Original Research Article

Effect of Insect Meals on the GrowthEfficiency, Survival and Total Carotenoid Content of an Ornamental fish *Xiphophorus maculatus*(Gunther, 1866)

ABSTRACT

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),* a feeding trial was conducted in Zoology laboratory, Vidyasagar College, West Bengal, India, between November 2021 and May 2022.A total of 250platy juveniles(0.04±0.002 gm) were stocked in 12 glass aquariums with 20 fishes for each experimental feed set. Each experimental diet was made per triplicate. The growth efficiency parameters of platy fish in terms of weight gain percentage, specific growth rate (SGR), Feed conversion ratio (FCR) were measured at the end of thestudy period.Protein content in percent is significantly higher in mealworm (46±0.58) and then in grasshopper (42.50±0.29). Final weightof fisheswas significantly higher in mealworm fed sets (2.134gm±0.008) and lower in commercial meal fed sets (0.827gm±0.003). The lowest FCR was found in commercial diet (0.458±0.002) and bestFCR values was found in cricketdiet(0.280±0.001).Higher value of SGR was obtained in mealworm fed sets (2.209±0.002) and lower SGR was in commercial diet (1.683±0.002).Mean survival percentage was 90% in mealworm diet and lowest survival value was 55% at commercial diet. Carotenoid content in fish body was significantly higher in mealworm diet (9.23µg/g±0.071), while lowest was observed in commercial diet (0.47µg/g±0.001).Present study shows that insect diets were better than commercial diet, but meal worm diet was better to increase survival and carotenoid content in *X. maculatus.*

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

1. INTRODUCTION

In recent times, aquarium fish culture is a 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 eight 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. 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. Platy (*Xiphophorus maculatus*) is a freshwater brilliantly coloured ornamental fish because of theirchromatophores and colour pigment containing cells which are found in skin of platy(2). Different shades of red and orange (carotenoid), yellow (xanthophylls), and brownof platy are the resultsfrom the utilization of carotenoids as principal resource of fish skin pigmentation (3,4) but they cannot be synthesized by fish *de novo* (5) and then they need taken through enriched diets with carotenoids, then absorbed and sometimes convert to other carotenoids, and later taken into different tissues and are deposited in their skin (6).

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 sourcesshould be easily available and have low cost or nearly costless.

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 theirgrowth and skin pigmentation.

Based on these aspects, the present study wasan 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 (six months period) 250 juveniles (average weight 0.04 gm and average length 0.6 cm) were acclimatizedin a glass aquarium (36"×12"×12") for 10 days in laboratory condition and fed with commercial diet. After this period, fishes were randomly collected and four experimental sets in glass aquariums (18"×12"×12") were prepared with three replicates 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.

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

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 manuallymicronizedand kept in airtight zipper plastic bags until further use in feeding trial and analysis.

2.2.1 Proximate Composition Analysis

Proximate analysis of experimental meals was carried out to obtain crude protein, fat, fibre and moisture contents using the method of AOAC (2006)(25).

2.3 Growth study

Final weight and length of fishes were recorded at the end of the experimental period of 6 months.The growth efficiency of platy fish was evaluated in terms of specific growth rate (SGR), total food consumption and feed conversion ratio (FCR).

2.4 Estimation of carotenoid

Carotenoid content of experimental feed samples were estimated by Cyanotech (2002) (26) method. At the end of the feeding trial, coloured region of skin and tissue of fishes from four experimental sets were collected to measure the total carotenoid content following the method ofTiewsoh et al. (2019) (27)

2.5 Water Quality Analysis

Physical and chemical parameters of water like temperature, dissolved oxygen, pH, free carbondioxide, hardness, alkalinity, and TDS of experimental aquariums were analyzedtwice a week using standard methods (28).

2.6 Statistical Analysis

Data were presented in tables and figures as mean ± SE. 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.

3. RESULTS AND DISCUSSION

3.1 Water quality parameters

Physical and chemical water quality parameters of different experimental aquarium setwere shown in Table 1.

Table 1. Physical and 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

Throughout the experimental period of six months, therange of values of physico-chemical parameters of water in the experimental aquariums were noted in table 1. Though the ground water of the experimental area, used in experimental sets, having higher water hardness and TDS, did not show any adverse effect on the fishes during the acclimatization period. This finding was asserted by earlier studies, where demonstrated that ornamental fishes have high adaptability and are capable of surviving in water with a wide range of hardness and TDS (29, 30).As all the experimental aquariums contained the same ground water, therefore all the experimental fishes encountered the same range of water hardness and TDS. Hence, it could be safely said that there was no such isolated influential effect of this high range of water hardness and TDS on growth efficiency and survival rate of platy fish.

