Growth and reproductive performance of female guppy, *Poecilia reticulata* (Peters) fed diets with different nutrient levels

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ABSTRACT

Three feeds used in guppy (*Poecilia reticulata*) farming in south Asia were evaluated for their effects on growth and reproductive performance of females. Feeds namely diet - 1, 2 and 3 contained 18.26%, 29.27% and 43.60% protein and 4.17%, 4.55% and 9.47% lipid respectively. The growth and reproductive performance were evaluated based on growth parameters, gonadal development and fry production. Fish fed diet-3 had significantly (p<0.05) higher specific growth rate compared to the other two groups. The ovary weight and ovary volume of guppy fed with diet-3 were also significantly (p<0.05) higher than those with diets 1 and 2. The absolute fecundity values were 4.28±1.61, 7.98±1.89 and 18.44±2.07 respectively for the fish fed diet-1, diet-2 and diet-3. The number of fry produced and their survival were also significantly (p<0.05) higher in fishes fed diet-3. The diet which resulted in better growth and reproductive performance of female guppy, was found to contain the recommended levels of protein and lipid compared to other diets. The results of the present study demonstrated that the use of feed with inadequate nutrients can lead to poor reproductive performance thereby necessitating more breeding area to fulfill the production targets especially in commercial guppy farming.

Keywords: Broodstock nutrition; FCR, Fecundity, Guppy, Specific growth rate

Introduction

Live bearing species of the family poeciliidae such as guppies (*Poecilia reticulata*), platies (*Xiphophorus maculatus*), swordtails (*Xiphophorus helleri*) and molly (*Poecilia latipinna, Poecilia sphenops*) are popular ornamental or aquarium fishes produced in many Asian countries (Chong *et al.*, 2004). The guppy is considered to be the most popular aquarium fish (Whitern, 1979) and three of the top 10 species in the world ornamental fish trade are members of the family poeciliidae (Singh, 2005). The farming of poeciliids is carried out in indoor cement tanks, outdoor earthen ponds or net cages (Fernando and Phang, 1994). Feeding may rely on live feed such as blood worm *Tubifex*, coupled with daily prepared feed consisting of mixtures of fish meal, skimmed milk powder, locally available ingredients such as rice bran and wheat flour. These feeds may not provide broodstock fish with adequate nutrients to promote optimal reproduction (Fernando *et al.*, 1991).

Broodstock nutrition is an important factor governing egg production and larval survival (Izquierdo *et al.*, 2001). An improvement in broodstock nutrition and feeding has been shown to improve not only egg quality but also enhance seed production. Gonadal development and fecundity are affected by certain essential nutrients (Izquierdo *et al.*, 2001). Dietary protein and lipid play major roles in growth and reproductive performance (Watanabe *et al.*, 1984; Watanabe and Kiron, 1995; Furuita *et al.*, 2002).

Dahlgren (1980) found that female guppy fed 31% protein levels showed the highest reproductive performance. Shim and Chua (1986) suggested that 30-40% dietary protein should be the optimal level for feeding guppy and the female fish fed 30%-40% dietary protein levels and 9-10.5% lipid levels gained the highest mean body weight, ovary weight, gonado-somatic index (GSI %) and number of yolky oocytes. Ling *et al.* (2006) proposed the dietary protein and lipid requirements for optimized growth and reproductive performance of female swordtails to be at 30% and 12% respectively. Chong *et al.* (2004) found that a minimum of 30% protein should be included in the diet of female swordtail broodstock.

The live bearing poeciliidae breed easily in captivity (Ling *et al.*, 2006) and in view of this, farmers do not pay much attention to provide the fish with nutritionally balanced feed. Owing to the use of unbalanced feed in guppy farming, problems relating to small brood size, deformed fry and low survival have been reported by...
farmers. Ultimately poor broodstock nutrition will lead to the production of inferior quality fry with low survival rate. Therefore, the current study was carried out to evaluate the effect of three types of feed presently being used in guppy farming on the growth and reproductive performance in female guppy.

