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Research Article

GROWTH AND SURVIVAL OF FINGERLINGS OF BLACK MOLLY (*POECILIA SPHENOPS*) WITH DIFFERENT ANIMAL PROTEIN BASED FORMULATED DIETS

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ABSTRACT

The efficacy of fingerlings of black molly, *Poecilia sphenops* to four types of formulated feeds containing different proportions of animal protein (fish meal, prawn meal and plankton meal) was evaluated in comparison with a commercially available pellet feed. Diet I was prepared in combination with fish meal and prawn meal, Diet II was prepared in combination with fish meal and plankton meal. Diet III and diet IV were prepared only with fish meal and plankton meal respectively. In 35 days feeding experiment, length gain (cm) of *P. sphenops* with Diet I, Diet II, Diet III , Diet IV and commercial diet was 0.47, 0.58, 0.74, 0.4 and 0.75cm, respectively while weight gain (g) was 0.12, 0.24, 0.25, 0.18 and 0.35 g, respectively. Specific growth rate of experimental diets ranged between 0.34 and 1.0 while that of commercial diet was 0.51. Higher mortality was recorded in commercial pellet feed (66.67%) compared to experimental diets (0 to 46.67%). Growth and development of *P. sphenops* suggest all the diets supported normal growth, nevertheless variation in the growth performance with different diets might be due to the difference in the composition of the macro-nutrient of these diets. In view of higher cost and mortality rate of commercial pellet feed, experimental diets could be used in commercial production of molly fish.

Keywords: *Poecilia sphenops*, Formulated diets, Growth performance, Survival.

INTRODUCTION

The black molly, *Poecilia sphenops*, is a common aquarium fish native in freshwater streams and brackish water habitats in Central and South America (from Mexico to Columbia), which often interbreeds with the sailfin molly (Shipp, 1986). This species has been introduced into parts of Southeast Asia. They are omnivorous and feed on various aquatic invertebrates, such as insects and worms, as well as plant and other organic debris. In natural conditions, fish can regulate and maintain their food intake and therefore their nutritional requirements, reducing the possibility of suffering nutritional deficiencies; however, this problem can be observed when the fish are subject to confinement conditions (Lovell, 2000).

The feed requirements of fish vary in quantity and quality according to their feeding habits and digestive anatomy as well as their size and reproductive state. Feed requirements are also affected by environmental variations such as temperature and the amount and type of natural food available (Gonzalez and Allan, 2007). It is important to highlight that the food intake in fish is closely related to its energy requirements, e.g. a decrease in feeding was observed in *Cichla* sp. at higher digestible energy (DE):brute protein (BP) ratios due to the fact that high dietary energy induce satiety (Sampaio e*t al.*, 2000). It was also observed that fish fed with low DE: BP ratio had a higher protein deposition due to the increase in the protein consumption (Sampaio *et al.*, 2000). Despite ornamental fish are known worldwide, the determination of nutritional requirements has not been studied deeply and it has based on the information from other species such as finfish used in aquaculture (Lovell, 2000). One advantage of ornamental fish over farmed fish is the low amount of food required, e.g. feeding requirements fluctuated from 3.8 mg feed/day/g BW in Neon tetra (*Paracheirodon innesi*) to 25.79 mg feed/day/g BW in goldfish (Pannevis and Earle, 1994). Fish food consists of natural food and artificial feeds. When fish have balanced diet to eat, they grow fast and stay healthy. In natural waters and well-fertilized

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ponds, microscopic plants (phytoplankton), microscopic animals (zooplankton), insects, crustaceans, copepods and molluscs are examples of natural foods. In the absence of natural foods, nutritionally complete manufactured feeds that contain all essential nutrients must be fed to fish.

