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The Effect of Ascorbic Acid on Hatching Performance and Tolerance against Environmental Stressor (High Temperature) by Immersion of Angel Fish (*Pterophyllum Scalare* Schultze, 1823) Fertilized Eggs

[1]Amin Farahi, [1]Milad Kasiri, [1]Mohammad Sudagar and [2]Amir Talebi

[1]Department of Fishery, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran
[2]Department of Fisheries Sciences, Islamic Azad University of Babol, Babol, Iran

Abstract: The aim of this study was to evaluate the influence of L-ascorbic acid (AA) in three levels (0, 100, 1000 and 2000 mg LG^{1}) on eyed egg and hatching rate, growth and viability of larvae and larval tolerance against high temperature stress of angel fish (*Pterophyllum scalare* Schultze, 1823). Fertilized eggs were placed in water containing different levels of AA for 3 h. The percentage of eyed egg and hatching were measured after 2 and 3 days respectively. After larvae absorbed their yolk sac, half of them were challenged by high temperature (36°C) and the others were reared for 20 days and growth and survival were calculated. The result showed that the highest eyed egg and hatching rate were in 2000 mg LG^{1} and had significantly difference with other treatments ($P<0.05$). The significant differences in larval tolerance against high temperature stress were observed in 1000 and 2000 mg LG^{1} compared to 0 and 100 mg LG^{1} treatments. No significant difference was observed between growth amount of treatment batches. Viability was different among experimental groups, but it was not significant between 0 and 100 mg LG^{1}. According to our results when broodstocks of angel fish do not have enough vitamin C in their ovaries, immersion of fertilized eggs in 2000 mg LG^{1} of AA may be beneficial.

Key words: Angel fish % Acid ascorbic % Fertilized eggs % High temperature stress

INTRODUCTION

Vitamin C is known to perform numerous biochemical and physiological functions in both plant and animal metabolism [1]. Fishes and shrimps require a dietary source of vitamin C to prevent or reverse scorbutic symptoms. Among these species, dietary essentiality of vitamin C in fish and shrimp probably results from an absence or insufficiency of L-gulonolactone oxidase [2,3].

Over the past 30 years a great deal of research has been conducted to study the function of ascorbic acid in aquatic species. Effects of dietary ascorbic acid on growth, morphogenesis, reproduction and adaptation have been studied extensively in carp [4], catfish [5,6], trout and salmon [7,8], shrimp [9,10], tilapia [11,12] and even snake heads [13].

When eggs absorb water, it is possible to introduce compounds and micronutrients, such as vitamins and mineral elements, into the eggs with the water solution before hardening. In rainbow trout, immersion the fertilized eggs in enrichment water by vitamin C had significantly effect on TAA (total acid ascorbic) concentration at the eyed stage and in hatched alevins [14]. Useful effects of complementary ascorbic acid in broodstock diets on fish fertility have been shown in rainbow trout, *Oncorhynchus mykiss* [7,15], tilapia, *Oreochromis niloticus* [12], cod, *Gadus morhua* [16], yellow perch, *Perca flavescens* [17] and guppy, *Poecilia reticulata* [18]. Also, AA is known to take part in several biochemical reactions within the cells, all related to its ability to undergo reversible oxidation and reduction [19]. AA has been shown to improve immune response [6] and tolerance to environmental stressors [20,21].

It has been cleared that vitamin C is required by all animals for body maintenance, growth and other biological performances and the vitamin C level needed for these functions varies with the species and culture environment [22].

Corresponding Author: Amin Farahi, Department of Fishery, Gorgan University of Agricultural Sciences and Natural Resources, Iran, Golestan, Gorgan, Shahid Beheshti Avenue, Postal code: 49138-15739.
E-mail: farahi2010@yahoo.com.

The present study was undertaken to observe the ascorbic acid on hatching performance and tolerance against environmental stressor (high temperature) by immersion of angel fish (*Pterophyllum scalare* Schultze, 1823) fertilized eggs.

MATERIAL AND METHODS

Collection of Fertilized Eggs and Vitamin Treatments: The experiments were conducted from June to August 2010 in Institute of Ornamental Fish Hatchery in Babol, Iran. 12 pairs of angel fish breeders acclimated for 2 weeks in 12 (80×30×40 cm) glass aquaria. Breeders were spawned during one month. After spawning breeders were separated of each aquarium and transferred to another tanks.

