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Note

Nutritional interventions to enhance reproductive performance and larval rearing of dwarf gourami *Trichogaster lalius* (Hamilton, 1822)

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Abstract

Considering the importance of broodstock nutrition, the reproductive performance and larval rearing of dwarf gourami *Trichogaster lalius* (Hamilton, 1822), were evaluated in response to nutritional interventions under captive conditions for 90 days. To evaluate reproductive performance, four dietary treatments were tested. Treatment 1 and 2 received formulated feed supplemented with 0 and 4% supplementary diet mix (SDM). SDM comprised, Refined lecithin, α Tocopheral acetate, L-ascorbic acid, Spirulina powder and L-tryptophan. Treatment 3 was fed with live feed (*Tubifex*), while Treatment 4 received a combination diet, comprising live feed (*Tubifex*) and 4% SDM-formulated feed. Among both male and female dwarf gourami, the highest gonad weight and gonadosomatic index (GSI) were observed in Treatment 4. Spawning was successful only in Treatment 3 and 4. After 60 days, fertilisation rates in Treatment 3 and 4 were 65.33±1.76 and 70.67±0.67% respectively, while hatching rates were 35.33±1.76 and 42.00±1.15% respectively. The combination diet in Treatment 4 yielded better results in terms of fertilisation and hatching success. For larval rearing, a 30 days experimental trial was conducted in triplicates, with a stocking density of 100 larvae per 10 l water. Three dietary treatments were used: Treatment 1 fed with live feed (Infusoria), Treatment 2 fed with 4% SDM-formulated feed and Treatment 3 fed with boiled egg yolk. The highest larval length gain (10.54±0.07 mm) and survival rate (66.33±0.88%) were recorded in Treatment 1, fed with live infusoria.

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Ornamental fishes are often referred to as 'living jewels' due to their vibrant colouration, distinct body shapes and their adaptive behaviour. They form an important commercial segment of the fisheries sector, fulfilling aesthetic and recreational demands. Globally, around 133 ornamental fish species representing 4 sub-families and 15 genera originating from Africa and Asia are recognised. Among these, labyrinth fish belonging to the order Anabantiformes and family Osphronemidae, commonly called gauramis have a huge market in the ornamental fish industry. Although a wide variety of gourami species are found in nature, some species grow to sizes that make them unsuitable for the general hobbyist. In contrast, the dwarf gourami *Trichogaster lalius* (Hamilton, 1822) is a popular gourami species in the ornamental fish industry due to its compact size, peaceful nature and brilliant colouration. Native to South and South-east Asia, it is popular in countries such as India (Jena *et al*., 2019), Sri-Lanka, Bangladesh, Nepal, Vietnam, Thailand, Malaysia, Pakistan, United States, Australia and Columbia (Zuanon *et al.,* 2013; Darshan *et al*., 2019). Dwarf gourami is indigenous to India and has great potential. The species is mainly sourced through wild capture, which may render them vulnerable to overexploitation in the near future. This necessitates the need for live gene banking and standardisation of protocols for captive breeding and rearing.

Nutrition has a significant influence on the growth and maturation of ornamental fish and several live feeds have been used for fish rearing (Shim *et al*., 1989). According to Manissery *et al*. (2001) and Muchlisin *et al*. (2006), nutrition plays a major role in the reproductive performance of fish. Watanabe *et al.* (1990), stated that nutrition has intense consequences on gonadal growth as well as fecundity and broodstock nutrition is an important factor that governs egg production and larval survival. Live feed plays a crucial role in the broodstock development of *Trichogaster lalius*. Studies have shown that live feed can significantly improve growth performance, reproductive success and overall health of the broodstock. Broodstock development for *T. lalius* involves careful management of environmental conditions, nutrition and breeding practices to ensure healthy and productive fish for ornamental aquaculture. Key aspects include providing a suitable environment, a nutritious diet, and optimising breeding conditions to maximise egg production and hatching rates (Izquierdo *et al.,* 2001). Live feed mimics the natural diet of *T. lalius*, ensuring they receive a balanced and complete nutritional profile for optimal development, and are often richer in essential nutrients like fatty acids and vitamins, that are crucial for egg and sperm quality. This can lead to increased egg production and higher hatching rates. This study reports the results of nutritional interventions on the reproductive performance and larval rearing of dwarf gourami, *Trichogaster lalius*.