In ornamental fish, a correct formulation of the diet improves the nutrient digestibility and supplies the metabolic needs, reducing the maintenance cost and at the same time the water pollution. The efforts in preparing suitable commercial feeds that will meet nutritional and physiological requirements of the fish and replace the natural food are still in progress. Such feed should contain around 25–50% protein, 10–15% carbohydrates and 12–15% of fat, as reported by Berka (1982) and Jirásek and Mare (2001a, 2001b). Main disadvantage of the industrially prepared feeds is dry matter content which is ten times higher compared to the natural food (Bogut *et al.,* 2010). In contrast with farmed fish, skin pigmentation is a mandatory characteristic in ornamental fish, and the use of dietary supplements with carotenoids is recommended (Velasco-Santamaría and Corredor-Santamaría, 2011).

Formulated feeds are complete feeds which supply all the nutrients necessary for optimal growth and health of the fish in culture practices. Fish nutrition has advanced dramatically in recent times with the development of new and balanced commercial feeds that promote optimal fish growth and health. In fish farming, nutrition is critical because feed represents 40–50% of the production costs. The development of new species-specific diet formulations supports the fish farming industry as it expands to satisfy increasing demand for affordable, safe, and high-quality fish. Artificial feeds are well-compounded mixture of feedstuffs and can be in mash or pellet form that could be fed to fish. Mash feeds are good for fries, and pellets (0.8 m–1 mm) for fingerlings, juveniles (2–3 mm) and adults (4.5 mm) depending on pellet sizes. Complete diets supply all the ingredients necessary for the optimal growth and health of the fish. Complete diets are used when the fish is totally dependent on the artificial diet, whereas, the diets used for fishes grown in natural condition requires the artificial feed for supplementary purpose. Supplemental diets do not contain a full complement of vitamins or minerals, but are used to help fortify the naturally available diet with extra protein, carbohydrate and/or lipid.

It is reported that the dietary protein level influences the body weight in several fish species (Chong *et al.,* 2004; Ling *et al.,* 2006). Protein requirements generally are higher for smaller fish. As fish grow larger, their protein requirements usually decrease. Protein requirements also vary with rearing environment, water temperature and water quality, as well as the genetic composition and feeding rates of the fish. According to Elangovan and Shim (1997), the comparison of protein requirements between fish species is complex since this can vary according to the size and life stage (Lochmann and Phillips, 1994), diet formulation or farming condition (Fiogbe and Kestemont, 1995). Protein is used for fish growth if adequate levels of fats and carbohydrates are present in the diet. If not, protein may be used for energy and life support rather than growth. The crude protein requirements in many fish species generally range between 25 and 55% (National Research Council, 1993). According to Sales and Janssens (2003), lipids are important sources of energy and fatty acids are essential for normal growth and survival of fish. Lipids supply about twice the energy as proteins and carbohydrates. Lipids typically comprise about 15% of fish diets, supply essential fatty acids (EFA) and serve as transporters for fat-soluble vitamins. In general, most fish require unsaturated fatty acids with long chains; nevertheless, the requirements vary according to the species with the most essential n-3 and n-6 fatty acids (Lovell, 2000). Freshwater fish require linoleic acid (18:2 n-6) or linolenic acid (18:3 n-3) whereas seawater species require eicosapentaenoic acid (20:5 n-3) and docosahexaenoic acid (22:6 n-3) Marine fish oils are naturally high (>30%) in omega 3 HUFA, and are excellent sources of lipids for the manufacture of fish diets (National Research Council, 1993).

In ornamental fish the information about carbohydrate metabolism and its function remain unknown compared to the broad knowledge in other fish species. The real role of CHO_n and glucose in fish is uncertain (National Research Council, 1993). Carbohydrates are the most economical and inexpensive sources of energy for fish diets. Although not essential, carbohydrates are included in aquaculture diets to reduce feed costs and for their binding activity during feed manufacturing. In fish, carbohydrates are stored as glycogen that can be mobilized to satisfy energy demands. Based on Lovell (2000), most fish species require vitamin supplementation which varies according to species, fish size, food rates, environmental factors, nutrient interrelationships or health condition. They often are not synthesized by fish, and must be supplied in the diet. Deficiency of each vitamin has certain specific symptoms, but reduced growth is the most common symptom of any vitamin deficiency. Scoliosis (bent backbone symptom) and dark colouration may result from deficiencies of ascorbic acid and folic acid vitamins, respectively. Minerals are inorganic elements necessary in the diet for normal body functions of fish. They can be divided into two groups (macro-minerals and micro-minerals) based on the quantity required in the diet and the amount present in fish. Fish can absorb many minerals directly from the water through their gills and skin, allowing them to compensate to some extent for mineral deficiencies in their diet. Considering the economic importance of mollies, the present paper reports results of four feeds formulated with different proportions of animal protein (fish meal, prawn meal and plankton meal) for economic production of this fish.

