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Effect of different diets on larval growth and juvenile production of Nilgiri Melon barb *Haludaria fasciata*

Efecto de dietas prácticas seleccionadas sobre el crecimiento larvario y la producción de juveniles de Nilgiri Melon barb *Haludaria fasciata*

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ABSTRACT

Larval feeds play a vital role in the production of juvenile fish in hatcheries. The present study evaluated the effect of six different diets on the growth and survival of larvae of Nilgiri Melon barb *Haludaria fasciata*. The selected experimental diets were Growfin (GF), Elarval 100 (EL), *Spirulina* powder (SP), Decapsulated *Artemia* eggs (DA), Live *Artemia* nauplii (LA) and a combination of live artemia and Prince Wean 300 (LAP). The larvae at 3 days post-hatch (dph) were collected and fed with the experimental diets for 35 days. The feeding was done thrice daily under each treatment group. The intermediate growth in total length and larval survival was measured every five days and the body weight of fish larvae on the initial and final days was calculated during and after the treatment. The larvae fed with LAP had a higher mean total length than all other treatments. The mean weight was observed to be higher in LA, which significantly ($P<0.05$) differs from other treatments. The average specific growth rate was higher in DA, LA and LAP. The heterogeneous growth was observed lower in treatment LAP than in other treatments. It is observed that live feeds are not compulsory for the larval feeding program of *H. fasciata*. Artemia nauplii can be replaced partially or fully with micro diets such as EL and Prince Wean without heavily affecting the growth and survival of larvae.

Keywords: Larval feeds, Inert feeds, Specific growth rate, *Haludaria*, Feeding protocol

RESUMEN

Los alimentos para larvas juegan un papel vital en la producción de peces juveniles en los criaderos. El presente estudio evaluó el efecto de seis dietas diferentes sobre el crecimiento y la supervivencia de larvas de Barbo melón *Haludaria fasciata*. Las dietas experimentales seleccionadas fueron Crecimiento de Aleta (GF), Estimulante

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larval 100 (EL), polvo de espirulina (SP), huevos de *Artemia* decapsulados (DA), nauplios de *Artemia* viva (LA) y una combinación de *Artemia* viva y Prince Wean 300 (LAP). Las larvas a los 3 días posteriores a la eclosión (dph) se recolectaron y alimentaron con las dietas experimentales durante 35 días. La alimentación se realizó tres veces al día en cada grupo de tratamiento. El crecimiento intermedio en longitud total y supervivencia larval se midió cada cinco días y se calculó el peso corporal de las larvas de peces en los días inicial y final durante y después del tratamiento. Las larvas alimentadas con LAP tenían una longitud total media más alta que todos los demás tratamientos. Se observó que el peso promedio fue mayor en LA, lo que difiere significativamente ($P<0.05$) de otros tratamientos. La tasa de crecimiento específico promedio fue mayor en DA, LA y LAP. El crecimiento heterogéneo se observó menor en el tratamiento LAP que en los demás tratamientos. Se observa que los alimentos vivos no son obligatorios para el programa de alimentación de larvas de *H. fasciata*. Los nauplios de *Artemia* se pueden reemplazar parcial o totalmente con microdietas como EL y Prince Wean sin afectar en gran medida el crecimiento y la supervivencia de las larvas.

Palabras clave: Alimentos para larvas, Alimentos inertes, Tasa de crecimiento específica, *Haludaria*, Protocolo de alimentación

INTRODUCTION

Larval feeding in fish hatcheries plays a vital role in the growth, survival, health and quality of the produced fish seeds. The growth rate is rapid in the larval development stages and the nutritional need at this stage is higher than in juveniles and adults (Velasco-Santamaría and Corredor-Santamaría, 2011). Appropriate feed size, attractiveness, nutritional content and digestibility are the requirements of fish larval feeds (Hamre et al., 2013). When these dietary requirements are not appropriately met, the growth will be stunted or reduced and cause deformity and mortality (Rønnestad et al., 2013). The growth and survival rates during larval rearing are critical for improving juvenile production and thereby stabilizing the economic viability of fish hatcheries.