The experiments were conducted at the wet laboratory of the old campus of ICAR-Central Institute of Fisheries Education (CIFE), Mumbai, India. The experiment on nutritional interventions for enhancing reproductive performance was carried out for a period of 90 days, including breeding trials for one month. Subsequently, the laboratory work was carried out in the laboratories of ICAR-CIFE, Mumbai. Two forty (240) advanced fingerlings of *T. lalius*, procured from Tribeni River, Hooghly, West Bengal, having an average weight of 1.13±0.07 g (ranging from 0.72-1.62 g) and an average length of 4.03±0.21 cm (ranging from 3.4-4.6 cm) were randomly distributed in four distinct experimental groups, keeping males and females separate for each treatment in triplicates following a completely randomised design (CRD). The setup consisted of 24 glass aquaria of 27 l (20 X 45 X 30 cm^3) capacity. The total volume of the water in each tank was maintained at 25 l throughout the experimental period with round-the-clock aeration. The experimental aquaria were cleaned manually at weekly intervals and the faecal matter was siphoned out every morning along with 20% water exchange.

The formulated feed used for the experiment is based on the previous experimental results for growth performance conducted on this particular stock of dwarf gourami (Upasana *et al.,* 2019). Formulated feed with 4% supplementary diet mix (SDM) gave the best result in growth for dwarf gourami *T. lalius* (Upasana *et.al.,* 2019). The experimental feed used in the study were: Treatment 1 - Formulated feed with no SDM; Treatment 2 - Formulated feed with 4% SDM; Treatment 3 - Live feed *Tubifex* and Treatment 4 - Combination diet (*Tubifex* + formulated feed with 4% SDM).

Ingredient composition of the experimental diet is given in Table 1 and the composition of the supplementary diet mix (SDM) is given in Table 2. All the ingredients were ground, weighed and mixed as per the formula (except oils and additives) and a dough was formed with the addition of distilled water. The dough was steam-cooked in a pressure cooker for 20-30 min followed by cooling the dough. After cooling the dough, vitamin-mineral premix, SDM and carboxymethyl cellulose (CMC) were added with soy oil and then mixed thoroughly. Pellets of 1.5 mm dia were prepared using a pelletiser machine and dried at room temperature under a ceiling fan for some time and kept in a hot air oven at 60°C until complete drying (<12% moisture). After drying and cooling, the pellets were packed in airtight polythene zipper bags, labeled properly and stored at 4°C until further use.

Feeding of fish fingerlings of each treatment group T1, T2, T3 and T4 was done with respective diets on a satiation basis (*ad libitum*) twice daily in the morning (09.00 hrs) and evening (18.00 hrs) throughout the feeding trial of 60 days as given in Table 3. Fish of T4 were fed with *Tubifex* worm in the evening and formulated feed in the morning. *Tubifex* worms were procured from the local wholesale market (Kurla, Mumbai, India) and these worms were also fed *ad libitum.* Regular siphoning was carried out daily, during the morning hours.

