in grasshopper and mealworm fed sets where data did not vary significantly (DMRT). On 75th day and 3rd month significantly higher value were found in commercial meal fed sets and remaining meal fed sets did not show significant variations. On 5th month onward significantly, higher values were noticed in cricket meal fed sets.

When data were compared within different meal fed sets between time period, a decreasing trend of data of CF were noticed up to 4th month and then data did not vary significantly. For all the four diet fed sets highest value was found in 15th day. In commercial meal fed sets lower values were found in last two time periods and in cricket meal fed sets, lower in last three time periods and in grasshopper meal fed sets lower in last five time periods were obtained where data did not vary significantly (DMRT). In case of mealworm fed sets, significantly lowest value was found in the last time period i.e. on 6th month.

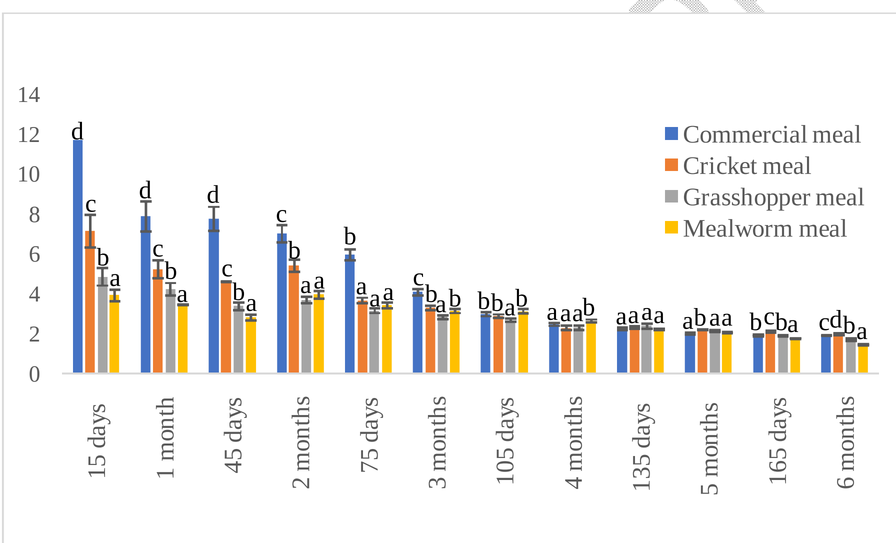

Fig. 4. Condition factor of *X. maculatus* in four experimental meals fed sets on 15 days interval time periods

Values are mean ± SE. Bars with different letters within growth stage are significantly different (P=.001) using DMRT

When data of SGR were compared within time period between meals, from start to 105th day of experiment, a similar trend of data i.e. lowest in commercial meal fed sets, then in cricket meal fed sets and then in grasshopper meal fed sets and significantly highest in mealworm fed sets were found. On 4th month higher values were noticed in both the sets of mealworm and grasshopper meal and on 135th day significantly higher values were observed in grasshopper fed sets and lower in mealworm fed sets. 5th month onwards significantly higher value were found in cricket meal fed sets and lower in mealworm fed sets.

When data were compared between time periods, for all the meal sets, up to 2nd month values of SGR were increased and 2nd month onwards values were decreased till the end of the experiment.