3.2 Nutrient Composition of experimental meals

The proximate nutritional composition of commercial feed, grasshopper meal, cricket meal and mealworms diets were listed in table 2.

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±0.31a	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 efficiency

The growth efficiency parameters of the platy fish at the end of the experiment are shown in table 3. The initial length of platy fish varied from 0.55 cm to 0.65 cm. After six months, the result of final length showed significantly higher value in mealworm meal fed set followed by grasshopper meal fed set and significantly lower value was foundin commercial meal fed set. The initial weight of platy fish ranged from 0.037 gm to 0.043 gm. At the end of the experiment, the best live final weight was recorded from mealworm meal fed set followed by grasshopper meal fed set and poorest value was noticed in commercial meal fed set. Similarly, significantly highest weight gain is noted in mealworm fed set and lowest in commercial diet fed set. In case of the result of specific growth rate (SGR) in percent, again significantly highest value was detected in mealworm fed set and second highest in grasshopper meal fed set whereas lowest value was obtained from commercial meal fed set.Total food consumption was significantly higher in mealworm fed set and lower in commercial meal fed set. Platy fishes from commercial meal fed set showed significantly higher value for feed conversion ratio (FCR) whereas lower value was obtained from cricket meal fed set followed by grasshopper meal fed set.

In the current study, it was noticed that all the three chosen insect meal played a positive role in improving weight gain, SGR (%) and FCR of *X. maculatus.* 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) (31) 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.

In the present study, a varied range of growth rate were obtained in the four experimental meal fed sets, mainly due to variation in nutrient composition of their diet, amount of consumed food, and also on utilization of food energy. Here Platy fishes from mealworm meal fed set showed higher value for weight gain, total food consumptionand SGR (%) and lower values for all these parameters were noted in commercial meal fed set. The results support the observation of Johnston et al. (2003) (32) and Sapkale et al. (2017) (33) where demonstrated that higher growth was obtained with the increased food consumption by *X. maculatus.* Higher consumption of diets by platy reflects higher weight gain and higher growth of selected fish rather than maintenance (34).

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) (35) 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.

Table 3.Growth parameters of platy fish, *X. maculatus*fed with different insect mealsin six months feeding trial

Growth parameters	Diets			
	Commercial meal	Cricket meal	Grasshopper meal	Mealworm meal
Initial length (cm)	0.60±0.029a	0.57±0.017a	0.58±0.017a	0.58±0.033a
Final length (cm)	3.500±0.000a	4.067±0.033b	4.933±0.067c	5.267±0.033d
Initial weight (gm)	0.04±0.002a	0.04±0.002a	0.04±0.002a	0.04±0.002a
Final weight (gm)	0.827±0.003a	1.346±0.003b	2.053±0.012c	2.134±0.008d
Weight gain (gm)	0.79±0.010a	1.29±0.014b	2.01±0.011c	2.09±0.014d
SGR (%)	1.683±0.002a	1.953±0.001b	2.188±0.003c	2.209±0.002d
Total food consumption (gm)	0.36±0.001a	0.37±0.001b	0.63±0.002c	0.68±0.001d
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 Survival percentage

Survival percentage was significantly higher in mealworm meal fed set (90%) and lower in commercial meal fed sets (55%) (Figure 1).This result was supported by the fact that when fish obtained the dietwith high nutritional value showed,in addition to higher growth efficiency and feed utilization, a positive impact on survival rate.The same opinion was reported by Sapkale et al. (33) for *X maculatus* when provided optimum nutrition.

