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Evaluated the Influence of Ascorbic Acid on Hatching Performance and Tolerance Against High Ammonia Concentration by Immersion of Kutum *(Rutilus frisii kuttum)* Fertilized Eggs

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Abstract: The effect of L-ascorbic acid (AA) in four levels (0, 500, 1000 and 2000 mg L^{-1}) on eyed egg and hatching rate, growth and viability of larva, and larval tolerance against high ammonia concentration stress in kutum *Rutilus frisii kutum* (Kamenskii, 1901) was evaluated. The fertilized eggs were placed in water containing different levels of AA for 4 h. The percentage of eyed egg and hatching were measured after 2 and 5 days respectively. After larva absorbed their yolk sac half of them were challenged by high ammonia concentration (5 mg L^{-1}) and the others were reared for 60 days and growth factors and survival were recorded. The result shown that with increasing AA, percentage of eyed egg and hatching were increased and the highest eyed egg and hatching rate were in 2000 mg L^{-1} and had significantly difference with other treatments ($P<0.05$). The significant differences in larval tolerance against high ammonia concentration stress were observed between 2000 mg L^{-1} comparison to 0 and 500 mg L^{-1} treatments. No significant different were observed between growth parameters of treatments ($P>0.05$). Viability was different between experimental groups, but was not significant between 0 and 500 mg L^{-1}. According to our results, immersion of fertilized eggs of kutum in 2000 mg L^{-1} of AA may be beneficial.

Key words: Kutum, Acid ascorbic, Fertilized eggs, High ammonia concentration.

INTRODUCTION

Kutum *Rutilus frisii kutum* Kamensky (Cyprinidae) is one of the economically important fishes of the Caspian Sea. They are mostly distributed in the southern part of the Caspian Sea, especially in the area from Astara to Gorgan River and migrate into rivers for spawning (Razavi, 1995, 1998). Kutum is in great demand by virtue of its high taste quality and the cuisine customs of the local residents and is consumed all year round. The Iranian fisheries organization (Shilat) produce and release up to 200 million fry (average weight, 1g) into the Caspian Sea annually (Dorafshan and Paykan Heyrati, 2006).

On the other hand vitamin C is an essential vitamin for normal physiological functions in animals including fish (Wilson and Poe, 1973; Lim and Lovell, 1978). The ascorbic acid requirement for different teleost fish has been well documented (Dabrowski, 2001). Tissues vary considerably in their concentration of ascorbic acid, but gonads represent one of the highest levels, several-fold higher than blood plasma (Blom and Dabrowski, 1995a; Ciereszko and Dabrowski, 1995). High concentrations of ascorbic acid in fish gonads indicate its particular relevance to reproduction. The lack of dietary AA also resulted in lower egg hatching rates (Sandnes *et al*., 1984), egg strength (Mangor-Jensen and Holm, 1994), and poor fry survival (Soliman *et al*., 1986).

On the other hand 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 (Falahatkar *et al*., 2006). Useful effects of complementary ascorbic acid in broodstock diets on fish fertility have been shown in rainbow trout, *Oncorhynchus mykiss* (Sandnes *et al*., 1984; Blom and Dabrowski, 1995b), tilapia, *Oreochromis niloticus* (Soliman *et al*., 1986), cod, *Gadus morhua* (Mangor-Jensen and Holm, 1994) and yellow perch, *Perca flavescens* (Lee and Dabrowski, 2004).

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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 (Conklin, 1997). AA has been shown to improve immune response (Li & Lovell, 1985), and tolerance to environmental stressors (Ishibashi *et al*., 1992; Merchie *et al*., 1995).

It has been established that vitamin C is required by all animals for body maintenance, growth and other biological performance and the vitamin C level needed for these functions varies with the species and culture environment (Delong *et al*., 1958; Lovell, 1972). Lee *et al*., (1998) reported that by increasing of vitamin C level in the diet to 1500 mg kg^{-1} diet, the best growth performance and feed utilization for Korean rockfish (*Segastes schlegeli*) were obtained. Similarly, the influence of dietary levels of vitamin C on growth rates of *Heterobranchus longifilis* fingerlings was studied by Ibiyo *et al*., (2007) who mentioned that the dietary level of vitamin C required for maximum growth of this species is 100 mg kg^{-1} diet.

The aims of this investigation were to evaluate the effect of immersion fertilized kutum eggs in enrichment water by different levels of L-ascorbic acid on hatching performance (eyed egg and hatching percent), tolerance against high ammonia concentration test, some of growth factors and viability of larva.

MATERIAL AND METHOD

The experiments were conducted from March to June 2010 in aquaculture research center in university of Gonbad kavous, Iran. 12 Kutum female (mean weight, 957±94.3 g) and 8 Kutum male (mean weight, 724.3±83.5 g) transferred to the place of experiment and acclimated for 2 days in 4000 L tanks. Broodstocks injected by 0.5 mg kg^{-1} Ovaprim (sGnRH+Dompridon) and 12 hours after injection treatment females were stripped and pooled. Fresh milt also was collected from males 12 h after injection, pooled and stored in syringe.