Modern larval feeding methods suggest the usage of live feeds, micro diets and other commercial feeds to satisfy the nutritional requirements of larvae as well as to maintain water quality in rearing tanks (Geffroy and Simon, 2013; Rønnestad et al., 2013; Prusińska et al., 2020; Lipscomb et al., 2022). The feed consumption and digestion ability of fish larvae vary with species and life stage (El-Dahhar et al., 2021; Rønnestad et al., 2013). Different micro diets and live feeds have been experimented during the larval rearing of freshwater fishes *Corydoras aeneus*, *Synodontis eupterus*, *Synodontis nigriventris*, *Epalzeorhynchos bicolor*, *Pterophyllum scalare*, *Pangasius bocourti, Osphronemus gourami,Tinca tinca L.* and *Trichogaster lalius and larval growth and survival rates were evaluated* (Hung, 1999; Mamcarz et al., 2011; Sukardi et al., 2018; Lipscomb et al., 2020, 2022) These studies reported larval fish diet

require either a live food or its combination with a micro diet or commercial diet or in certain case an inert diet alone throughout larval rearing. The dietary experiments affected the larval growth rate, deformity and larval survival.

The Nilgiri Melon barb *Haludaria fasciata* belongs to the family cyprinid and is endemic to freshwater streams and rivers of southern India (Jayaram, 1991). They are collected from the wild and traded as ornamental fish (Daniels, 2002; Sureshkumar et al., 2013). The hatchery production of such wild-collected fishes under the trade can meet the supply in the market and thereby reducing stress on the ecosystem (Mercy et al., 2007; Kujawa and Piech, 2021) and be useful for restitution purposes when threatened or endangered in their environment (Wang et al., 2001; Ross et al., 2008). There are no studies conducted on the effect of feeds on the larval growth and production of *H. fasciata*. In this study, six diets including artemia nauplii have experimented on larval feeding of *H. fasciata* and the juvenile production was analysed.

MATERIALS AND METHODS

The adult fishes of *H. fasciata* were collected from freshwater streams of the Western Ghats region (12°05'19.5_N 75°34'24.3_E) in Kannur district, Kerala. The experiment was conducted at the laboratory of School of Industrial Fisheries, CUSAT, Ernakulam. The brood fishes were stocked in glass tanks (100 liters) for a month to getacclimatized to the semi-controlled conditions of the lab and artificial feeding. The fishes were fed with commercial pellet feed (Growfin™ Feed No.4) having 32% protein and 5% fat. For spawning, the brood fishes were stocked in a glass tank two days before the experiment in a ratio of 3 female and 1 male fish. A breeding box containing artificial plants was placed inside the breeding tank to collect their eggs. Five sets of broodstocks were kept for egg production, After spawning, the 300 viable eggs were collected and transferred to ten poly polypropylene plastic tubs with a dimension of 12 cm x 8 cm x 6 cm and incubated for 28 h. The hatchlings were reared in the same tubs for initial three days.

On the third day evening, 270 numbers of healthy larvae ie; similar size without any deformity and that are actively swimming were collected and 15 larvae were stocked in each experimental tub (18 nos.). The tubs are made of transparent poly polypropylene and had a dimension of 30cm x 25cm x 15cm (l x b x h). At the beginning stage, the water was kept up to a height of 5 cm, later increasing up to 12 cm on 10days post hatch (dph). The fish larvae were reared in a controlled condition and the water quality of the fish larval rearing tub was maintained at pH- 7.2, ammonia - >0.02 ppm, Dissolved oxygen .- >5 ppm, general hardness - 80 ppm and temperature 26º C - 28º C. Water exchange was done every day at 20% and the fecal matter and food waste were siphoned out. Mild aeration was provided from the 8th day onwards as the larvae attained better swimming movements. Artificial light was provided for 13 h using white light providing 750 lux and 11 h dark condition on each day (Fig. 1).

Six dietary treatments were carried out in this study. All of the selected feeds in the treatments were available globally and were purchased from online merchants and local aquaculture feed dealers. The feeds selected for the larval rearing experiment were Growfin™ (GF) of Growel Feeds Pvt. Ltd., Elarval 100 (EL) and Prince wean 300 (P) of Lucky Star Holdings Pte. Ltd., Neotea™ Spirulina powder (SP) of Neoteric DCBA ideas, Decapsulated artemia eggs (DA) and Live artemia nauplii (LA). The EL and P were micro coated diets having a size of 60-150µm and 250-400µm and bought from local aquaculture dealer at 900 and 800 Indian National Rupees (INR) / Kg respectively. The GF was a commercial pellet feed having 0.6 mm size bought for 130 INR / Kg from local aquaculture dealer. It was ground and powdered before feeding the larvae in this experiment. The DA was the artemia embryo/eggs processed from the decapsulation of artemia cysts and it was purchased in wet condition at a rate of 2500 INR /Kg and stored in frozen condition during the experiment. The LA was live artemia at the nauplii instar 1 stage, which is hatched daily from the dry artemia cyst purchased from the local market at a rate of 5000 INR / Kg. The spirulina powder was bought from an online merchant at a rate of 1200 INR / Kg. The GF, EL, SP, DA and LA were fed continuously for 35 days in five treatments. In the sixth treatment LAP, LA was fed for the first five days and P was fed for the remaining 30 days of the treatment. The feeding was done thrice a day at 8:00 am, 01:00 pm and 05:00 pm and performed manually. The larvae were fed at a satiation rate by observing their feeding behavior and stomach fullness. Excess feed was siphoned or removed after one hour. The treatment was carried out in triplicate ie; in eighteen tubs.

