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Effect of vitamin C on egg hatching and spawn survival of blue gourami, *Trichopodus trichopterus* (Pallas, 1770)

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Abstract

The effect of L-ascorbic acid as a source of vitamin C was assessed on the egg hatching performance and spawn survival of blue gourami, *Trichopodus trichopterus*. The eggs were procured from nests and immersed in L-ascorbic acid at the concentrations of 250 mg L⁻¹ (T₁), 500 mg L⁻¹ (T₂), 1000 mg L⁻¹ (T₃) and 2000 (T₄) mg L⁻¹ with control (T₀) in the experiment. Egg incubation period was ranged from 18 to 21 h with 100% hatching rate. The eggs were hatched out after 18 h in the treatment T₃, followed by T₂ (19 h), T₁ and T₀ (20 h) and T₄ (21 h). The maximum survival (97.5%) of spawn was recorded in T₃ and the minimum in T₄ (62.5%) after 20 days of spawn rearing. Results showed that a treatment of 1000 mg L⁻¹ L-ascorbic acid decreased egg incubation time and significantly better ($P < 0.05$) survival of spawn.

Keywords: Blue gourami, vitamin C, egg incubation, spawn survival

1. Introduction

Vitamin C plays a significant role in fish reproductive biology such as antioxidant effect on gametes, capacity to prevent DNA damage, recovery in multiple spawners, endocrine regulations, maturation and fertility (Dabrowski and Ciereszko, 2001) [1]. Ascorbic acid, as a source of vitamin C, is optically active and its L-form is physiologically active form. Deposition of ascorbic acid in the growing oocytes is important for the hydroxylation of protein bound proline and lysine to give optimum collagen strength through the embryonic stage (Waagbo *et al.*, 1989) [2]. Some fish species cannot synthesize vitamin C in their bodies due to the lack of L-gulonolactone oxidase and this enzyme is required for biosynthesis of ascorbic acid from glucose or other simple precursors (Sales and Janssens, 2003; Adewolu and Aro, 2009) [3, 4]. Therefore, ascorbic acid is needed to be provided externally to the fishes. The requirement varies among fish species, but intra-species differences such as fish strain, size and age also affect the dietary requirement (Li and Robinson, 1999) [5]. Blue gourami (*Trichopodus trichopterus*) is one of the most popular egg-layers among hobbyists (Degani and Gur, 1992) [6]. It is a labyrinth fish and has good acceptance to artificial diets. Considering the influence of vitamin C in the reproductive performance and nutritional studies, the present study was attempted to evaluate the effect of different levels of L-ascorbic acid on egg hatching performance and larval survival of blue gourami.

2. Materials and Methods

2.1 Materials

Broodstock of blue gourami was reared and conditioned in 54 L capacity aquarium tanks for procurement of eggs in the Wet Laboratory of College of Fisheries, Ratnagiri. It was fed clam meat at 8% of body weight twice a day. Circular plastic containers of 3 L capacity were used for the egg incubation experiment. Spawn of blue gourami were fed green culture dominating *Chlorella* cells and *Infusoria* culture.

2.2 Methods

Plastic containers were arranged following completely randomized design with four replicates for each treatment. Plastic containers of 3L capacity were filled with 1 L water of pH 8.5 – 9.5. The L-Ascorbic acid levels of 0 mg L⁻¹ (T₀), 250 mg L⁻¹ (T₁), 500 mg L⁻¹ (T₂), 1000 mg L⁻¹ (T₃) and 2000 mg L⁻¹ (T₄) were added in it and water pH changed to 6.5 – 7.5. Eggs of *T. trichopterus* were randomly stocked in the experimental containers at the rate of 30 eggs

container⁻¹ for the immersion treatment of three hours. After the treatment, the same eggs were transferred to water of pH 6.5-7.5 with same stocking density in the plastic containers containing 1 L volume of water. The experiment was continued for a period of 20 days. After egg incubation and hatching, spawns were fed with green water and *infusoria* at the rate of 15 ml container⁻¹ twice a day. Water quality parameters such as temperature, pH, dissolved oxygen (DO), total alkalinity and total hardness were determined by following standard methods given in APHA (2005) [7] and Boyd (1981) [8].

3. Results

3.1 Egg incubation period

Eggs were incubated in the plastic containers at 25.4–26.2 °C. The hatching rate of 100% was noticed in the experiment. Eggs in T₃ treatments comprising 1000 mg L⁻¹ of L-Ascorbic acid were hatched out after 18 h. while, eggs in T₂ treatment were hatched out after 19 h. Eggs in T₀ and T₁ treatments were hatched out after 20 h; whereas, eggs in T₄ treatment were hatched out late as compared to other treatments i. e. after 21 h.

3.2 Hatching percentage

The analysis of hatching rate (%) was calculated by using following formula:

$$\text{Hatching rate (\%)} = \frac{\text{Total number of hatchling produced}}{\text{Total number of eggs incubated}} \times 100$$

After the incubation, all eggs in each treatment were hatched out after 18 h to 21 h of incubation time, meaning 100% of hatching rate was observed.

