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Investigation of the hatching performance, growth, survival and tolerance against high ammonia concentration by enrich water incubation of carp (*Cyprinus carpio*) eggs with α-tocopherol

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Abstract. The effect of α-tocopherol (Vitamin E) on eyed egg and hatching rate, growth and viability of larva, and larval tolerance against high ammonia concentration stress of common carp *Cyprinus carpio* (Linnaeus, 1758) was evaluated. The fertilized eggs were placed in water containing 4 levels of α-tocopherol (0, 300, 500 and 1000 mg L^1) for 5 h. The percentage of eyed egg and hatching were measured after 2 and 3 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 the highest eyed egg and hatching rate were in 1000 mg L^{-1} but had not significantly difference with other treatments ($P<0.05$). The significant differences in larval tolerance against high temperature stress were not observed between treatments. No significant different were observed between growth parameters of treatments ($P>0.05$). Viability was different between experimental groups and was not significant between 0 and 300 mg L^{-1}. According to present results, enrich incubation water of carp eggs by 1000 mg L^{-1} of α-tocopherol may be beneficial.
Key Words: Carp, α-tocopherol, fertilized eggs, high ammonia concentration.

چکیده: اثر α توکوفرول (ویتامین E) بر روی میزان چشم زدگی و تخمه گشایی، رشد و بقای لارو، تحمل لاروی در برابر استرس حاصل از غلظت بالای آمونیاک در ماهی کپور *Cyprinus carpio* (Linnaeus, 1758) مورد بررسی قرار گرفت. تخم های بارور شده در آب حاوی چهار سطح مختلف α توکوفرول (0، 300، 500 و 1000 میلی گرم در لیتر) و به مدت 5 ساعت قرار داده شد. درصد چشم زدگی و تخمه گشایی پس از 2 و 3 روز اندازه گیری شد. پس از جذب کیسه زرده، نیمی از لاروها در معرض غلظت بالای آمونیاک (5 میلی گرم در لیتر) قرار گرفته و مابقی آنها به مدت 60 روز پرورش داده شدند و پارامترهای رشد و بقا محاسبه و ثبت شد. نتایج نشان داد که بالاترین نرخ چشم زدگی و تفریخ در تیمار 1000 میلی گرم در لیتر مشاهده شد، اما تفاوت معنی داری با سایر تیمارها نداشت ($P>0/05$) . تفاوت معنی داری در مقاومت لاروها در برابر استرس حاصل از غلظت بالای آمونیاک در بین تیمارها مشاهده نشد. هیچ تفاوت معناداری میان شاخص‌های رشد تیمارها مشاهده نشد ($P>0/05$). نرخ زنده مانی بین گروه های آزمایشی متفاوت بود ولی این اختلاف بین تیمارهای 0 و 300 میلی گرم در لیتر معنی دار نبود. با توجه به نتایج مطالعه حاضر می توان عنوان کرد که غنی سازی آب انکوباسیون تخم های کپور با 1000 میلی گرم در لیتر α توکوفرول می تواند سودمند باشد.
کلمات کلیدی: ماهی کپور، α توکوفرول، تخم های بارور شده، غلظت بالای آمونیاک.

Introduction. Vitamin E is a fat-soluble vitamin that consists of a group of tocopherols and tocotrienols with a hydrophobic character. It has a major function in its action as a lipid antioxidant to protect the polyunsaturated membrane lipids against free radical attacks (Wang & Quinn 2000). Vitamin E activity is present in a group of naturally occurring closely related tocopherols. Among them, α-tocopherol has the highest vitamin E activity.

α-tocopherol is absorbed from the intestine along with dietary fats (Bjorneboe et al 1990). As a fat-soluble antioxidant, the major function of vitamin E is to prevent peroxidation of polyunsaturated fatty acids of phospholipids and cholesterol in cellular and subcellular membranes. α-tocopherol can donate a hydrogen atom to a lipid peroxyl radical to generate lipid hydroperoxide, thus terminating the progression of lipid peroxidation. Other functions of α-tocopherol have also been proposed, e.g. in maintaining membrane protein thiols, stabilizing membrane structure, preventing certain diseases (Lii et al 1996), activating fish immune functions (Anderson 1992, Sakai 1999)

and protecting cells with other antioxidants from damage and lysis induced by oxidative stress (Konar et al 2000). As a membrane-bound antioxidant, vitamin E appears to scavenge free radicals at the site of their formation.