MATERIALS AND METHODS

Experiments were conducted to study growth and survival of fingerlings of *P. sphenops* using formulated diets. Five batches of fingerlings having 15 numbers in each 100 L fibre tank with 50 L of water were provided with five different types of feed. Four different types of formulated feeds were prepared using different proportions of fish meal, prawn meal and plankton meal. Whereas quantity of wheat flour, corn flour, starch, fish oil, vitamins and minerals were kept constant to make 1 kg diet. The dried trash fish and prawn that was not used for cooking formed the fish meal and prawn meal, respectively. Diet I was prepared in combination with fish meal and prawn meal, Diet II was prepared in combination with fish meal and plankton meal. Diet III and diet IV were prepared only with fish meal and plankton meal respectively. The quantity of the animal products, plant products, vitamins and minerals used to prepare these diets are presented in Table 1. The feeds were pelletized following standard procedure in a feed mill. Along with these four diets another batch of fishes was experimented with imported commercial pellet feed (Aini Pellet feed for Ornamental fishes-China). At the end of experiment length, weight and survival rate of the fish were measured and subjected to statistical analysis. During the experiment, the water quality parameters were analyzed periodically to monitor the health of the fish larvae. The mean values for temperature, dissolved oxygen, hydrogen ion concentration and salinity were 27 ± 2°C, 4.5- 5.2 mg/L, 6.8-7.5 and 0.2 g/L, respectively.

Length, weight and survival of fingerlings from all the tanks were measured and recorded on day 1 of the experiment, followed by once in seven days and then at the end of the experiment. The length gain, weight gain, survival rate, mortality rate and specific growth rate was calculated following standard procedure.

Length gain (mm) = Final length – initial length

Weight gain (g) = Final live weight – initial live weight

$$\text{Survival rate (\%)} = \frac{\text{Final no. of fishes}}{\text{Initial no. of fishes}} \times 100$$

$$\text{Mortality rate (\%)} = \frac{\text{Difference between initial and final no. of fishes}}{\text{Initial no. of fishes}} \times 100$$

$$\text{Specific Growth Rate (\%/day)} = \frac{\text{Final weight} - \text{Initial weight}}{\text{experimental period}} \times 100$$

The influence of different experimental feed on growth and survival were compared using one-way ANOVA at 0.05 level of significance using SPSS software version 10.

RESULTS

Results pertaining to length and weight gain, specific growth rate (SGR) and survival of feeding trials of fingerlings of *P. sphenops* with experimental diets and commercial pellet diet are presented in Table 2. The initial and final length of fishes fed with Diet I were 2.55 ± 0.24 and 3 ± 0.22 cm, showing a length gain of 0.47 cm. Similarly, the initial and final weight of fishes were 0.23 ± 0.05 and 0.36 ± 0.08 g with a weight gain of 0.12 g. The specific growth rate of these fishes was 0.34% and their survival rate and mortality rate were 100% and 0% respectively.

Mollies fed with Diet II had an initial and final length of 2.71 ± 0.27 and 3.29 ± 0.36 cm and initial and final weight of 0.23 ± 0.06 and 0.47 ± 0.17 g. Thus, the length and weight gain was 0.58 cm and 0.24 g, respectively. Their specific growth rate was 0.69% and their survival rate and mortality rate was 66.67% and 33.33%, respectively.