Four different concentrations of ascorbic acid including 0 (control), 500, 1000 and 2000 mg L^{-1} of L-ascorbic acid (AA) (Sigma, St Louis, MO, USA) were added to each experimental aquarium (with 80 liter aerated water). Each treatment was performed in three replicate.

Approximately 1 g (~1000 oocytes) were used for each replicate and placed in petri dish (10 cm diameter). Sperm motility was checked before experimentation (Ciereszko and Dabrowski, 1993), and semen samples with > 90% initial motility were pooled and used for fertilization. Ova from the all of 12 females were mixed together. 200 microlitres of semen was used for each replicate. Obtained eggs were fertilized by milt and were placed in aquarium containing different levels AA for 3 h, after hardening their water emptied and used fresh water (without AA) and aeration was performed. Eggs were incubated in these aquariums at 20°C. The percentage of eyed egg and hatching rate was measured after 2 and 5 days respectively.

After yolk sac absorption, larva were divided to 2 group. For evaluated the newly hatched larval quality, the tolerance of them was estimated to ammonia stress. In this propose, half of newly hatched larvae were exposed to 5 mg L^{-1} total ammonia (TAN; $NH4^{+}$+NH3) in glass aquarium containing with 10 L of no aeration water and survival duration was recorded (Jafaryan *et al*., 2009). The solution of ammonia was obtained by dissolving reagent grade ammonium chloride (NH4Cl). No feed was offered during exposure. Larvae not responding to mechanical stimuli were considered dead.

The other half of larva were reared for 60 days. Larva were fed by artemia naupli and diet during this period. Fish from each aquarium were counted and weighed at 2-week intervals to monitor growth and mortalities were recorded.

The following variables were calculated:

Body weight increase (BWI) = $W_t - W_0$ (Tacon, 1990)

Specific growth rate (SGR) = $(\ln W_t - \ln W_0) \times 100\ t^{-1}$ (Hevroy *et al*., 2005)

Daily growth rate (DGR) = $[(W_t - W_0)/ t] \times 100$ (De Silva and Anderson, 1995)

Survival = $N_t \times 100\ N_0^{-1}$ (Ai *et al*., 2006)

W_t and W_0 were final and initial larva weights (g), respectively; N_t and N_0 were final and initial numbers of larva 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 shown that eyed egg and hatching rate were increased with increasing the level of vitamin C and were significant between 2000 mg L^{-1} with other treatments ($P<0.05$). The highest percentage of eyed egg and hatching (87.42±7.72 and 89.64±8.45) and lowest percentage of eyed egg and hatching (75.54±6.35 and 79.14±6.84) was observed in 2000 and 0 mg L^{-1} respectively. Differences were not significant between 0, 500 and 1000 mg L^{-1} treatments ‘‘see figure 1”.

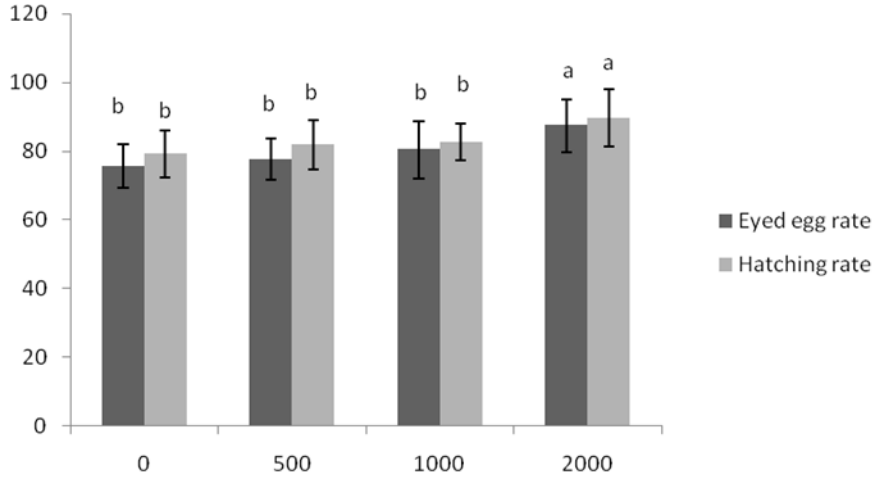

Fig. 1: The percentage of eyed egg and hatching in treated groups by vitamin C.

Effect of Vitamin C on Larval Tolerance:

As see in the figure 2 differences in larval tolerance against high ammonia concentration stress (5 mg L^{-1}) were observed between experimental groups, and they were significant between 0 and 500 with 1000 and 2000 mg L^{-1} treatments. Highest and lowest times of survival in 5 mg L^{-1} ammonia were observed in 2000 and 0 mg L^{-1} respectively.