Gonadosomatic index (GSI), was calculated after 60 days of experimental trial as:

$$GSI = \frac{\text{Gonad weight}}{\text{Total body weight}} \times 100$$

Breeding trials were performed after 60 days of the feeding experiment. The breeding setup had a single male and a single female dwarf gourami, from each treatment. The spawning success was calculated after the breeding trials for every treatment. Spawning success (%) was calculated using the formula:

$$\text{Spawning success (\%)} = \frac{\text{No. spawning success}}{\text{No. of spawning trials}} \times 100$$

Fertilised eggs are marked with a clear and transparent colour, whereas unfertilised eggs are characterised by feculent white colour. Fertilisation rate was calculated a few hours after the spawning activity. using the formula:

$$\text{Fertilisation \%} = \frac{\text{No. of eggs fertilised}}{\text{No. of eggs in the batch}} \times 100$$

Hatching rate was calculated as:

$$\text{Hatching \%} = \frac{\text{No.of eggs hatched}}{\text{No.of eggs in the batch}} \times 100$$

Newly hatched larvae of *T. lalius* were utilised for the present study. The experiment was conducted for a period of 30 days with three treatments in triplicates following CRD, at a stocking density of 100 larvae per 10 l water, in aquaria. Feeding was done after 3 days of hatching. Gentle aeration was provided in the experimental tanks. A daily water exchange rate of 20% was done during the experimental period. During larval rearing, dead larvae and waste were siphoned off daily.

The experimental diet used in Treatment 1 is live feed Infusoria, which was cultured using banana peelings and filtered water in

27 l aquarium, covered with cloth from above to prevent flies and mosquitoes, but allow passage of air. To get a regular supply of Infusoria, periodic harvesting was done and drops of milk were added regularly. In Treatment 2, formulated diet (Table 1) with 4% SDM (Table 2) was used for feeding, after the pellets were ground to powder form. In Treatment 3, boiled egg yolk was used for feeding. Boiled egg yolk was used to make yolk emulsion with water and used for feeding.

Length gain was calculated as:

Length gain = Final length-Initial length

Survival rate was calculated as:

$$\text{Survival (\%)} = \frac{\text{Total no.of harvested fish}}{\text{Total no.of stocked fish}} \times 100$$

Table 1. Ingredient composition of the experimental diets

Ingredients (%)	Experimental diet	
	T1	T2
Fish meal	25	25
Soybean meal	22	22
GNOC	15	15
De-oiled rice bran	20	16
Wheat flour	10	10
Starch powder	3	3
Soya oil	2	2
Vitamin and mineral mix	1	1
Carboxy methyl cellulose	2	2
Supplementary diet mix (SDM)	0	4
Total	100	100

Table 2. Composition of Supplementary Diet Mix (SDM)

Ingredients	Composition (%)
Refined lecithin	40
α Tocopheral acetate	1.5
Celin (L-ascorbic acid)	2.5
Spirulina powder	50
L-tryptophan	6
Total	100

Gouramis are popular among aquarium hobbyists for their peaceful nature and their unique reproductive behaviour, which involves building floating bubble nests to protect and nourish their eggs. For evaluation of the reproductive performance of *T. lalius*, the body weight, gonad weight and gonadosomatic index of both male and female fishes were recorded at the end of the 60 days feeding experiment. Highest GSI, in both males and females were recorded in Treatment 4, which was fed with a combination diet (Fig. 1). The combination diet gave better results, which could be attributed to the omnivorous feeding habits of *T. lalius*. Kasiri *et al.* (2012) observed similar results in GSI of angelfish, *Pterophyllum scalare*.

Spawning success, egg fertilisation and egg hatching was observed only in Treatment 3 and Treatment 4, which were fed with live feed

Table 3. Experimental design for reproductive performance

T1	Male (T1R1 ,T1R2,T1R3) Female (T1R1,T1R2,T1R3)	Formulated diet (0% SDM)
T2	Male (T2R1,T2R2,T2R3) Female (T2R1,T2R2,T2R3)	Formulated diet (4% SDM)
T3	Male (T3R1,T3R2,T3R3) Female (T3R1,T3R2,T3R3)	Live feed (*Tubifex*)
T4	Male (T4R1,T4R2,T4R3) Female (T4R1,T4R2,T4R3)	Formulated diet (4% SDM)+ *Tubifex*