**Materials and methods**

**Experimental setup, animals and diets**

The experiment was conducted over a period of seven months from October 2005 to April 2006 at the Central Institute of Fisheries Education, Mumbai, India. There were three treatment groups, each with three replicates, in nine 160 L rectangular plastic tubs (763 X 521 X 410 mm).

The sexes were separated to obtain virgin females for the growth experiment. Each tub was stocked with 60 *P. reticulata* female fry of almost uniform size (0.077-0.087g). Aeration was provided using a 2 HP air blower. Tubs were cleaned by siphoning fecal matter and uneaten food and 50% of the water was replaced with fresh chlorine-free borewell water every alternate days.

Three types of feed, which are used in the guppy farming, were evaluated in the study. Diet-2 is a commercially available diet whereas diet-1 and diet-3 were prepared using ingredients which are being used by farmers in guppy farming. Diet-1 was made mainly with wheat flour, rice bran and maize bran. Diet-3 was made of rice bran, wheat flour, fish meal and ground chicken feed.

**Feeding of fish**

Feeding was carried out until satiation, twice a day at 0800 and 1700 h throughout the experimental period. At each reading a small amount of feed was dropped into the tank and this process was repeated until satiation was observed. At the end of the week, the amount of feed left in each polythene pouch was weighed and the food consumed was calculated.

**Physico-chemical parameters of water**

The water quality parameters (water temperature, dissolved oxygen, pH, ammonia, nitrite and hardness) were monitored weekly throughout the experimental period. The dissolved oxygen and temperature were measured using a portable meter (Merck, Germany) and the other parameters were measured using colourimetric methods (APHA-AWWA-WEF, 1998).

**Growth parameters**

The growth performance in terms of specific growth rate of female guppy fry was assessed by recording their body length and weight initially and after two months rearing period. Before body weight measurements, the animals were kept overnight without food. The specific growth rate and food conversion ratio were determined using the following formulae

Specific growth rate (SGR %/day) = \( \frac{\ln W_f - \ln W_i}{T} \) \times 100

where \( W_f \) = mean final weight, \( W_i \) = mean initial weight and \( T \) = Total experimental days

Food conversion ratio (FCR) = total feed fed (g)/total wet weight gain (g)

**Reproductive performance**

Reproductive performance was determined in terms of ovary weight, ovary volume, ova diameter, absolute fecundity, GSI % and number of fry produced throughout the breeding period. Twenty fishes from each treatment were used to determine the reproductive performance. Dissections were carried out under a binocular stereoscopic microscope. After excision of the gonad, the oviduct and mesovarium were separated and removed. The ovarian length, width, height and weight were measured using digital vernier calipers (Zoom digimatic caliper) and ovary volume (length \times width \times height) was calculated. After taking all ovary measurements, one portion of ova were preserved in 4% formalin for the measurement of egg diameter using a microscope aided with computer software package (Motic Image Conversion 3.1). The other portion of the ovary was used to extract the lipids. The reproductive performance was calculated using the following formula:

Absolute fecundity = Number of mature oocytes in the ovary prior to spawning (Bagenal, 1973)

\[
GSI \% = \frac{\text{Weight ovary (g)}}{\text{Weight of fish (g)}} \times 100
\]

**Preparation of tanks for breeding and fry collection**

The 20 female fishes, which were separated from the experimental tanks, were used for breeding purpose. Three replicates of each diet group were used and each tank was stocked with 20 female fishes and 10 males of the same size. Breeding tanks were provided with polythene strips arranged in bundles to offer shelter for the new swimming fry from parental cannibalism. Each bunch of polythene strips was tied to a weight, which helped to keep it in a fixed position in the tank. After the gestation period, the newly born fry in each tank were collected daily using a hand net during the feeding time and kept in separate tanks used for fry collection and the number was recorded. The fry collected from each replicate breeding tank during one month period was put into one tank to avoid cannibalism and three tanks were used to collect fry from each replicate tanks during the three months breeding period.
Biochemical analysis of tissues and diets

At the beginning of the experiment, 30 fishes were selected, weighed and subjected to proximate analysis (AOAC, 1995) to determine the moisture, crude protein, crude fat and ash content of the carcasses. On termination of the experiment also the fishes were sampled and subjected to proximate analysis in order to determine the body composition.