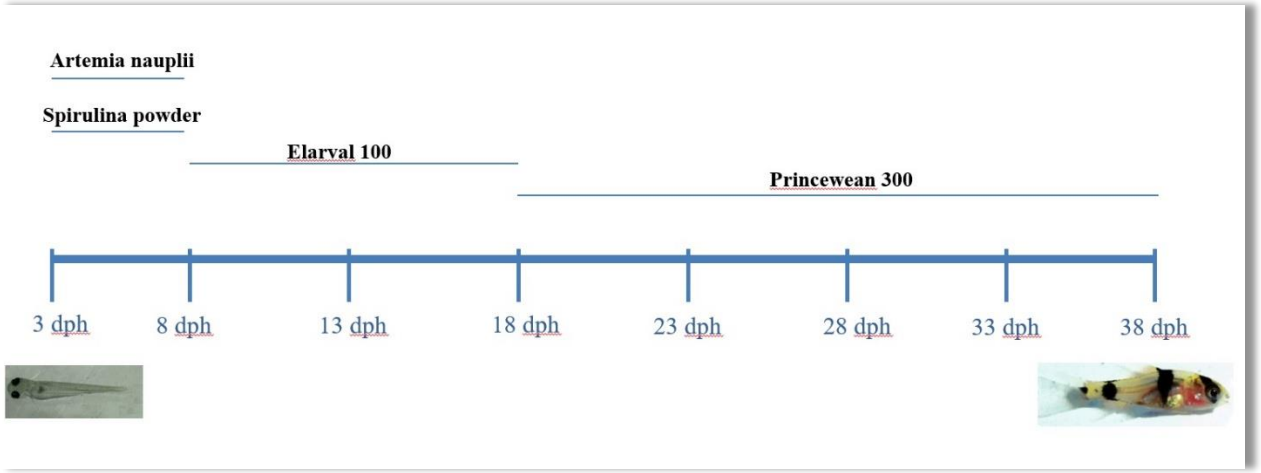

Figure 1. Hypothesized feeding protocol for the larval rearing of *H. fasciata*

Proximate analysis of the feeds was done based on the Association of Official Analytical Chemists (AOAC) method 20th edition to estimate protein, fat, ash, carbohydrate and moisture. The calorie/energy content was

calculated by Pearsons's composition and analysis of food 9th edition. The dry weight proximate analysis values of LA and DA were obtained by converting the values into wet weight by the following calculation.

$$\text{Nutritional content in dry weight (\%)} = \frac{\text{Nutritional content (\%) in wet weight}}{(100 - \text{\% of moisture})} \times 100$$

Initial and final growth parameters were collected from 15 larvae and intermediate growth on every 5th day was collected from 8 larvae randomly selected from the triplicate rearing tubs of each treatment. The initial weight of the larvae was taken by weighing 15 larvae together and the average weight is noted. The final weight was determined by weighing larvae individually using analytical balance (CAS 54, Contech, India). The weighing procedure was conducted based on the method described by Krejszeff et al., (2013). The digital images of larvae (±0.1 mm) from initial stages up to 8mm TL were taken using a Pathological microscope (ESAW, India) connected with a camera (MD500) along with a stage micrometer for obtaining measurements during the first eight days. After eight days of rearing, the digital photographs of large larvae along with a measuring scale were taken using camera (Canon 700D). The total length of larvae was measured from the images using software ImageJ 1.52. The juvenile stage is determined by the loss of larval character and attaining the morphological features of adult fishes. The average daily weight gain, length gain and Fulton's condition coefficient were estimated from the following equations. The Wt and WT represent the weight (mg) of fish larvae at the initial and final stages respectively. The tL and TL represent initial and final total length (mm) respectively for values at intervals and end of the treatment. The T-t represents the number of days between the measurements were taken.