When fed with Diet III, mollies showed initial and final length as 2.62 ± 0.20 and 3.36 ± 0.25 cm and initial and final weight as 0.23 ± 0.04 and 0.48 ± 0.17 g, respectively. The length and weight gain was 0.74 cm and 0.25 g, respectively. The specific growth rate was 0.71% and survival rate and mortality rate was 53.33% and 46.67%, respectively.

Mollies fed with Diet IV showed length and weight gain as 0.74 cm and 0.25 g, respectively, with initial and final length of fishes as 2.62 ± 0.20 and 3.36 ± 0.25 cm and initial and final weight of fishes as 0.23 ± 0.04 and 0.48 ± 0.17 g, respectively. Their specific growth rate was 0.71%. Their survival rate and mortality rate was 53.33% and 46.67%, respectively.

Mollies fed with commercial pellet feed had the survival rate as 33.33% and mortality rate as 66.67%. The specific growth rate was 1%. Their length and weight gain was 0.75 cm and 0.35 g, respectively, with initial and final length as 2.75 ± 0.21 and 3.50 ± 0.33 cm and initial and final weight as 0.23 ± 0.05 and 0.58 ± 0.20 g, respectively (Figure 1-5).

One-way ANOVA for the length of the fishes fed with formulated feed also shows significant difference at p=0.05 level of confidence in the length gain (Control feed: df=79, F=15.61, p=0.00; Diet I: df=89, F=9.33, p=0.00; Diet II: df=84, F=5.76, p=0.00; Diet III: df=80, F=23.62, p=0.00 and Diet IV: df=84, F=3.16, p=0.01). One-way ANOVA for the weight of the fishes fed with formulated feed also shows significant difference at p=0.05 level of confidence in the length gain (Control feed: df=79, F=22.15, p=0.00; Diet I: df=89, F=6.84, p=0.00; Diet II: df=84, F=9.99, p=0.00; Diet III: df=80, F=36.19, p=0.00 and Diet IV: df=84, F=9.01, p=0.00). Variation with regard to length and weight gain, SGR and survival rate of mollies was observed with experimental and commercial pellet diets.

Table 1. Composition of different macro-nutrients and micro-nutrients (g/kg) in the four formulated diets for finger lings of *P. sphenops*

SI. No.	Ingredients	Diet I	Diet II	Diet III	Diet IV
1	Fish meal	300	300	400	-
2	Prawn meal	100	-	-	-
3	Plankton meal	-	100	-	450
4	Wheat flour	400	400	400	450
5	Corn flour	100	100	100	-
6	Starch	50	50	50	50
7	Fish oil	40	40	40	40
8	Vitamins	5	5	5	5
9	Minerals	5	5	5	5

Table 2. Growth performance (Mean ± SD) of fingerlings of *P. sphenops* fed with four different formulated diets and commercial pellet feed in 35 days experiment.

Growth performance	Diet I	Diet II	Diet III	Diet IV	Pellet feed
Initial length of fishes (cm)	2.55 ± 0.24	2.71 ± 0.27	2.62 ± 0.20	2.73 ± 0.22	2.75 ± 0.21
Final length of fishes (cm)	3 ± 0.22	3.29 ± 0.36	3.36 ± 0.25	3.13 ± 0.34	3.50 ± 0.33
Initial weight of fishes (g)	0.23 ± 0.05	0.23 ± 0.06	0.23 ± 0.04	0.24 ± 0.05	0.23 ± 0.05
Final weight of fishes (g)	0.36 ± 0.08	0.47 ± 0.17	0.48 ± 0.17	0.42 ± 0.14	0.58 ± 0.20
Gain in length (cm)	0.47	0.58	0.74	0.4	0.75
Gain in weight (g)	0.12	0.24	0.25	0.18	0.35
Specific growth rate (SGR) (% /day)	0.34	0.69	0.71	0.51	1
Survival rate (%)	100	66.67	53.33	66.67	33.33
Mortality rate (%)	0	33.33	46.67	33.33	66.67