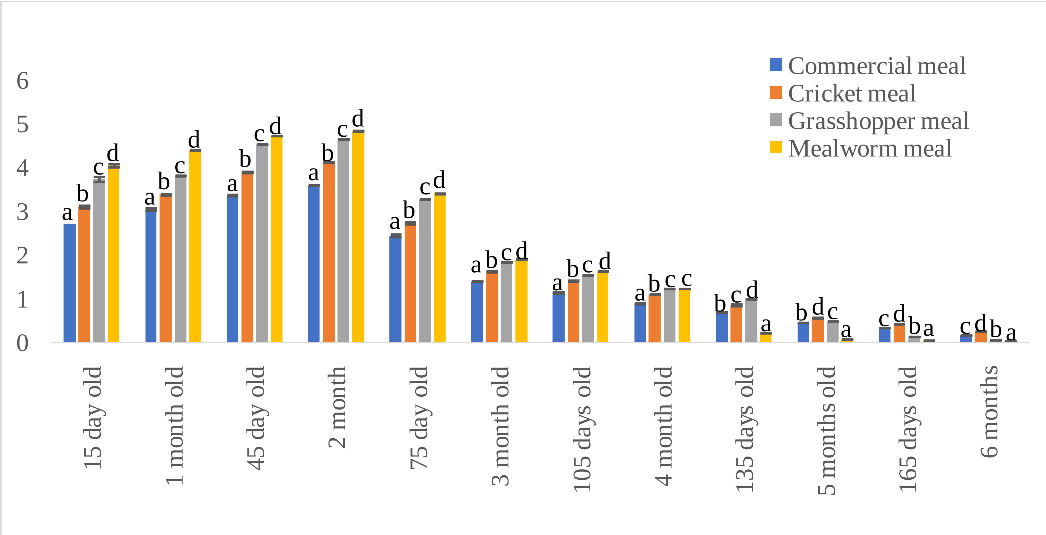

Fig. 5. SGR of *X. maculatus* in four experimental meals fed sets on 15 days interval time periods

Values are mean ± SE. Bars with different letters within growth stage are significantly different (P=.001) using DMRT

When data were compared between meal within time period, significantly highest value of FCR in commercial meal fed sets, then cricket meal fed sets and then grasshopper meal fed sets, and lowest in mealworm meal fed sets were observed from start to 4th month of the experiment. On 135th day the trend of data was similar as mentioned except in case of mealworm fed sets which showed significantly highest value. On 5th month onwards, significantly highest value of FCR was found in mealworm meal fed sets and lowest in cricket meal fed sets.

When data of FCR were compared between time period within meals, it was found that values of FCR showed an increasing trend throughout the experimental periods for all the four meals fed sets.

Table 3. FCR of *X. maculatus* in four experimental meals fed sets on 15 days interval time periods

Time period	Experimental diets			
	Commercial meal	Cricket meal	Grasshopper meal	Mealworm meal
15th day	0.138±0.0017d	0.042±0.0006c	0.034±0.0006b	0.030±0.0003a

1 month	0.174±0.0015d	0.056±0.0008c	0.051±0.0003b	0.045±0.0007a
45th day	0.210±0.002d	0.073±0.0005c	0.067±0.0002b	0.061±0.0001a
2nd month	0.245±0.0019d	0.095±0.0002c	0.085±0.0005b	0.080±0.0004a
75th day	0.286±0.0051d	0.115±0.0005c	0.100±0.0011b	0.085±0.0003a
3rd month	0.315±0.0037d	0.168±0.0006c	0.156±0.0019b	0.144±0.0003a
105th days	0.390±0.0006d	0.226±0.0027c	0.217±0.0014b	0.180±0.0002a
4th month	0.552±0.013d	0.329±0.0022c	0.266±0.0013b	0.245±0.0005a
135th days	0.743±0.0143c	0.381±0.007b	0.338±0.0022a	1.372±0.0101b
5th months	0.781±0.0007c	0.455±0.0042a	0.689±0.0021b	4.293±0.0077d
165th days	0.981±0.0046b	0.671±0.0042a	2.583±0.0167c	6.565±0.0021d
6th months	1.952±0.0017b	1.223±0.013a	5.317±0.0167c	11.423±0.0145d

**Values are mean ± SE. Bars with different letters within growth stage are significantly different (P=.001) using DMRT*

When the data of overall growth parameters taking the last data under consideration were compared between different selected meals it was observed that final length, final weight and SGR (%) were significantly higher in mealworm fed sets followed by grasshopper fed sets and lower in commercial meal fed sets. Values of FCR was significantly higher in lower in cricket meal fed sets followed by grasshopper meal fed sets and higher in commercial meal fed sets.

Table 4. Overall growth details on effect of different insect meal on juveniles of platy fish, *X. maculatus* for a period of six months

Growth parameters	Diets			
	Commercial meal	Cricket meal	Grasshopper meal	Mealworm meal
Final length (cm)	3.500±0.000a	4.067±0.033b	4.933±0.067c	5.267±0.033d
Final weight (gm)	0.827±0.003a	1.346±0.003b	2.053±0.012c	2.134±0.008d
CF (%)	1.928±0.008c	2.004±0.052d	1.713±0.061b	1.462±0.026a
SGR (%)	1.683±0.002a	1.953±0.001b	2.188±0.003c	2.209±0.002d
FCR	0.458±0.002d	0.280±0.001a	0.312±0.001b	0.325±0.001c

**Values are mean ± SE. values with different letters are significantly different (P=.001) using DMRT.*

3.4 Carotenoid content of fish meal and in fish skin and tissue

The spectrophotometric analysis of pigment content of platy fish meals was estimated and the highest value was found in mealworm, followed by grasshopper and lowest in commercial meal (Fig 6).