$$\text{Average daily weight gain (mm/day)} = \frac{(WT - Wt)}{(T-t)}$$

$$\text{Average daily length gain (mm/day)} = \frac{(TL - tL)}{(T-t)}$$

$$\text{Fulton's condition coefficient (K)} = 100 \times WT \times TL^{-3}$$

The specific growth rate of length and weight at final day and the specific growth rate of length at every five days was calculated using the equation

$$\text{SGR in weight (\%/ day)} = \frac{(\ln WT - \ln Wt)}{(T-t)} \times 100$$

$$\text{SGR in length (\%/ day)} = \frac{(\ln TL - \ln tL)}{(T-t)} \times 100$$

Survival rate and deformity rate were calculated at the end of the study from all triplicates using the following equation

$$\text{Survival rate \%} = \frac{\text{Total number of larvae survived}}{\text{Total number of larvae stocked}} \times 100$$

$$\text{Deformity rate \%} = \frac{\text{Total number of larvae deformed}}{\text{Total number of larvae stocked}} \times 100$$

The descriptive analysis was carried out using Microsoft Excel. The normality and homoscedasticity of the growth parameters were analyzed for the parameters studied using the Shapiro–Wilk test. The differences between treatments in TL, WT and Fulton's K were analyzed with one way ANOVA (non-parametric) Kruskal–Wallis test and Dwass-Steel-Critchlow-Fligner (DSCF), test for pair wise comparisons were carried out using Jamovi 2.0. A P value ≤0.05 is considered statistically significant.

RESULTS

Proximate composition of experimented feeds: The results of the proximate analysis are shown in table 1. The protein content was higher in SP (56%) and EL (55.8%) than in other feeds. LA and DA had the lowest protein content on wet weight basis. The micro diets EL (9.4%) and P (9.7%) had the highest fat content. The GF (43.2%), SP (18.1%) and EL (17.8%) had the highest carbohydrate content. The moisture content was higher in the live feeds LA (78%) and DA (73%). The energy and fat content were higher in LA and DA on the calculated dry weight basis.

Larval growth under dietary treatments

Table 1. Proximate composition of experimented feeds

	SP	EL	GF	DA (in dry weight basis)	LA (in dry weight basis)	P
Protein (gm/100gm)	56	55.8	34.8	14 (51.85)*	14.3 (52.72)*	53
Fat (gm/100gm)	5.8	9.4	4.8	2.96 (10.96)*	3.1 (14.09)*	9.7
Ash (gm/100gm)	12.5	11.6	11.2	4.3 (15.92)*	3.1 (14.09)*	12.6
Carbohydrate (gm/100gm)	18.1	17.8	43.2	5.74 (21.52)*	4.2 (19.09)*	19.6
Moisture (gm/100gm)	7.6	5.3	6	73	78	5.1
Energy/ Calorie (Kcal/100gm)	349	379	355	106 (392.59)*	88 (400)*	378

*Nutritional content in dry weight basis

gm – gram Kcal – Kilo calorie

Spirulina powder (SP), Elarval 100 (EL) GrowfinTM (GF), Decapsulated artemia eggs (DA) and Live artemia nauplii (LA) and Prince wean 300 (P)

All the larvae (n=270) attained the juvenile stage at 35 days of dietary treatment. The larvae fed with LAP had a higher mean TL (14.61 ± 0.07 mm) than all other treatments (Table 2). The lower mean TL was observed in SP (11.38 ± 0.69 mm) (Fig.3). The mean WT was observed higher in LA (44.76 ± 2.56 mg) and lower in SP (16.77 ± 1.04 mg). The mean SGR and Fulton condition coefficient was found higher in LA than in other treatments. The heterogeneous growth in length was lower in treatment LAP while for SP it was higher (Fig. 2). The heterogeneous growth in weight was lower in treatment LAP, EL, and SP (Fig. 3). The dietary treatments had a significant difference in TL between treatments. The LA, DA and LAP have significant difference in TL ($P<0.05$) with treatments GF, EL and SP. There is no significant difference ($P>0.05$) between treatments LAP, LA and DA and between GF and SP. The treatment EL significantly differed in TL ($P<0.05$) from GF and SP.