(*Tubifex*) and a Combination diet (Formulated feed with 4% SDM + *Tubifex*) respectively. No spawning was observed in Treatment 1 and Treatment 2, which were fed with formulated feeds. In hatcheries, the adequate supply of live feed has a vital role in feeding fish larvae, fry and fingerlings. The results of the present study suggest that dwarf gourami, *T. lalius* matures and breeds well if fed on live feed during its maturation phase. Similar results were reported by Das and Kalita (2006) in *Colisa fasciata*. Treatment 4 fed with a combination diet showed better results during fertilisation and hatching. The breeding success may be attributed to ready bioavailability of nutrients in live feed. Farahi *et al.* (2010) reported similar results in hatchability of angelfish, *P. scalare*. James *et al.* (2006) revealed that the fishes fed with Spirulina diet (30%) exhibited higher feed consumption, growth, fertility, colouration and leucocyte count in the ornamental red swordtail, *Xiphophorus hellerii*. The authors proposed that 30% of Spirulina can boost reproductive performance in ornamental fishes. Kasiri *et al.* (2012) reported a significant increase in the GSI, fecundity and hatchability of freshwater angelfish when fed with a combination of live earthworm, dried *Tubifex*, dried *Gammarus* and granulated feed. According to Ahilan and Kumaran (2003), live feeds are the best broodstock booster dietary source for ornamental fishes which are effective in producing good quality eggs, increasing fertilisation, hatching and survival rates in ornamental fishes. According to Patino and Thomas (1990) and Degani and Boker (1992) live feed might possibly affect steroid production which is essential for oogenesis and the reproductive cycle. Kolkoski *et al.* (1997) reported that the addition of live feed along with a formulated feed enhances the efficiency of formulated feed by promoting the assimilation of dietary nutrients in seabass. The present study demonstrated that the use of formulated feed along with live feed enhanced breeding performance in *T. lalius*.

For evaluation of larval rearing of dwarf gourami, *T. lalius* with different experimental feeds, larval length gain and survivability was recorded after 30 days. The highest value of length gain of 10.54±0.07 mm was observed in Treatment 1, which was fed with live feed, Infusoria and the lowest value of length gain of 5.84±0.07 mm was observed in Treatment 2, which was fed with formulated feed (Fig. 2a). Significant differences ($p<0.05$) were observed in length gain in all the treatments. In the case of larval rearing, the survival rate plays an important role. The highest value of survival rate of 66.33±0.88% was observed in Treatment 1 and the lowest was observed in Treatment 3, which was fed with boiled egg yolk (Fig. 2b). Das and Kalita (2006) achieved similar results in the feeding of larval stages of *Trichogaster fasciata*. Similar results were observed by Degani (1991), in blue gourami, *Trichogaster trichopterus,* wherein, larvae fed on Infusoria grew faster (1.24±0.25 cm in 35 days) than larvae fed with egg-yolk

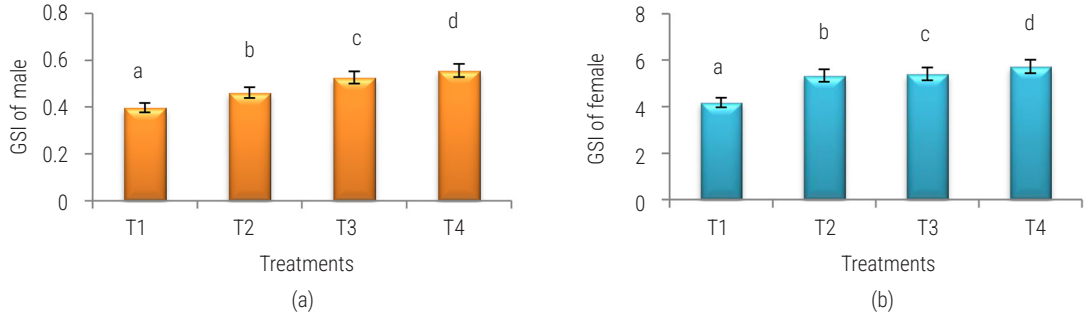

Fig 1. Gonadosomatic Index of (a) male and (b) female *T. lalius* in different experimental groups. Values are presented as Mean±SE. Treatments with different superscripts indicate significant differences ($p<0.05$)