Four different concentrations of ascorbic acid including 0 (control), 100, 1000 and 2000 mg LG^{1} of L-ascorbic acid (AA) (Sigma, St Louis, MO, USA) were added to each experimental aquarium. Each treatment was performed in three replicate. Gentle aeration was provided by air stones. During the experiment, the water quality parameters were monitored during the trial and average value for temperature, dissolved oxygen, hydrogen ion concentration (pH) and salinity were 26±2 ºC, 5.7-7.7 mg lG^{1}, 6.9-7.8 units and 0.1 mg lG^{1} respectively. Dark cycle of 12:12 h was maintained during the experiment.

Incubation: Eggs were incubated in the aquariums at 26°C. The percentage of eyed egg and hatching rate was measured after 2 and 3 days respectively.

Larva Cultivation and High Temperature Challenge: After yolk sac absorption, larvae were divided in 2 groups. For evaluation of the newly hatched larval quality, half of the larvae were challenged by high temperature. In this propose, larvae were transferred to other aquariums and temperature was increased to 36°C (10°C higher than incubation temperature) and survival duration was calculated.

The other halves of larvae were reared for 20 days. Larvae were fed with artemia naupli diet during this period. Fish from each aquarium were counted and their length was measured to monitor growth and mortalities were recorded.

Calculations and Statistical Analysis
The Following Variable Was Calculated

$$\text{Survival} = N_t \times 100\ N_0\ G^{1} \quad [23]$$

N_t and N_0 were final and initial numbers of larvae in each replicate, respectively; and t is the experimental period in days.

Results are presented as means±SD. Significant differences among treatments were determined by analysis of variance (ANOVA) and the differences between means were tested with Duncan's multiple-range test using SPSS 16.0 programme. Differences were considered significant at $p<0.05$.

RESULTS

Effect of Vitamin C on Eyed Egg and Hatching Rate: The result demonstrated that eyed egg and hatching rate were increased with increasing the level of vitamin C and were significant between 2000 mg LG^{1} with other treatments ($p<0.05$). The highest percentage of eyed egg and hatching (94.10±3.25 and 96.25±1.40) and the lowest percentage of eyed egg and hatching (77.92±3.88 and 82.70±1.20) were observed in 2000 and 0 mg LG^{1} respectively. Differences were not significant between 0, 100 and 1000 mg LG^{1} treatments (Figure 1).

Effect of Vitamin C on Larval Tolerance: As seen in the figure 2, differences in larval tolerance against high temperature stress (36°C) were observed among experimental groups and they were significant between 0 and 100 with 1000 and 2000 mg LG^{1} treatments. The highest and lowest times of survival in 36°C were observed in 2000 and 0 mg LG^{1} respectively.

Effect of Vitamin C on Growth and Viability: Growth amount was not significantly different among treatments. Growth amount in 2000 mg LG^{1} was higher than 0 and 100 mg LG^{1} and lower than 1000 mg LG^{1} treatments but these differences were not significant (Figure 3).

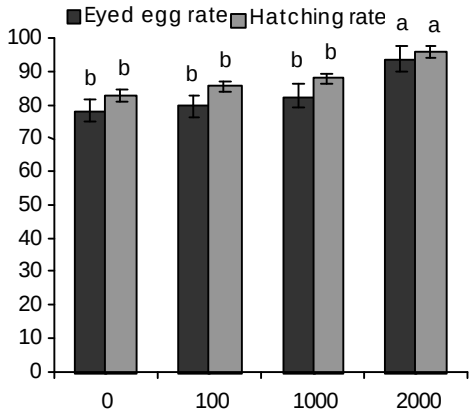

Fig. 1: The percentage of eyed egg and hatching rate in groups treated by vitamin C.

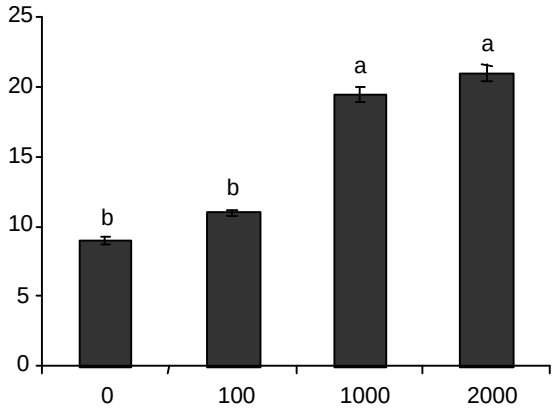

Fig. 2: Effect of vitamin C on larval survival duration against high temperature (36°C).