Table. 2. Growth performance of *H. fasciata* on selected diets

Parameters	SP	EL	GF	DA	LA	LAP
Mean tL (mm)	4.53 ± 0.02	4.53 ± 0.03	4.53 ± 0.03	4.51 ± 0.03	4.53 ± 0.02	4.53 ± 0.16
Mean TL (mm)	11.38 ± 0.69^{a}	13.59 ± 0.52^{b}	11.89 ± 0.49^{a}	14.15 ± 0.57^{c}	14.46 ± 0.4^{c}	14.61 ± 0.07^{c}
Mean Wt (mg)	1.6 ± 0.24	1.6 ± 0.25	1.6 ± 0.21	1.6 ± 0.27	1.60 ± 0.29	1.60 ± 0.22
Mean WT (mg)	16.77 ± 1.04^{a}	28.79 ± 0.96^{b}	19.16 ± 0.489^{c}	33.69 ± 3.25^{d}	44.76 ± 2.50^{e}	40.38 ± 0.29^{f}
Average daily weight gain	0.43 ± 0.03	0.78 ± 0.03	0.48 ± 0.04	0.92 ± 0.09	1.23 ± 0.07	1.10 ± 0.01
Average daily length gain	0.20 ± 0.02	0.26 ± 0.01	0.21 ± 0.01	0.28 ± 0.02	0.28 ± 0.01	0.29 ± 0.00
SGR % length d^{-1} (mm±SD)	2.6 ± 0.18	3.13 ± 0.11	2.72 ± 0.12	3.26 ± 0.11	3.31 ± 0.08	3.25 ± 0.09
SGR % weight d^{-1} (mg±SD)	6.69 ± 0.18	8.24 ± 0.09	6.98 ± 0.21	8.68 ± 0.27	9.48 ± 0.16	9.21 ± 0.02
Fulton Condition Coefficient	1.16 ± 0.15^{a}	1.15 ± 0.1^{ab}	1.15 ± 0.03^{abc}	1.19 ± 0.04^{abc}	1.50 ± 0.04^{d}	1.30 ± 0.01^{ae}
Larval deformity (%)	6.67	0	0	0	0	0
Larval survival rate (%)	88.89	97.78	80	97.78	97.78	95.55

The values with different superscript letters (a,b,c,d&e) in a row are significantly different ($P<0.05$) based on Dwass-Steel-Critchlow-Fligner (DSCF)test for pair wise comparisons.

Spirulina powder (SP), Elarval 100 (EL) GrowfinTM (GF), Decapsulated artemia eggs (DA) and Live artemia nauplii (LA) and Live artemia for five days and there after Prince wean 300 (LAP)

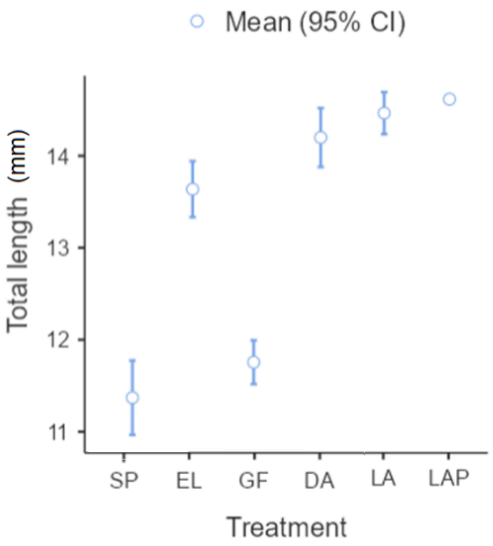

Figure 2. Final total lengths (mm) of larvae under each treatment.