Vitamin E has been used to improve sperm quality and to prevent male infertility both in humans and in animals (Halver 1989) and has been known to improve the reproductive performance of fish. Increasing fertilized eggs and survival of fry in ayu, *Plecoglossus altivelis* (Temminck and Schlegel, 1849) (Takeuchi et al 1981), high fecundity and higher percentage of normal eggs in gilthead seabream *Sparus aurata* (Linnaeus, 1758) (Izquierdo et al 2001) were reported. Vitamin E deficiency hindered ovarian growth in common carp *C. carpio* and led to a reduction in spawning success (Watanabe & Takashima 1977).

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). Also, enrichment of incubation water of goldfish fertilized eggs by AA, shown that vitamin C influenced on eyed egg, hatching rate and increased the larval tolerance against high temperature challenge, but had no effect on growth parameters (Taati et al 2010).

The significance of vitamin E in fish reproduction was confirmed in earlier studies. In a study of the effects of vitamin E and growth hormone on gonadal maturity in the common carp (*C. carpio*), dietary vitamin E resulted in a higher gonadosomatic index, larger ova, and more eggs with higher hatchability than the control (Gupta et al 1987). Further, spawning was complete in fish fed a diet supplemented with vitamin E but partial in the majority of fish fed diets lacking vitamin E (Gupta et al 1987). Vitamin E is essential for fertility and reproduction in fish and fish cannot synthesize vitamin E, so the maternal dietary content of each prior to oogenesis is an important determinant of reproductive fitness (NRC 1993). Beneficial influences of complementary vitamin E in broodstock diets on fish fertility have been shown in crayfish, *Astacus leptodactylus* (Eschscholtz, 1823) (Harlioglu & Barim 2004), goldfish, *Carassius auratus* (Bloch, 1782) (James et al 2008, 2009), Black Sea trout, *Salmo labrax* (Pallas, 1814) (Serezli et al 2010) and guppy, *Poecilia reticulata* (Peters, 1859) (Mehrad & Sudagar 2010).

This study evaluated the effect of enrichment incubation water of carp fertilized eggs by different levels of α-tocopherol on hatching performance (eyed egg and hatching percent), tolerance against ammonia stress, some of growth factors and viability of larva.

Material and Method

Gametes collection and vitamin treatments. The experiments were conducted in May and June 2009. 10 reared carp female (mean weight, 1735.89±624.6 g) and 20 reared carp male (mean weight, 1164.±487.12 g) were transferred to the place of experiment and acclimated for 1 week in 4000 L tanks. Broodstocks were injected with 0.5 mg kg-1 Ovaprim (sGnRH+Dompridon) and 12 hours after injection treatment females were stripped. Fresh milt also was collected from males 12 h after injection and stored in syringe. Four different concentrations of vitamin E 0 (control), 300, 500 and 1000 mg L^{-1} of α- tocopherol (Sigma, St Louis, MO, USA) were added to each experimental aquarium (with 80 liter aerated water). Each treatment was performed in three replicate.

Fertilization and incubation. Approximately 1 g (~900 oocytes) were used for each replicate and placed in petri dish (10 cm diameter). Sperm motility was checked before experimentation (Ciereszko & Dabrowski 1993), and semen samples with >90 % initial motility were pooled and used for fertilization. Ova from the all of 10 females were mixed together. 300 Microliters of semen was used for each replicate. Obtained eggs were fertilized by milt and were placed in aquarium containing different levels α-tocopherol for 5 h, after hardening their water emptied and used fresh water (without vitamin E) and aeration was performed. Eggs were incubated in these aquariums at 22°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, larva were divided in 2 groups. The tolerance of newly hatched larval quality was evaluated 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 45 days. Larva were fed with *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.