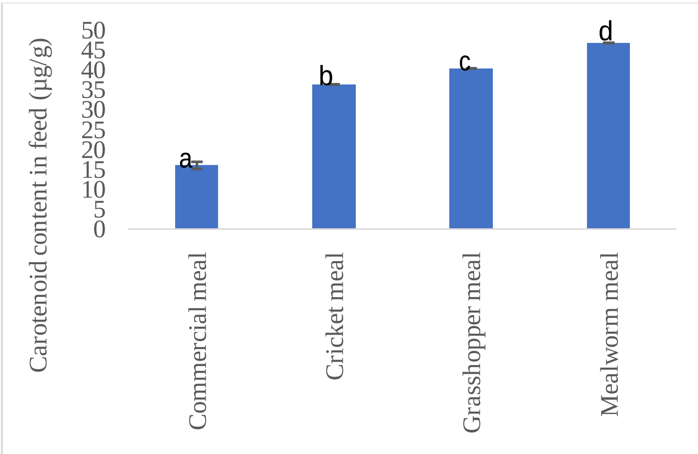

Fig. 6. Carotenoid content (µg/g) in four experimental meals

**Values are mean ± SE. Bars with different letters are significantly different (P=.001) using DMRT*

Among the four experimental meal sets, the concentration of carotenoid deposition in the body of platy fishes was significantly higher in mealworm diet fed experimental fish sets, whereas lowest value was reported in commercial diet fed fish sets (Fig 7).

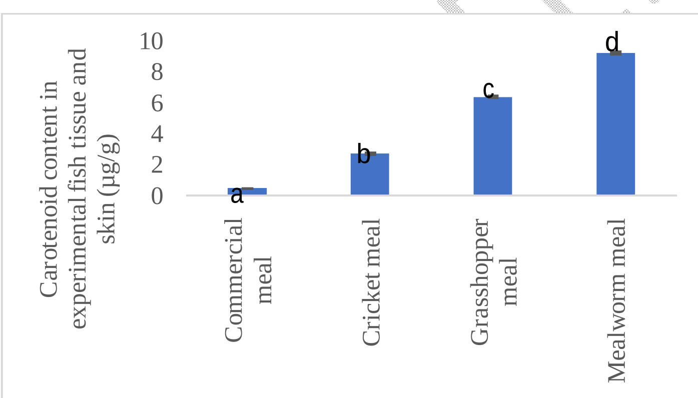

Fig. 7. Carotenoid content (µg/g) in four experimental meal fed fish tissue and skin

Values are mean ± SE. Bars with different letters are significantly different (P=.001) using DMRT

3.5 Survival percentage

Survival percentage was significantly higher in mealworm meal fed set (90%) and lower in commercial meal fed sets (55%).

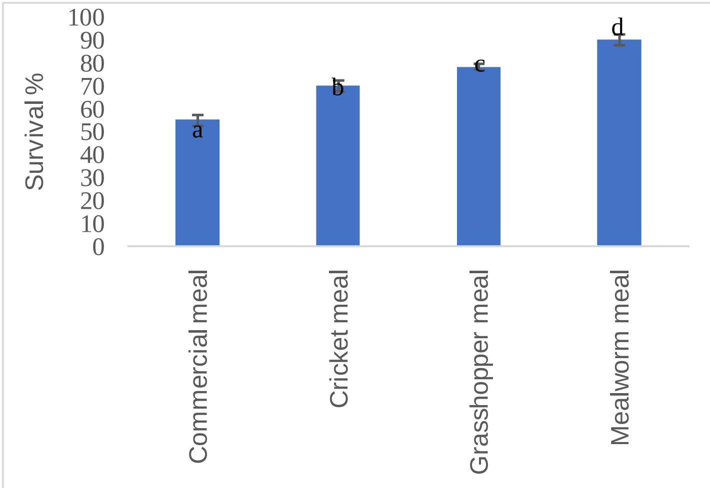

Fig. 8. Survival percent of *X. maculatus* fed with four experimental meals

Values are mean ± SE. Bars with different letters are significantly different (P=.001) using DMRT

In the current study, it was noticed that all the three chosen insect meal played a positive role in improving the weight gain percent, SGR and FCR of *X. maculatus.* A significant weight gain increase and concurrent FCR decrease was observed in the mealworm meal fed fishes, followed by grasshopper meal and then cricket meal fed fishes which were more acceptable than commercial meals. According to Weisburger and Chung (2002) (29) the improved weight gain and FCR is an indicator of improved utilization of nutrients. Results of the present study revealed that insects as fish feed significantly affect growth as well as FCR of platy fishes.