Statistical analysis

Comparison of various growth and reproductive parameters from different dietary treatments was carried out using analysis of variance (ANOVA) and Duncan’s test by SPSS (2005) wherever applicable. The data are presented as mean ± S.E. of three replicate groups.

Results and discussion

The water temperature recorded in the experimental tanks were within the acceptable range (23.5-28.3 °C). Optimal temperature for reproduction in all live bearers are reported to be between 22 and 26 °C (Dawes, 1991). Dissolved oxygen (5.6-6.8 mg l⁻¹), pH values (7.5-8.1) and hardness (225-242 mg l⁻¹) were also within the acceptable range throughout the experimental period. Total ammonia (<0.34 mg l⁻¹) and nitrite levels (<0.003 mg l⁻¹) were maintained below the sub-lethal levels by changing water on alternate days. The water quality parameters were maintained within the acceptable range for the growth and reproduction of guppy and hence there was no effect of water quality parameters on the growth and reproduction.

The proximate compositions of the diets are given in Table 1. The three diets contained three different levels of nutrients. Diet-1, 2 and 3 contained 18.26%, 29.27% and 43.60% protein and 4.17%, 4.55% and 9.47% lipid respectively. Initial proximate composition of the fishes selected, weighed and subjected to proximate analysis inorder to determine the body composition.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Percentage composition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diet-1</td>
</tr>
<tr>
<td>Moisture</td>
<td>6.40</td>
</tr>
<tr>
<td>Crude protein</td>
<td>18.26</td>
</tr>
<tr>
<td>Crude lipid</td>
<td>4.17</td>
</tr>
<tr>
<td>Total carbohydrates</td>
<td>69.14</td>
</tr>
<tr>
<td>Ash</td>
<td>8.43</td>
</tr>
</tbody>
</table>

Growth performance

The nutritional requirements for the ornamental fishes have been reported by Swain (1999). The fish fry can be fed with 40 - 50% protein, 4 - 6% lipid and 40 - 50% carbohydrates while the adult or brood fishes can be fed with 30 - 35% protein, 6 - 8% lipid and 40 - 50% carbohydrates after maturation, the values varied between 53.95 and 56.85%. Satia (1974) observed a general increase in the protein content in the carcass of rainbow trout (Oncorhyncus mykiss) in relation to the amount present in the diet. Ogino and Saito (1979) also reported that there was a linear relation between protein content of the diet and the body protein content of the young carp Cyprinus carpio. However, in the present study, significant difference (p<0.05) was observed in the fish fed diet-3 compared with the fish fed diet-1. Ling et al. (2006) also found dietary protein and lipid significantly influenced muscle protein content of female swordtail Xiphophorous helleri. There was no significant difference between the protein levels in the muscle of the fish fed diet-2 and diet-3, although the protein levels were significantly different in the diets. Crude fat level in fish fry was 24.97% and after maturation, the fat content varied between 26.56 and 29.83%. In the present experiment, the dietary lipid levels significantly influenced the crude fat levels of carcass. The highest amount of lipid was found in carcass of the fish fed diet-3. It is known that there is a strong relationship between the dietary lipid levels and the levels of lipid in the carcass of fish (Cowey, 1993). Earlier studies have also reported increased lipid concentration in fish carcass corresponding to increased levels of dietary lipids (Chou and Shiau, 1996; Chaiyapechara et al., 2003; Lee and Sang, 2005).