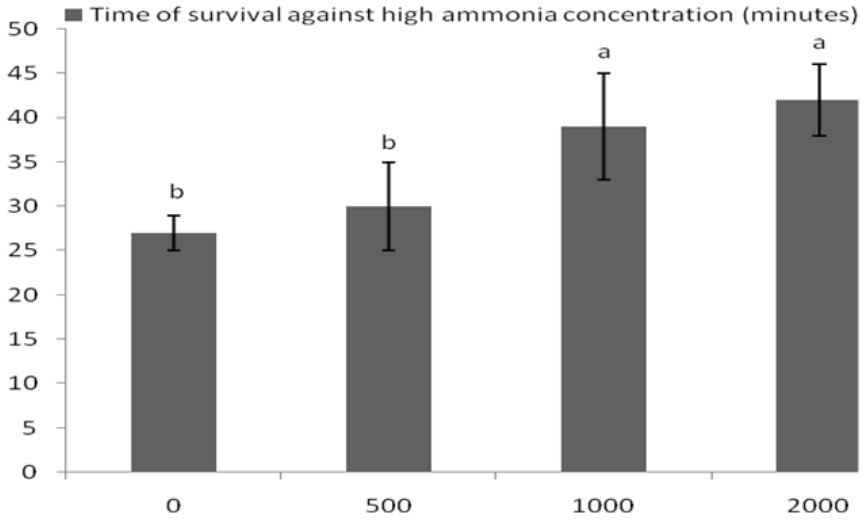

Fig. 2: Effect of vitamin C on larval survival duration against high ammonia concentration (5 mg L^{-1}) stress.

Effect of vitamin C on growth and viability:

Growth parameters (BWI, SGR and DGR) were not significantly different between treatments. Highest and lowest BWI, SGR and DGR were observed in 1000 and 500 mg L^{-1} respectively. Growth parameters in 2000 mg L^{-1} were higher than 0 mg L^{-1}, and lower than 500 and 1000 mg L^{-1} treatments but these differences were not significant ‘‘see table 1”.

Table 1: Growth factors of obtained fry kutum from treated groups by vitamin C 60 days (Mean ± SD).

Vitamin (mg L^{-1})	0	500	1000	2000
BWI	312±5.12[a]	310±4.34[a]	322±4.74[a]	316±3.64[a]
SGR	9.57±2.73[a]	9.56±3.42[a]	9.62±5.69[a]	9.59±4.56[a]
DGR	520.54±14.61[a]	516.37±9.2[a]	536±11.24[a]	526.72±8.26[a]

Values of different superscripts in a row are significantly different at (P<0.05).

As see in the figure 3 survival rate were increased with increasing the level of vitamin C. But significant difference was not observed between 0 and 500 mg L^{-1}. Highest survival (67.57% ± 8.64) and lowest survival (51.84% ± 6.29) were observed in 2000 and 0 mg L^{-1} treatments.

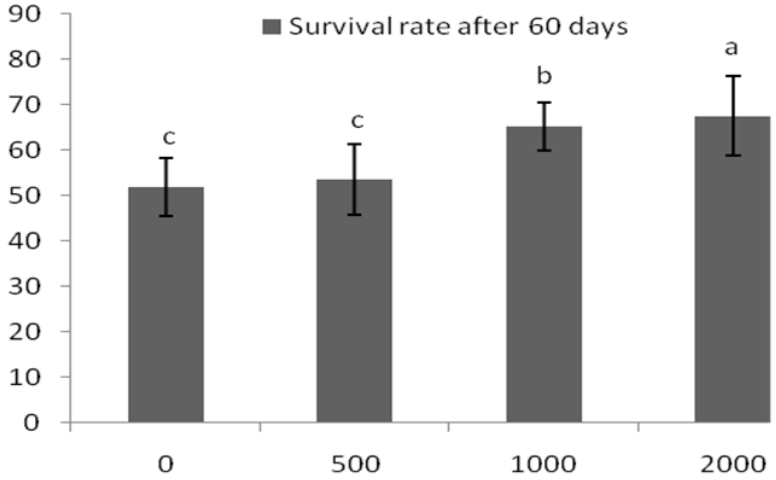

Fig. 3: Survival rate of obtained fry kutum from treated groups by vitamin C after 60 days.

Discussion:

The present study confirmed that additional AA is useful for the propagation of Kutum 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 (Dabrowski, 1991; Blom and Dabrowski, 1995a; Dabrowski and Ciereszko, 2001).

Ascorbate transfer actively from yolk reserves into larval fish body. For instance, Terova *et al*., (1998) 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 (Knox *et al*., 1988; Blom and Dabrowski, 1998). 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 the present 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 L^{-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, (1995), Fitzsimons, (1995), Fisher *et al*., (1996) and Amcoff *et al*., (1998) 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 results 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*., (2006) suggested that when broodstock rainbow trout do not have enough vitamin C in their ovaries, immersion of eggs in1000mg L^{-1} of neutralized AA (with NaOH) may be useful.

Treatment of Kutum fertilized eggs with ascorbic acid increased larval tolerance against high ammonia concentration (5 mg L^{-1}) stress. *Cavalli et al*., (2003) 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 α-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 indicates that survival increased with increasing AA but had not affect on growth parameters. Ibiyo *et al*., (2007) 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:

This study suggested that vitamin C can be used for increasing hatching performance and larval viability against environmental stressors. According to result, we suggested a dose of 2000 mg L^{-1} to enrich water of Kutum eggs incubation.

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