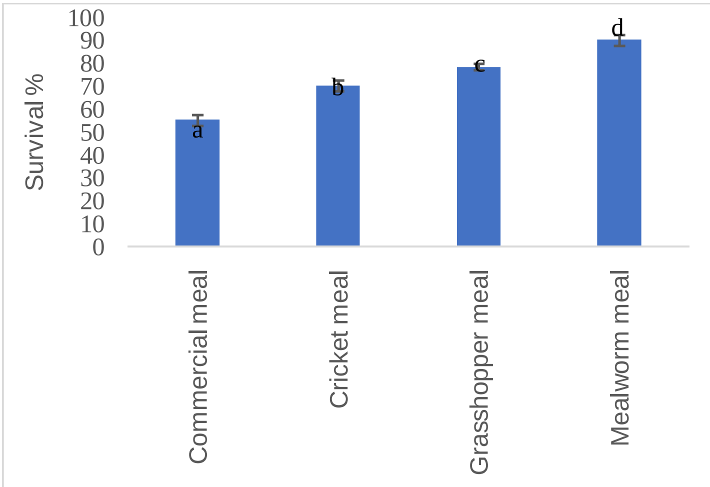

Fig. 1. Survival percent of *X.maculatus*fed with four experimental meals

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

3.5Carotenoid 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 (Figure2).

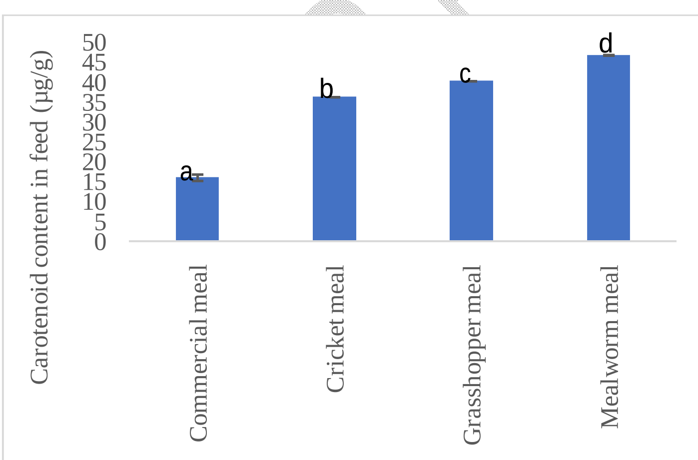

Fig. 2. Carotenoid content (µg/g) in four experimental meals

**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 mealwormdiet fed experimental fish sets, whereas lowest value was reported in commercial diet fed fish sets (Figure3).

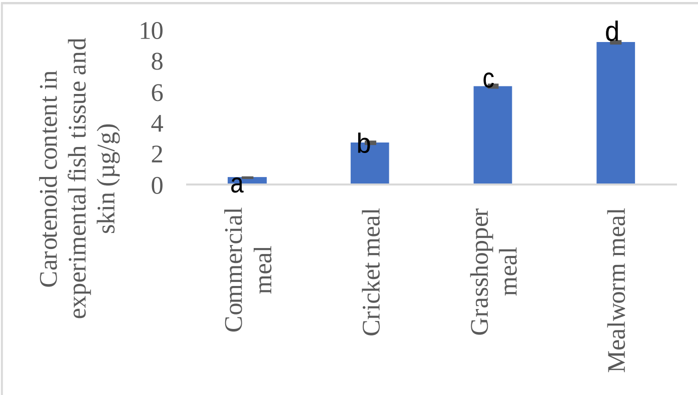

Fig. 3. Carotenoid content (µg/g) in four experimental meal fed fish tissue and skin

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

Carotenoid play an effective role in fish intermediary metabolism (36) which may responsible for enhancing nutrient metabolism that ultimately could improve growth of fish (37). 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 (38), rainbow trout (39), goldfish (40) and in guppy fish (41) which indicate that amount of carotenoid content in fish feed linked to the growth enhancement of fishes.

Storebakken and No (42) and Torrissen et al. (43) 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 (44), red yeast (45) and alfalfa (46).

The amount of carotenoid deposition in fish tissue and skin is species specific (47). The maximum level of carotenoid content in fish tissue and skinis definite which is attained by taking an optimum quantity of carotenoid through feed (48). Over that quantity of carotenoid in feed did not show any increase in carotenoid deposition in fish tissue and skin (49).

It was found that several carotenoid source like blue green algae and spirulina and some plant sources like beet root (38), tomato and carrot (41) and paprika (50) 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. maculatus.*

4. CONCLUSION

To conclude, the recent study revealed that among the three insect meals, mealworm and then grasshopper meal increased the growth efficiency and feed utilization of platy fish. Moreover, enhanced skin colour was observed in mealworm fed fishes, followed by grasshopper fed sets. This result suggests that platy fish, *X. maculatus* 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 expensive, 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 obtain most profile value with these fishes.

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