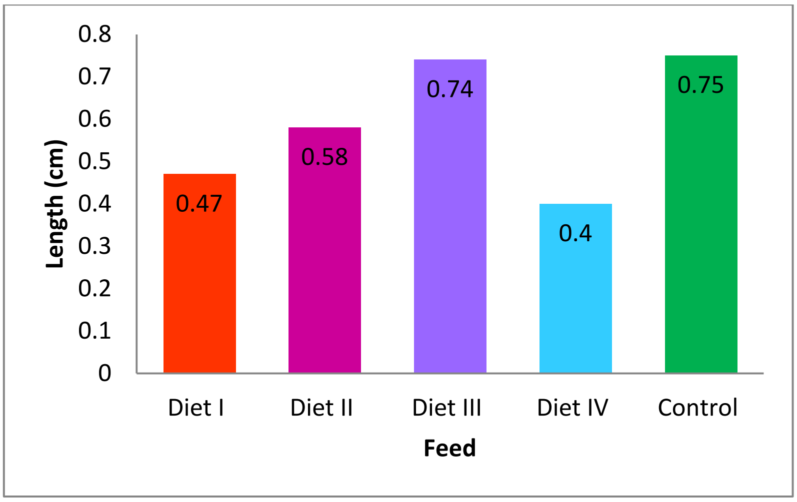

Figure 1. Length gain of fries and fingerlings of *P. sphenops* with different formulated diets.

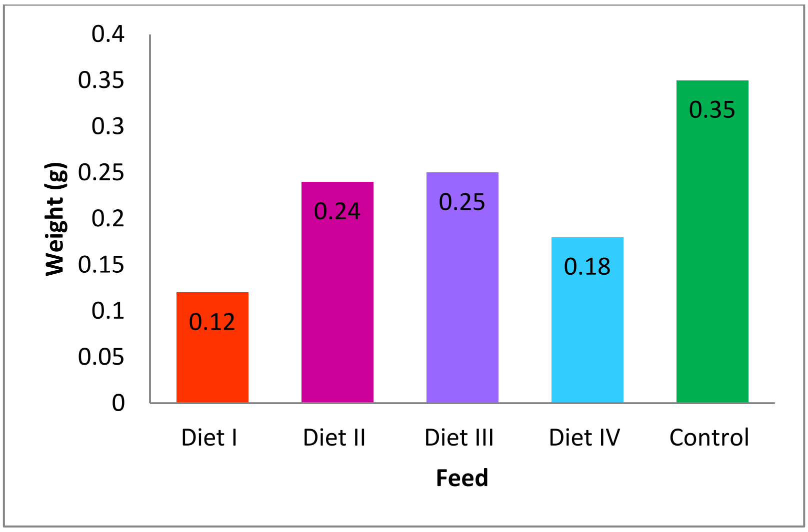

Figure 2. Weight gain of fries and fingerlings of *P. sphenops* with different formulated diets.

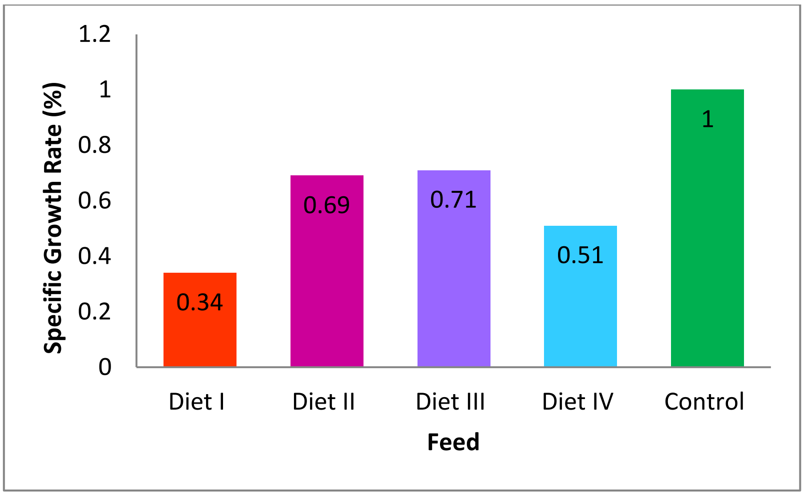

Figure 3. Specific growth rate of fries and fingerlings of *P. sphenops* with different formulated diets.