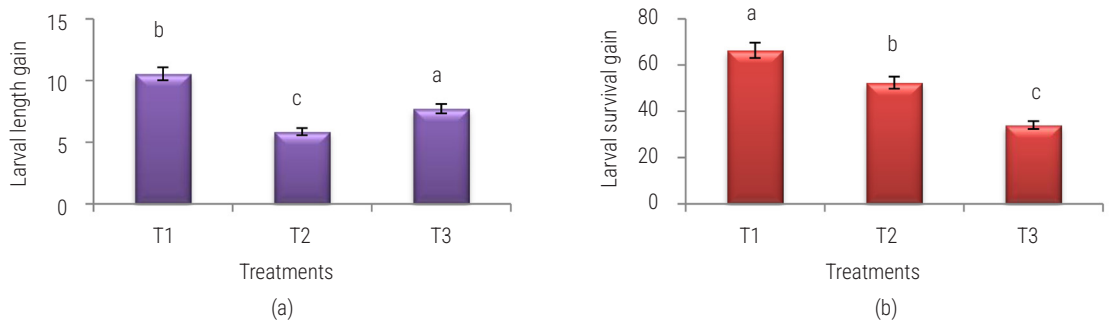

Fig. 2. Evaluation of experimental diets during larval rearing of dwarf gourami, *T. lalius.* (a) Length gain and (b) Survival rate. Values are presented as Mean±SE. Treatments with different superscripts indicate significant differences ($p<0.05$)

(0.64±0.20 cm in 35 days). Patra and Ghosh (2015) also recorded lesser survival rate (31.19%) in freshwater Angelfish *Pterophyllum scalare* larvae fed on egg custard in comparison to other live feed. Nagano *et al.* (2000) also demonstrated the importance of ciliates as initial food for first-feeding *Epinephelus septemfasciatus* for both growth and survival. Live feed is an important factor in larval rearing of several fish species (Girri *et al*., 2002; Wolnicki *et al*., 2003; Wang *et al.,* 2005). The necessity of a huge amount of live feed in aquaculture and mainly in ornamental fish culture is essential to rear its larvae from endogenous to exogenous feeding phases. Providing appropriate live feed through this phase is vital which governs the survival rate (Altaff and Janakiraman, 2013). One of the major responsible factors for failure in larval growth and survival is malnutrition (Luis *et al*., 2010).

Nutrition plays an important role in all life stages of fish, particularly in enhancing reproductive performance and larval survival. The experimental results of the present study demonstrated that, even though formulated feeds are useful in ornamental fish rearing, live feeds are indispensable for successful spawning and fry production under captive conditions. *Tubifex* effectively induced spawning, while freshwater Infusoria proved as an ideal starter feed for early larvae with small mouth, owing to its ease of culture, high digestibility and minimal impact on water quality. The use of Infusoria would enable intensive larviculture of this freshwater indigenous ornamental fish and would lead to better larval performance and an exponential increase in the production yield. Even though the nutrients in the formulated diet supported the reproductive performance of *T. lalius*, the nutrients of the live feed *Tubifex* helped in inducing spawning under captive conditions and Infusoria proved to be a suitable starter feed for effective larval survival. The use of formulated feed along with live feed enhanced the captive breeding performance of *T. lalius*. Also the nutrients present in Infusoria, are better digested and utilised in comparison to boiled egg yolk and formulated feed, by the larvae of *T. lalius,* without affecting the water quality. If harnessed as an additional source of income, ornamental fish farmer guilds could look into the associated auxiliary occupation of setting up production units for availability of Infusoria and *Tubifex* at all times. This would not only offer farmers and exporters a better alternative live food organism for feeding fish larvae, but also more importantly, open avenues of enhancing growth performance and quality through bioencapsulation. The use of live feed discussed in this paper is likely to have a positive impact to the ornamental fish industry.

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