Table 2. Carcass (muscle) analysis (mean values ±SE) of female guppy after 3 months rearing period

<table>
<thead>
<tr>
<th>Diet type</th>
<th>Percentage on wet weight basis</th>
<th>Percentage on dry weight basis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Moisture</td>
<td>Dry matter</td>
</tr>
<tr>
<td>At beginning of the experiment</td>
<td>61.15±0.19</td>
<td>38.21±0.16</td>
</tr>
<tr>
<td>At end of the experiment</td>
<td>57.83±0.16</td>
<td>41.50±0.25</td>
</tr>
<tr>
<td>Diet-1</td>
<td>59.76±0.29</td>
<td>38.52±0.16</td>
</tr>
<tr>
<td>Diet-2</td>
<td>57.38±0.10</td>
<td>42.22±0.17</td>
</tr>
</tbody>
</table>

Values within a column with different superscript letters are significantly different (p<0.05)
carbohydrates. Dahlgren (1980) conducted an experiment with three types of feed with different protein levels and recorded higher growth and reproductive performance in the fishes fed 35% to 47% protein levels. Compared to the recommended nutritional requirements, diet-1 did not contain the required protein and lipid levels while diet-2 did not contain the required lipid level. Whereas, diet-3 contained higher protein percentage and required amount of lipid. Therefore, by comparing the results of the present study with that of the required dietary protein and lipid levels reported, it is possible to establish the effect of three diets used on growth and reproduction of female guppy.

It has been reported that decrease in body weight occurs when the fish is fed with low protein diets (Dabrowski, 1977; Jauncey, 1982). Shim and Chua (1986) noted that guppy fed with diets having more than 20% protein levels had significantly greater gains in mean body weight than the fish fed with less than 20% of dietary protein levels. They recommended 30-40% dietary protein levels for feeding of guppy. The mean values of total length, standard length and final weight after 3 months rearing period are given in Table 3. The total and standard lengths of the fish fed diet-3 was significantly (p<0.05) higher than that of the fish fed diets 1 and 2, since diet-3 contained all the dietary nutrient requirements especially protein and lipid for the growth of fish. The mean initial weight of fry used for the experiment was in the range of 0.077-0.087 (Table 3) and final mean weight after three months rearing period ranged from 0.32 to 0.89 g. The feed conversion values of the fish fed with diet-1 had a very high value (Table 3) indicating the non-availability of required nutrients for the growth of fish. The survival (%) of fishes fed diet-3 was found significantly (p<0.05) higher than the other two groups during the study.

### Table 3. Growth parameters (mean values ±SE) of female guppy fed different diets

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Diet-1</th>
<th>Diet-2</th>
<th>Diet-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total length (mm)</td>
<td>21.8±0.87</td>
<td>29.14±0.79</td>
<td>36.03±0.76</td>
</tr>
<tr>
<td>Standard length (mm)</td>
<td>17.71±1.17</td>
<td>23.94±0.75</td>
<td>29.38±0.76</td>
</tr>
<tr>
<td>Initial weight (g)</td>
<td>0.077±0.01</td>
<td>0.081±0.01</td>
<td>0.087±0.02</td>
</tr>
<tr>
<td>Final weight (g)</td>
<td>0.27±0.01</td>
<td>0.35±0.01</td>
<td>0.48±0.01</td>
</tr>
<tr>
<td>Weight gain (g)</td>
<td>0.193±0.01</td>
<td>0.269±0.01</td>
<td>0.393±0.01</td>
</tr>
<tr>
<td>SGR (%)</td>
<td>0.90±0.03</td>
<td>1.06±0.02</td>
<td>1.24±0.02</td>
</tr>
<tr>
<td>FCR</td>
<td>3.50±0.10</td>
<td>2.21±0.11</td>
<td>1.70±0.01</td>
</tr>
<tr>
<td>Survival (%)</td>
<td>79.29±0.94</td>
<td>82.89±0.57</td>
<td>92.54±0.89</td>
</tr>
</tbody>
</table>