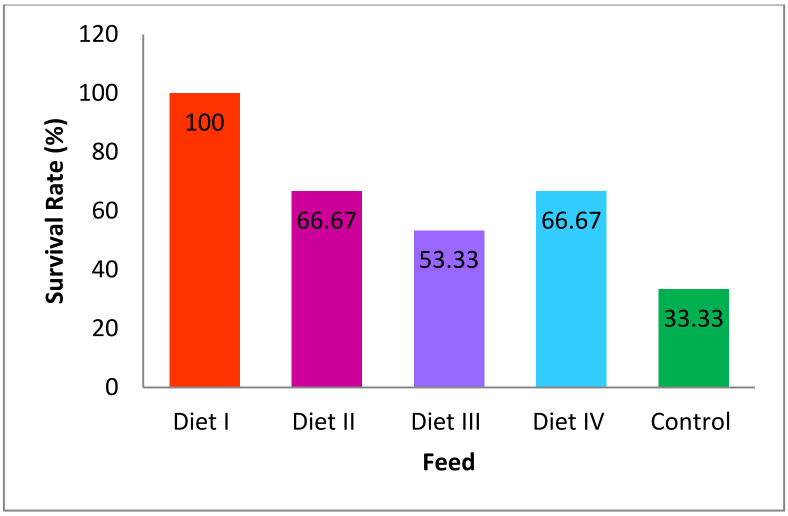

Figure 4. Survival rate of fries and fingerlings of *P. sphenops* with different formulated diets.

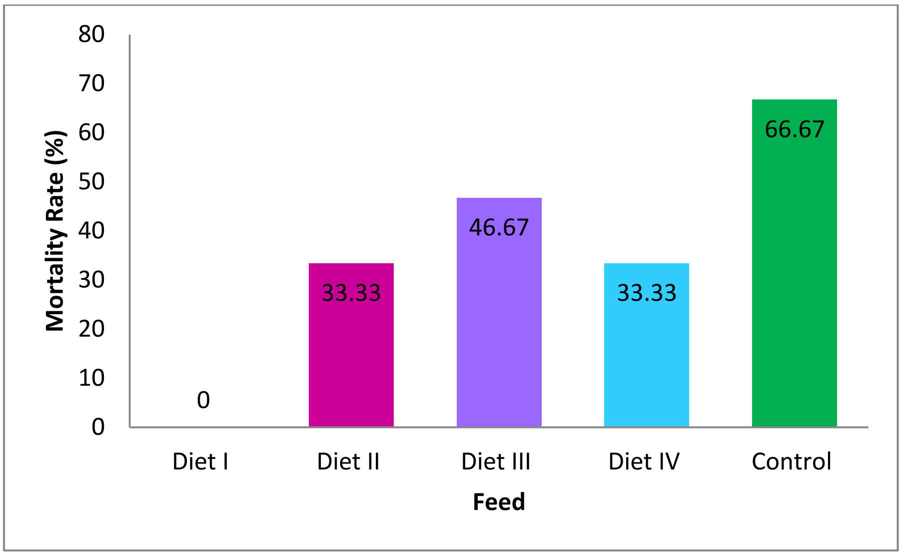

Figure 5. Mortality rate of fries and fingerlings of *P. sphenops* with different formulated diets.

DISCUSSION

Ornamental fishes are reared in high density in indoor systems and cannot forage freely on natural feeds, they should be provided with a complete diet. Complete diets supply all the ingredients necessary for the optimal growth and health of the fish. In general, complete diet formulated with organic and inorganic components constitute the following proportions, protein (18-50%), lipid (10-25%), carbohydrate (15-20%), ash (<8.5%), phosphorus (<1.5%), water (<10%), and trace amounts of vitamins, and minerals. In the present study fishes were reared in aquarium tanks and hence complete artificial diet was formulated. All the experimental diets supported the growth of the mollies as evidenced by their growth parameters.