Calculations and statistical analysis. The following variables were calculated:

Body weight increase (BWI) = Wt – W0 (Tacon 1990)

Specific growth rate (SGR) = (ln Wt – ln W0)×100 t^{-1} (Hevroy et al 2005)

Daily growth rate (DGR) = [(Wt – W0)/ t]×100 (De Silva & Anderson 1995)

Survival = Nt×100 $N0^{-1}$ (Ai et al 2006)

Wt and W0 were final and initial larva weights (g), respectively; Nt and N0 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 and Discussion

Effect of vitamin E on eyed egg and hatching rate. The result shown that eyed egg and hatching rate were increased with increasing the level of vitamin E but no significant between treatments ($P<0.05$). The highest percentage of eyed egg and hatching (86.54±6.27 and 91.63±5.47) and lowest percentage of eyed egg and hatching (79.36±8.53 and 84.1±3.2) was observed in 1000 and 0 mg L^{-1} respectively (see Figure 1).

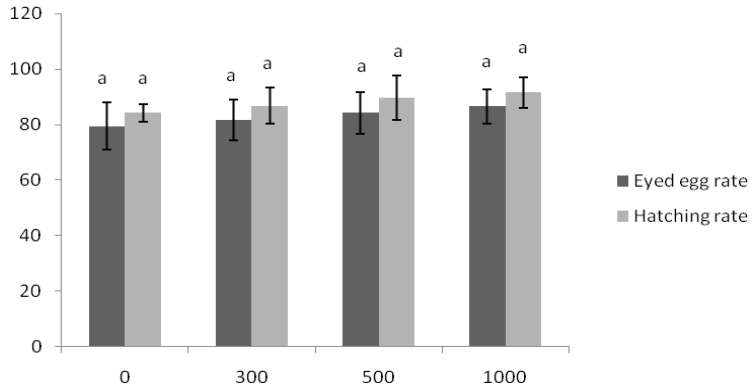

Figure 1. The percentage of eyed egg and hatching rate in groups treated by vitamin E.

Effect of vitamin E on larval tolerance against high ammonia concentration stress. As see in the Figure 2, differences in larval tolerance against high ammonia concentration stress (5 mg L^{1}) were not observed between experimental groups. Highest and lowest times of survival in 5 mg L^{1} were observed in 500 and 300 mg L^{-1} respectively.

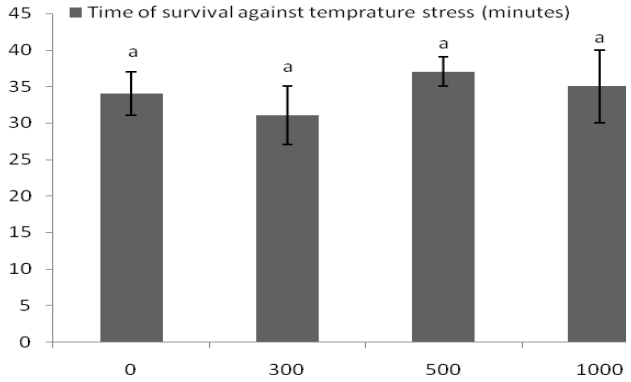

Figure 2. Effect of vitamin E on larval survival duration against high ammonia concentration (5 mg L^{-1}).

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 0 mg L^{-1} respectively. Growth parameters in 500 mg L^{-1} were higher than 0 and 300 mg L^{-1}, and lower than 1000 mg L^{-1} treatments but these differences were not significant (see Table 1).

Table 1

Growth factors of Carp fry after 60 days (Mean ± SD)

Vitamin	0 (mg L^{-1})	300 (mg L^{-1})	500 (mg L^{-1})	1000 (mg L^{-1})
BWI	298±13.45^a	314±26.34^a	320±15.28^a	329±8.69^a
SGR	9.49±3.64^a	9.58±2.57^a	9.61±4.13^a	9.66±3.25^a
DGR	495.52±14.3^a	521.46±5.67^a	531.27±26.746^a	546.72±17.534^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 E. But significant difference was not observed between 0 and 300 mg L^{-1}. Highest survival (75.69%± 5.74) and lowest survival (47.32%± 7.56) were observed in 1000 and 0 mg L-1 treatments.