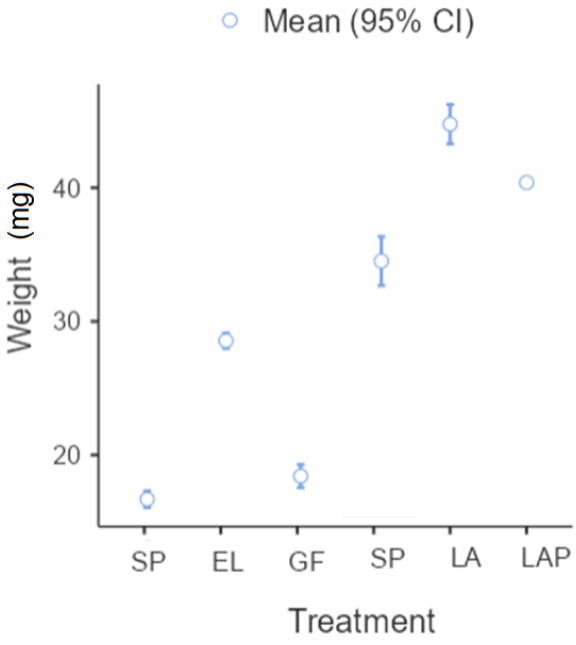

Figure 3. Final body weights (mg) of larvae under each treatment

Intermediate growth and mortality of larvae under the treatments: The larvae LA and LAP had higher SGR than other treatments in the first 5 days (Table 3). The EL showed a higher SGR than other treatments in 5-10 days (Fig. 4). The SP and DA showed higher SGR than other treatments between 10-15 days. LAP and GP showed higher SGR than other treatments between 15-20 days (Fig.4). Except for the treatment LA, all others exhibited lower SGR between 20-25 days. The EL, DA and LAP showed higher SGR at 25-35 days. The growth in treatment EL had a peak SGR value during the 5-10 days and for DA it was 10-15 days. GF had mortality during the first 5 days, 10-15 days and 20-25 days. LAP first 5 days and 5-10 days, SP had higher mortality during 5-10 days, 10-15 days, and 30-35 days. LA and EL had mortality during the first five days. DA had mortality during 10-15 days.

Larval survival, mortality and deformity rate: The larval survival rate was higher (97.78%) in LA, DA and EL than in other treatments (Table 3). The treatment GF had the lowest survival rate (80%). The larval deformity was observed only in treatment SP (6.6%). The larval deformity was observed during 10-15 days of treatment. The deformed larvae have exhibited spinal bent. All the other treatments had no larval deformities observed during the treatment period.

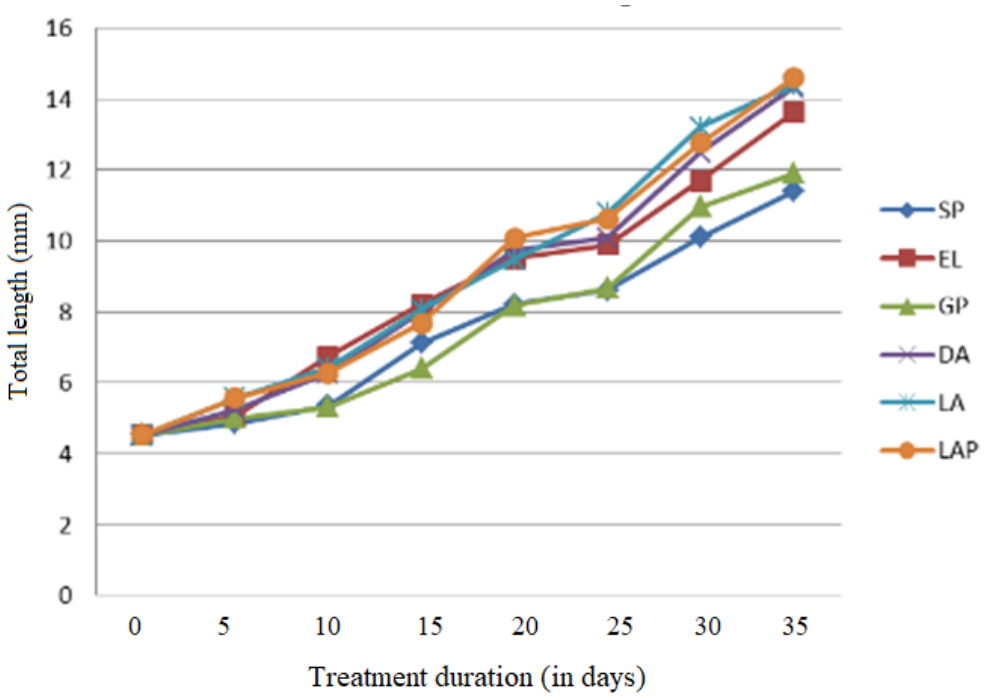

Figure 4. Average growth of larvae (TL) under each treatment during the rearing period

Spirulina powder (SP), Elarval 100 (EL) GrowfinTM (GF), Decapsulated artemia eggs (DA) and Live artemia nauplii (LA) and Live artemia for five days and there after Prince wean 300 (LAP)

Table 3. Average (Mean ± SD) SGR (% length d^{-1}) of larvae under the dietary treatments at each interval