3.3 Survival (%)

The average spawn survival was calculated for each replicate of each treatment of the experiment for analysis of survival (%) by using the following formula (Kashani *et al.*, 2010) [9]. The results are shown in Fig. 1.

$$\text{Survival (\%)} = \frac{\text{Number of fry obtained}}{\text{Number of spawn stocked}} \times 100$$

The maximum survival of 97.5% was observed in T₃ (1000 mg L⁻¹); whereas, T₄ (2000 mg L⁻¹) showed the minimum survival of 62.5%. One way ANOVA showed significant difference ($P < 0.05$) in the survival (%) of *T. trichopterus* spawn stocked at different levels of Vitamin C. The Student-Newman-Keuls multiple range test (SNK) revealed that the survival in T₃ (1000 mg L⁻¹) was significantly higher ($P < 0.05$) than T₀ (0 mg L⁻¹) and T₄ (2000 mg L⁻¹).

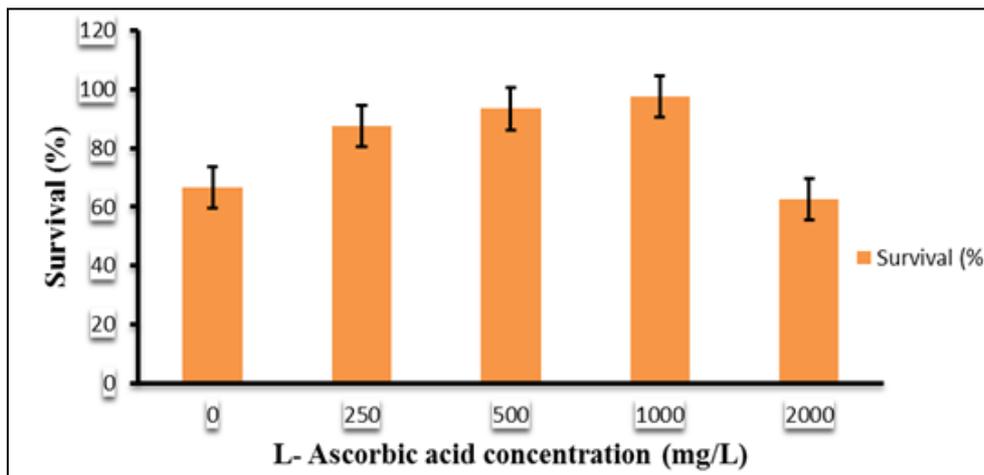


Fig 1: Average survival (%) of *T. trichopterus* spawn

3.4 Water quality parameters

During the experiment, the values of water quality parameters such as temperature, pH, dissolved oxygen (DO), total alkalinity and total hardness were ranged between 25.1 – 26.2 °C, 6.3 – 6.9, 3.4 – 4.13, 27.5 – 40 and 34.5 – 43.5 respectively.

4. Discussion

Generally, vitamin C is an indispensable and multifunctional micronutrient. Many previous studies have shown the role of vitamin C in improvement of fish breeding performance and increased larval survival rate. Gavriilidou *et al.* (2003) [10] indicated that the addition of 1200 mg AA kg⁻¹ into rainbow trout broodstock diet increased not only hatchability, but the viability of fry and the percentage of the total fry produced. Falahatkar *et al.* (2006) [11] suggested that when broodstock of rainbow trout did not have enough vitamin C in their ovaries, immersion of eggs in at least 1000 mg L⁻¹ of neutralized ascorbic acid (with NaOH) for 3 h and 24 h after fertilization, applied during water hardening were useful which showed

54.9% of survival rate. Langroudi *et al.* (2009) [13] observed that the diets of angel fish containing *Artemia urmiana* + fatty acid emulsion + 0.5 g vitamin C resulted in highest fertilization rate of 98.8%, hatching rate of 96.8% and the highest larvae survival (93.3%) which was significantly higher than the control group ($P < 0.05$). Egg hatching and survival rates could be increased by addition of ascorbic acid at the rate of 2000 mg L⁻¹ in Prussian carp, kutum and angel fish as observed by Taati *et al.* (2010 & 2011) [14-15] and Farahi *et al.* (2011) [12]. Results of the present study also indicated that the dose of 1000 mg L⁻¹ L-ascorbic acid give better results in terms of percentage hatchability and survival rates. As such, the findings of the study showed a level of vitamin C can be added to the extent of 1000 mg L⁻¹ to 2000 mg L⁻¹. In the present study, a level of 2000 mg L⁻¹ did not evince better performance. On the basis of study, it is concluded that immersion of blue gourami eggs in a concentration of 1000 mg L⁻¹ of L-ascorbic acid is required for egg hatching and better survival of its spawn.

5. Conclusion

It is concluded that the concentrations of 1000 mg L⁻¹ of vitamin C level applied during eggs hardening was useful for enrichment of vitamin C concentration in the eggs. It represented a clear advantage of decreasing egg incubation time and maximizing spawn survival percentage of blue gourami, *Trichopodus trichopterus*.

6. Acknowledgements

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