A varied range of growth rate of each of the four experimental meals were obtained, mainly due to their nutrient composition, amount of consumed feed, experimental time period and also on utilization of food energy. Study have shown that feed consumption of *X. maculatus* were increased till 2nd month of period for all the four experimental sets and also up to that period weight gain percent and SGR were also displayed an increasing trend for all sets. The results support the observation of Johnston et al. (2003) (30) and Sapkale et al. (2017) (31) where demonstrated that higher growth was obtained with the increased feed consumption by *X. maculatus.* Higher consumption of diets by platy reflects growth of selected fish rather than maintenance (32). In the present study, the highest weight gain and specific growth rate were obtained in mealworm meal which contain 46% protein followed by grasshopper containing 42.50% protein and then in cricket meal with 37.67% protein and lowest in commercial meal as diet containing least percentage of protein (33.83%). This finding is comparable with the relevant works conducted by Chong et al. (2004) (33) on *Xiphophorus helleri* where they proved that higher weight gain and better SGR were obtained when the fish were fed with diets containing more than 40% protein. The result of the study revealed that till 2nd month, selected consumed meals were well utilized by *Xiphophorus helleri* to increase their weight and also to enhance the specific growth rate. But after 2nd month, weight and SGR of platy fishes were decreased till the end of the experiment may be due to utilization of food energy for reproduction.

Carotenoid play an effective role in fish intermediary metabolism (33) which may responsible for enhancing nutrient metabolism that ultimately could improve growth of fish (34). In the present study, significantly highest growth percentage reported in mealworm fed sets might be due to the amount of diet's natural carotenoid that may have growth promoting role. Vitamin A and some nutrients are formed in the carotenoid metabolism in fishes which act as growth promoter (11). A similar type of results was noticed in some previous findings in red sword tail (35), rainbow trout (36), goldfish (37) and in guppy fish (38) which indicate that amount of carotenoid content in fish feed linked to the growth enhancement of fishes.

Storebakken and No (39) and Torrissen et al. (1989) (40) reported that the absorption and deposition of carotenoid in platy fishes is steadily influenced by the nature of carotenoids, carotenoid concentration in diets and the size of fishes. In the present study, mealworm diets increased the carotenoid level in the tissue and skin of platy fishes, then fed with grasshopper meal followed by cricket meal. This observation clearly depicted that carotenoid deposition in fishes directly linked with dietary carotenoid content which ultimately increase colouration. Goldfish showed a similar trend of results when fed with various natural carotenoid sources like Spirulina (41), red yeast (42) and alfalfa (43). It was found that several carotenoid source like blue green algae and spirulina and some plant sources like beet root (35), tomato and carrot (38) and paprika (44) as fish feed greatly influenced colour development in ornamental fishes. Still now, various natural carotenoid sources from plant origin have been successfully tried in ornamental fishes. However, the study on ornamental fishes with natural carotenoid sources from animal origin are very limited and hence, in the present investigation, nonconventional sustainable insect protein sources with favourable amount of carotenoid were tried as fish feed which showed not only significant effect in skin colouration but also on growth, FCR and survival percentage of platy fishes, *X. helleri.*

4. CONCLUSION

To conclude, the recent study revealed that among the three insect meals mealworm and then grasshopper meal enhanced the growth performance and feed utilization till 135 days and then cricket meal improve these mentioned parameters till the end of the experiment. Moreover, enhanced skin colour was observed in mealworm fed fishes, followed by grasshopper fed sets. This result suggests that platy fishes, *X. helleri* has the potentiality to efficiently utilize the carotenoid of insect tissue and then to store in the fish skin and tissue and ultimately enhancing skin colouration. As synthetic carotenoids are very costly, easily available insects with natural carotenoid such as mealworm and grasshopper would be the alternative cheap natural protein and carotenoid source as feed for the ornamental fish seller to fetch superior cost in trading of this fishes.

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