Treatments	0-5 days	5- 10 days	10-15 days	15-20 days	20-25 days	25-30 days	30-35 days
SP	1.42 ± 0.52	1.99 ± 0.53	5.62 ± 0.64	2.85 ± 0.96	1.18 ± 1.13	3.18 ± 1.07	2.40 ± 1.35
EL	2.20 ± 0.44	5.81 ± 0.34	3.92 ± 0.54	2.87 ± 0.83	1.22 ± 0.47	3.33 ± 0.48	3.06 ± 0.85
GF	2.01 ± 0.83	1.22 ± 0.93	3.67 ± 0.91	4.95 ± 0.91	1.15 ± 0.72	4.69 ± 0.7	1.63 ± 1.02
DA	2.97 ± 0.73	3.58 ± 0.42	4.92 ± 0.64	3.89 ± 0.52	0.68 ± 0.38	4.26 ± 0.51	2.72 ± 1.04
LA	4.02 ± 0.32	2.78 ± 0.84	4.61 ± 1.01	3.21 ± 0.70	2.59 ± 0.96	4.05 ± 0.45	1.72 ± 0.58
LAP	4.12 ± 0.4	2.36 ± 1.14	4.08 ± 1.28	5.44 ± 0.74	1.33 ± 0.26	3.66 ± 0.45	2.69 ± 0.36

SD standard deviation. % length d^{-1} % growth in length per day

Spirulina powder (SP), Elarval 100 (EL) GrowfinTM (GF), Decapsulated artemia eggs (DA) and Live artemia nauplii (LA) and Live artemia for five days and there after Prince wean 300 (LAP)

DISCUSSION

Identifying a suitable feed is vital for larval rearing of any species under controlled conditions of ornamental fish hatchery (Chen et al., 2019; Lipscomb et al., 2022). This study was designed to identify practical diets that are suitable for the juvenile production of an ornamental fish *H. fasciata* during their initial 35 days larval rearing period. The exogenous feeding of *H. fasciata* was begun within 60- 72 hours post-hatch. In the ontogeny of many fishes, exogenous feeding generally commences before the yolk is fully exhausted (Balon, 1989; Wilson, 2012; Rønnestad et al., 2013) which was also observed in *H. fasciata*. Such species will have a comparatively higher survival rate than other species that have lesser yolk during their ontogeny (McCasker et al., 2014).

Protein and fat are important nutritional factors in larval feed affecting ontogenic growth. The increment in length and weight of fish larvae is essentially the deposition of the protein content of feeds in tissues (Carter and Houlihan, 2001). The protein content was higher in feeds SP (56%) and EL (55.8%); however, the diets have not resulted in higher growth than in other treatments. Apart from comparing the protein content of experimented feeds, it is the quantity and quality of the amino acid pool in the protein of feed that promotes larval growth (Velasco-Santamaría and Corredor-Santamaría, 2011). The EL, DA, P, and LA had fat content above 9% on dry weight basis. The fats are energy sources of larvae for growth and metabolism. The quantity and quality of HUFA and phospholipids in the fat content of larval feeds affect the growth (Watanabe et al., 1983; Sargent et al., 1999). The present study has not quantified various amino acids and fatty acids in the experimental diets to evaluate the effect of these nutrients on larval growth.

The growth parameters observed in the study were higher in LAP and LA than in other treatments. Both of these treatments had live artemia as their feed for the initial five days. Artemia nauplii is a smaller-sized live feed widely used in fish hatcheries (Lavens and Sorgeloos, 1996; Watanabe et al., 1983). The size, nutritional quality and digestibility of artemia nauplii are the main features for selecting them as a larval feed in fish hatcheries. Previous studies reported growth acceleration was found higher in *Barbus barbus* and *C. auratus* when fed with artemia nauplii than in other experimented feeds (Abi-Ayad and Kestemont, 1994; Prusińska et al., 2020). The motility of the artemia nauplii attracts the fish larvae to their food and therefore results in consuming more food. For example, Larvae of *Cyprinus carpio* fed the artemia nauplii at an amount of 200-250% of their body weight per day during the initial days of larval rearing (Bryant and Matty, 1980). In the present study, the final growth of larvae fed with DA was comparatively lower than LA fed larvae which may be either due to the lower attractiveness to larvae or lower protein and fat content than in LA. However, the application of DA reduces the labor and time involved in the hatching of artemia cysts (Lavens and Sorgeloos, 1996).

Replacing live feeds with inert diets has been a highly preferred research for the last four decades in the area of fish larviculture (Dabrowski, 1984; Lavens and Sorgeloos, 1996; Callan et al., 2003; Curnow et al., 2006; Printzi et al., 2021). Since, many of the commercial inert feeds can be stored for long time, easier to handle and time-effective feeding is possible. The application of inert feeds could reduce the usage of artemia nauplii which is generally costlier than inert larval feeds, thereby reducing the cost of seed production (Callan et al., 2003; Curnow et al., 2006). In this study, it is observed that the body weight and Fulton's condition coefficient were higher in LA (live artemia fed for 35 days) and the parameters significantly differed from other treatments. In treatment LAP, artemia nauplii was replaced with inert diet Prince Wean after the 5th day of treatment. The growth parameter TL for both LA and LAP treatments had no significant difference but was higher in LAP. Size heterogeneity among juveniles produced is a critical problem faced in fish hatchery(Kim et al., 2020). It is also observed that the treatment LAP has the lower heterogeneity in total length and body weight and resulted in even sized juveniles.