Fish requires two to four times more dietary protein compared to warm-blooded animals, due to relatively higher need of essential amino acids in fish. Further, there exists a difference in optimum level of protein requirement between carnivorous and omnivorous fishes. Typically, carnivorous fish requires 40-55% dietary protein against only 35-45% protein requirement in omnivorous fishes. On dry weight wet basis, proteins contribute about 65-75% of the total weight of animal tissue. The dietary protein should be just enough for growth and repair as it is more expensive than carbohydrates and fats. Moreover, any excess amount of protein can be utilized for energy production (Shim and Chua, 1986). Preliminary study on guppy (*Poecilia reticulata*) under controlled conditions indicated a dietary protein level of 30-40% for maximum growth, efficient feed conversion. Protein levels in aquaculture feeds generally average 28-32% for catfish, 32-38% for tilapia, and 38-42% for hybrid striped bass. Protein requirements usually are lower for herbivorous fish and omnivorous fish than they are for carnivorous fish. Protein requirements generally are higher for smaller fish. As fish grow larger, their protein requirements usually decrease. Protein requirements also vary with rearing environment, water temperature and water quality, as well as the genetic composition and feeding rates of the fish.

Carbohydrates are needed for energy in body activities. In artificial feed, it is usually used as a binder. It has good water binding capacities, reduced leakage of water soluble substances. Though in fishes, carbohydrates supply less energy per gram than that of lipids or proteins, they are still the cheapest source of dietary energy. In fact carbohydrates are not essential in the fish diet; these are included in feeds to increase the bulk, reduce the feed costs and to improve binding activity of ingredients. Carnivorous fishes are less well adapted to metabolise more amount of carbohydrate as it may be low in natural diets. Absence of carbohydrate in fish diet does not elicit any ill effect as these can be synthesized from dietary lipid sources. The dietary carbohydrate level up to 25% is as effective as fat as an energy source for most species. It also exerts protein-sparing effect in fish. It appears that sufficient carbohydrate in the diet obviates the need for gluconeogenesis. Besides lowering the cost of diets, carbohydrates also improve the pellet binding capacity of the fish feed Dietary fat ranges 3-15% (9-40% dry weight). Dietary lipids are usually added in some formulated feeds for two purposes – as a source of metabolic energy and to maintain structure and integrity of cellular membranes. Generally, the commercial feeds contain a minimum of 12% fishmeal and additional animal by-products, which probably ensures them against serious fatty acid deficiencies (Cowey and Sargent, 1972).

In all the four diets experimented in the present study the proximate composition, vitamins and minerals were included as per the requirements of these components in fish feed. Growth and development of *P. sphenops* suggest all the diets supported normal growth, nevertheless variation in the growth performance with different diets might be due to the difference in the composition of the macro-nutrient of these diets.

Length and weight gain and specific growth rate was maximum when fishes were fed with control pellet feed, followed by Diet III feed. The Diet I showed highest percentage of survival percentage. The length gain of fishes fed with Diet I was approximately similar to that of fishes fed with Diet IV and lesser than the fishes fed with Diet II, III and pellet feed. Similarly, their weight gain was not as high as that of fishes fed with other three diets and pellet feed. The survival rate of fishes fed with Diet II and Diet IV was similar. The survival rate of fishes fed with Diet III was lesser than that of Diet I, II and IV. When mollies were fed with commercial pellet feed the mortality rate of fishes was the highest and the survival rate of fishes was less compared to the four different types of formulated feed.

CONCLUSIONS

Of the four formulated experimental diets, diet III with fish meal exclusively as major macro-nutrient has given growth performance similar to the commercial pellet diet, while diet I and diet II formulated in combination of prawn meal and plankton meal, respectively as well as diet IV exclusively with plankton meal showed relatively lower length and weight gain. Nevertheless, significantly higher percent of survival rate of *P. sphenops* was recorded in the experimental diets than in the commercial pellet diet. In view of higher cost and mortality rate of imported commercial pellet feed compared to experimental diets. Experimental diets formulated with locally available animal protein sources could be used in commercial production of molly fish.

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