SGR= Specific growth rate; FCR= Feed conversion ratio
Values within a row with different superscript letters are significantly different (p<0.05)

The specific growth rates (SGR) for the fish fed with diets 1, 2 and 3 were 0.69, 0.86 and 1.12 respectively (Table 3). The SGR value of the fish fed with diet-3 was found significantly (p<0.05) higher than that of the other two groups which indicates that the nutrients available in the diet-3 fulfilled the dietary requirements of guppy to grow faster than other two groups. The fish fed diet-1 had the lowest SGR value indicating that the diet did not have the basic nutritional requirements for growth. Feed conversion ratio in the group of fish fed with diet-3 was significantly (p<0.05) lower than the fish fed diet-1 and diet-2. The significantly low FCR recorded in the group fed with diet-3 clearly indicates that those fishes were fed with a diet having required amounts of protein and lipid. The survival (%) of fishes fed diet-3 was found significantly (p<0.05) higher than the other two groups.

### Reproductive performance

Protein and lipid levels of broodstock diet have been identified as major dietary factors that determine successful reproduction and survival of off-spring (Samath and Pandian, 1984; James and Sampath, 1993; Jobling, 1998). Shim and Chua (1986) found that the diets with 30 to 40% protein appeared to be the best for gonadal development since those diets resulted in the greatest mean ovary weight and in the largest mean number of yolky oocytes in the ovary of guppy. The fish fed with diet-3, which contained 43.6% crude protein and 9.47% crude lipid had a significantly (p<0.05) higher breeding performance than other two groups.

### Ovary development

The ovarian weight and volume, ova diameter, absolute fecundity and mean percentage GSI values are given in Table 4. Variations in size of ovaries of matured female guppy fed different diets are presented in Fig. 1. Oocyte maturation involves transportation and accumulation of protein and lipid in the oocytes (Tyler and Sumpter, 1996). Feeding higher levels of protein and lipid resulted in better gonadal development in female guppy. Fish fed with diet-3 had significantly (p<0.05) higher ovary weight, ovary volume, number of ova, ova diameter,
absolute fecundity and mean GSI % compared to fish fed with diet-1. The optimized growth rate in broodstock has been positively linked with reproductively related factors such as earlier oocyte maturation, higher rate of vitellogenesis, better spawning performances and larger egg size (Milton and Arthington, 1983; Shim et al., 1989; Seghal and Toor, 1991; Gunasekara et al., 1995; EI-Sayed et al., 2004). Dahlgren (1980) observed that guppy females fed on a high protein diet developed comparatively heavier gonads indicating a higher ability to mobilize nutrients for the protective reproductive tissue. The fish fed diet-2 had significantly (p<0.05) higher ovary weight, ovary volume, absolute fecundity and % mean GSI than the fish fed with diet-1. Probably only when feeding with extremely high or low protein amounts, obvious influences on fecundity may appear (Dahlgren, 1980). The inferior ovarian mass recorded in the fish fed dietary protein of 18.26%, indicate that 18.26% dietary protein level could be below the requirement for proper oocyte development in female guppy. However, no significant difference was observed in the ova diameter of the fish fed with diet 1 and 2. Compared to the fish fed with diet-3, the poor development of ovary in fish fed diet-1 and 2 indicates that the nutrient levels of these diets could be below the optimal requirement for gonadal development in female guppy. Parameters such as egg size and composition have been proposed to be useful indicators of seed production in terms of hatchability and fry quality. Several studies have shown that larger egg size will eventually result in larger fry at hatching (Seghal and Toor, 1991).