Feed size, attractiveness, nutritional content and digestibility are the major quality requirements for developing inert larval feeds, especially at the initial stages. It is previously reported that among various cyprinids, the feeding aptitude of larvae differs and many thrive well on inert feeds from first exogenous feeding (Sales, 2011). But for species such as *Leuciscus leuciscus*, *L. idus* and *L. cephalus*, it is reported that the live feeds should be fed at least up to 8 to 12 dph and thereafter inert diets could be fed without negatively affecting the larval survival (Kujawa, 2004). Comparable growth and survival were achieved in the larvae of *Puntigrus tetrazona* in the initial 14 days of feeding with inert feed when compared to feeding with Artemia nauplii (Lipscomb et al., 2022). The present study has identified *H. fasciata* larvae fed with micro diets such as Prince Wean and E larval have comparable final growth and survival with that fed with Artemia nauplii alone. Based on these facts it is understood that live feeds are not necessary for larval rearing of *H. fasciata*.

The intermediate growth rate of larvae can vary with different diets fed to the larvae. The larval growth is associated with the ability of larvae to forage, ingest, digest and assimilate the feeds provided. In this study, the peak

growth rate in length per day or attaining an SGR value above 4 was observed in the treatments at different interval periods (5 days). The LA and LAP had higher growth rates on the initial days which indicates the artemia nauplii are suitable for the first five days of growth. But, except for SP all other treatments including LAP and LA showed mortality during the first five days. The mortality of larvae at first exogenous feeding is generally due to the inability of a few larvae in the group to consume and digest the feeds when introduced for the first time. The zero mortality in treatment SP indicates that the spirulina powder might be consumed by the larvae and effectively improved larval survival in the first five days. The EL showed a peak growth rate between 5-10 days. The labeled feed particle size of EL was 60-150 µm and the small feed particle might have improved feed consumption and thereby increased the SGR of *H. fasciata*. When compared with treatment LA, the LAP had mortality when larvae were weaned from live artemia to Prince Wean after the 5th day and this might be due to the larger particle size of Prince Wean (250-400 µm). The LAP had higher SGR during 15-20 days. Based on this observation, we hypothesize that feeding artemia nauplii along with spirulina at the first five days and weaning of larvae to Elarvae 100 at the 5th day onwards and Prince Wean at 15th day could reduce mortality due to starvation at the initial stage, improve growth and reduce feed cost of juvenile production (Fig.3).

Feeds that had poorly performed were the Growfin and Spirulina powder which had resulted in a significantly lower survival rate and growth rate of larvae than other treatments. Few larvae (6.67%) in the treatment SP were identified with spinal bent deformity. Fish larval deformities commonly occur during early life stages such as metamorphosis and organ development and malnutrition at these stages may result in the skeletal formation and ossification (Cahu et al., 2003; Hamre et al., 2013). The deformity was observed in 10-15 days, ie; during the flexion period of *H. fasciata* at which major morphological developments occur. Both feeds were in powdered form and might have leached nutrients into the water column before being eaten by the larvae or less consumed by the larvae due to their lower attractiveness (Geffroy and Simon, 2013). Therefore, Growfin and Spirulina powder are not recommended for larval feeding of *H. fasciata* for the whole period of larval rearing.

As conclusion, the present study have direct application in the hatchery production of commercial valuable ornamental fish *H. fasciata*. There was a difference of approximately 12- 17% in the larval survival rate, 2-3mm in the total length and 25-28mg in the body weight of larvae between the best-performed diets and poorly performed diets under the juvenile production experiment. The live feed artemia nauplii have performed best among the larval feeds experimented and is recommended for attaining better plumpness among larvae. However, live feed is not compulsory for their larval rearing. It is hypothesized that feeding dried spirulina powder along with artemia nauplii at the initial five days may reduce the larval mortality due to starvation. To reduce the feed cost during larval rearing, it is suggested that inert feeds such as Elarvae and Prince Wean can be fed after initial five days and fifteen days respectively. Both the feeds had comparable final growth and larvae survival with that of the larvae fed with Artemia nauplii. However, more trials are needed to confirm the suggested feeding protocols. The findings of this study are beneficial for choosing feeds for larval rearing in commercial hatcheries and aquarium fish hobbyists.

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