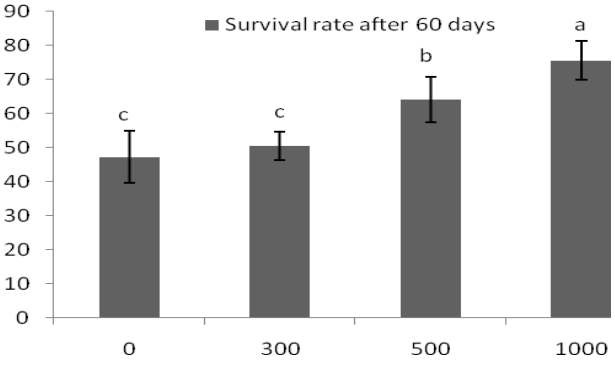

Figure 3. Survival rate of fry after 60 days.

Discussion. Although vitamin losses during processing, storage and leaching may be of significance, these are usually not considered. This study indicated that additional vitamin E to incubation water synchronic with hardening eggs is useful for the propagation of carp broodstock and affected positively on percentage of eyed egg, hatching rate and larval performance. In fish, higher dietary levels of vitamin E resulted in an increased percentage of buoyant eggs (considered an indirect parameter of normal egg development), improved hatching rates, and a higher production of viable larvae of red sea bream (Watanabe et al 1985). Similarly, Cahu et al (1991) found a linear correlation between the hatching rate of *Fenero penaeus indicus* (Milne-Edwards, 1837) eggs and vitamin E concentration. The levels of α-tocopherol in the eggs increase along with the dietary provision of α-tocopherol (Watanabe et al 1985; Cahu et al 1991, 1995; De Caluwe´ et al 1995). Watanabe et al (1985) indicated that the incorporation of dietary vitamin E into the eggs occurred together with lipids. Cavalli et al (2001) found a high correlation between the deposition of lipids and vitamin E in the ovary of *Macrobrachium rosenbergii* (De Man, 1879) and concluded that this was in line with the antioxidative function of this vitamin.

In the present study, we found increased eyed egg and hatching rate in the eggs after immersion with α-tocopherol solutions and they were maximum in 1000 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 E deficiency in broodstocks fish. Bylund & 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* (Linnaeus, 1758) 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 in 1000mg L^{-1} of neutralized AA (with NaOH) may be useful. Treatment of Carp fertilized eggs with α-tocopherol had not significantly effect on larval tolerance against ammonia 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 (*M. rosenbergii*). They tested the tolerance of newly hatched and 8-day-old larvae of prawn 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. The exposure to ammonia results in an increased cellular uptake and use of acylglycerols (Racotta & Herna´ndez-Herrera 2000). Therefore, higher levels of vitamin E, the major antioxidant present in cell membranes, could potentially enhance the use of these lipids by protecting them from peroxidation and, as a result, larvae would be better equipped to cope with higher levels of ambient ammonia. Also eggs treated during water hardening indicates that survival increased with increasing vitamin E levels but had not affect on growth parameters. The effects of vitamin E on fish growth has been reported. For example, James et al (2009) indivated that vitamin E had positive influence on growth, gonad weight, fecundity, and leukocyte count in goldfish (*C. auratus*). In this experiment, fish fed the 300 mg vitamin E kg^{-1} diet had the best feeding rate, weight gain, and specific growth rate. α-tocopherol had significantly effect on survival rate and this parameter were increased with increasing the level of vitamin E. In a study, the effect of dietary α-tocopherol on growth factors, survival, reproductive performance and sex ratio in guppy was investigated. Result shown in vitamin E treatments the body weight increase (BWI), percent body weight increase (PBWI), specific growth rate (SGR), daily growth rate (DGR) and reproductive performance of guppies were increased significantly with increasing the amounts of vitamin E and highest BWI, PBWI, SGR and DGR were observed in treatment 1000 mg L^{-1} and were no significant differences observed in survival rate and sex ratio between the treatments. This study indicated that BWI, PBWI, SGR and DGR and reproductive performance can be improved by dietary vitamin E supplementations and also may be

concluded that the vitamin E requirement of guppies fish for optimum growth and reproductive performance is 1000 mg kg^{-1} of dry diet (Mehrad & Sudagar 2010).

Conclusion. Result shown that vitamin E had not significantly effect on eyed egg and hatching rate, tolerance against high ammonia concentration, growth parameters but it's effect on survival in rearing duration was significant and highest viability in 1000 mg L^{-1} was observed. Altogether, can be express that enrich incubation water of carp eggs by 1000 mg L^{-1} of α-tocopherol may be useful.

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