**Fry production**

Mean total fry production and fry survival rate values recorded are given in Table 5. Mean total fry production was significantly (p<0.05) higher in fish fed with diet-3 (43.6% protein and 9.47% lipids) than the other treatments. Fry survival had no significant difference between diets 1 and 2 as well as 2 and 3. Ling et al. (2006) also observed that total fry production of female swordtail was influenced by dietary protein levels and was generally higher in 30% dietary protein diets as compared with the protein diets. They also observed that dietary protein and lipid levels affected total fry production by female swordtail broodstock. The results of the present study also indicated that the diet-3, which contained highest levels of protein and lipid, showed the maximum fry production in guppy female compared to the fish fed other two diets. In addition to the supply of essential fatty acids for reproductive physiology, dietary lipid can also provide energy to perform spawning activities. Another possible reason for the highest fry production in the fish fed with diet-3 could be due to the provision of optimal dietary fatty acids for regulation of reproductive physiology. The diet-1 and diet-2 probably had inadequate levels of essential fatty acids for proper maintenance of growth and reproduction. The mean values of weekly fry production are depicted in Fig. 2. The mean weekly fry production was found to be higher in the fish fed with diet-3 compared to the other two groups throughout the study period. Further, the fish fed higher levels of nutrients (diet-2 and 3) produced fry, weeks earlier than the fish fed with diet-1. It has been reported that the dietary protein and lipid levels play a major role in weight gain in fishes and provision of adequate levels will lead to higher fry production (Milton and Arthington, 1983). Significant positive correlations have also been observed between fry production and final weight of female swordtail (Chong et al., 2004; Ling et al., 2006). Though the absolute fecundity of females fed diet-2 and 3 were significantly different, the diameters of ova were not significantly different. This may

<table>
<thead>
<tr>
<th>Feed type</th>
<th>Ovarian weight (g)</th>
<th>Ovarian volume (mm$^3$)</th>
<th>Ova diameter (mm)</th>
<th>Absolute fecundity</th>
<th>Mean GSI %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet-1</td>
<td>0.02±0.011</td>
<td>30.50±3.39</td>
<td>1.35±0.08</td>
<td>4.28±0.61</td>
<td>6.25±1.63</td>
</tr>
<tr>
<td>Diet-2</td>
<td>0.05±0.013</td>
<td>73.52±5.40</td>
<td>1.50±0.01</td>
<td>7.98±0.89</td>
<td>10.41±0.91</td>
</tr>
<tr>
<td>Diet-3</td>
<td>0.12±0.012</td>
<td>120.53±13.97</td>
<td>1.67±0.02</td>
<td>18.44±2.07</td>
<td>13.48±1.24</td>
</tr>
</tbody>
</table>

Values within a column with different superscripts are significantly different (p<0.05)

<table>
<thead>
<tr>
<th>Diet type</th>
<th>Mean total fry production</th>
<th>Fry survival rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet-1</td>
<td>43.00±3.0</td>
<td>82.8±1.6</td>
</tr>
<tr>
<td>Diet-2</td>
<td>85.5±5.5</td>
<td>85.8±0.19</td>
</tr>
<tr>
<td>Diet-3</td>
<td>133.5±3.5</td>
<td>90.37±1.7</td>
</tr>
</tbody>
</table>

Values within a column being different superscripts are significantly different (p<0.05)

Fig. 2. Mean weekly production of fry (± SE) of female guppy fed different diets
due to fact that the amount of nutrient present in the mature fish contributes to the development of fish oocyte taking care not the number, but the quality of the ovum. The fish fed diet-1 produced the lowest number of fry in order not to compromise the quality of the young fry, a strategy reportedly observed in other poeciliids (Milton and Arthington, 1983).

It is evident from the results of the present study that the diet-3, which contained the recommended amount of dietary protein and lipid levels resulted in better growth and reproductive performance of female guppy compared to other the diets. Therefore, when a feed is formulated for guppy, the basic dietary requirements especially protein and lipid levels should be taken into account since these two components play a crucial role on growth and reproductive performance of fishes. The present study also demonstrated that the use of feeds with inadequate nutrients could lead to poor reproductive performance which in turn will lead to allocation of more breeding area to fulfill the production target especially in the commercial level guppy farming.

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