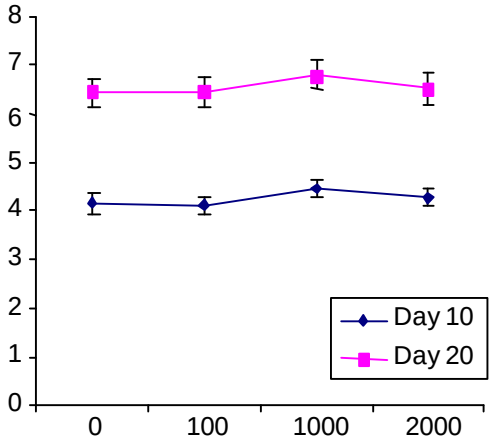

Fig. 3: Growth amount among treatments after 10 and 20 days (mm)

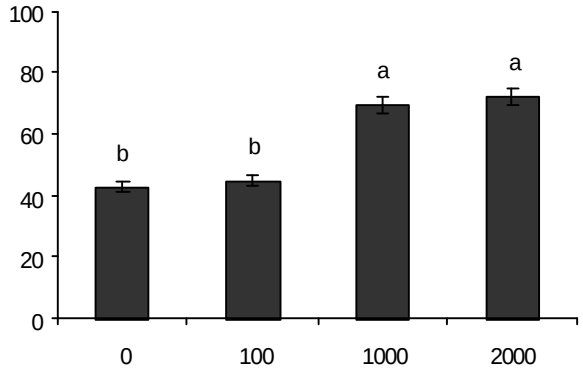

Fig. 4: Survival rate of fry after 20 days.

As see in the figure 4, survival rate were increased with increasing the level of vitamin C. But significant difference was not observed between 0 and 100 mg LG^{1}. The highest survival and lowest survival were observed in 2000 and 0 mg LG^{1} treatments.

DISCUSSION

The present study confirmed that additional AA is useful for the propagation of angel fish broodstock and affected positively on percentage of eyed egg, hatching rate and larval performance. The importance of high ascorbic acid concentrations in female fish gonads for embryo vitality has been reported [24,25].

Ascorbate transfer actively from yolk reserves into larval fish body. For instance, Terova *et al.* [26] argued that in some scenarios, ascorbate concentration increases significantly between unfertilized egg and yolk sac larvae. In other species, a decrease of approximately 20-50% was observed during embryonic development and endogenous feeding [27,15]. The most likely need for the ascorbic acid storage in egg yolk reserves is for the synthesis of collagens during the development of the embryo and for proline and lysine hydroxylation.

In this study, we found increased eyed egg and hatching rate in the eggs after immersion with ascorbic acid solutions and they were maximum in 2000 mg LG^{1} treatment. The application of this procedure may be helpful in balancing out individual variations among different females and may decrease susceptibility to vitamin C deficiency in broodstocks fish. Bylund and Lerche [28], Fitzsimons [29], Fisher *et al.* [30] and Amcoff *et al.* [31] used different concentrations of thiamin to prevent M74 disease (Baltic Sea salmon), EMS (salmonids in Great Lake) or CS (Cayuga syndrome; *Salmo salar* in Finger Lakes region). Their findings indicated that the concentration of thiamin after immersion of eggs in thiamin solutions was increased and the mortality of eggs and embryos decreased. Falahatkar *et al.* [14] suggested that when broodstocks rainbow trout do not have enough vitamin C in their ovaries, immersion of eggs in 1000 mg LG^{1} of neutralized AA (with NaOH) may be useful.

Treatment of angel fish fertilized eggs with ascorbic acid increased larval tolerance against high temperature stress at 30°C. Cavalli *et al*. [32] evaluated the effect of dietary supplementation of vitamins C and E on maternal performance and larval quality of the prawn *Macrobrachium rosenbergii*. They tested the tolerance of newly hatched and 8-day-old larvae of *M. rosenbergii* to ammonia exposure. Their results shown newly hatched and 8-day-old larvae tolerance tended to increase with increasing levels of AA and higher dietary levels of á-tocopherol acetate did not affect the tolerance to ammonia of newly hatched larvae, but it positively augmented the ammonia tolerance of 8-day-old larvae.

Also eggs treated during water hardening indicate that survival increased with increasing AA but had not affect on growth parameters. Ibiyo *et al.* [33] evaluated the requirements of vitamin C (ascorbic acid) in *Heterobranchus longifilis* fingerlings and indicated the survival and growth of *H. longifilis* fingerlings improved significantly with increasing supplementation of dietary ascorbic acid and its growth reached a plateau at between 100 to 200 mg AA kg^{-1} diet.

CONCLUSION

According to our results, we suggested a dose of 2000 mg AA L^{-1} to enrich water of angel fish eggs incubation.

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