Journal of Oceanography and Marine Science Vol. 3(1), pp. 1-7, January 2012
Available online at http://www.academicjournals.org/JOMS
DOI: 10.5897/JOMS11.022
ISSN 2141-2294 ©2012 Academic Journals

Full Length Research Paper

Assessment of the effects of dietary vitamin E on growth performance and reproduction of zebrafish, *Danio rerio (*Pisces, Cyprinidae*)*

Bahareh Mehrad*, Hojatollah Jafaryan and M. Mehdi Taati

Department of Fishery, Gorgan University of Agricultural Sciences and Natural Resources, Iran.

Accepted 8 November, 2011

The aim of this study was to evaluate the effects of dietary vitamin E (α-tocopherol) in 4 levels (100, 300, 500 and 1000 mg ascorbic acid kg^{-1}) with 1 control group (0 mg kg^{-1}) on growth, survival, fecundity and egg diameter in the zebrafish, *Danio rerio*. Zebrafish were divided into 5 treatments each triplicated, and fed with one of the five diets for 20 weeks. There were no significant differences in egg diameter observed between the treatments. The survival rate of zebrafish fed the diet containing 1000 mg kg^{-1} vitamin E was higher than other groups. In the vitamin E treatments, the body weight increase (BWI), specific growth rate (SGR) and fecundity of zebrafish increased significantly with increasing levels of vitamin E ($P < 0.05$). The significance of the results herein obtained underlined the importance of diet in the reproductive process, supporting the hypothesis that feed additives can improve fecundity. Considering that the zebrafish has been clearly established as a vertebrate model for biomedical research, these results support the potentiality of feed additives such as vitamins can improve reproduction in all vertebrates, including humans.

Key words: Zebrafish, vitamin E, growth performance, egg diameter, survival, fecundity.

INTRODUCTION

Nutrients like fatty acids, amino acids, minerals and vitamins have clear affects on reproduction as well as growth in fish.

Vitamin E is a lipid-soluble vitamin that comprises four tocopherols and four tocotrienols in nature. Among them, α-tocopherol has the highest vitamin E activity (NRC, 1993). Other than its vitamin E activity, α-tocopherol is a potent biological antioxidant that can protect biological membranes and lipid components containing unsaturated fatty acids against attack from oxygen free radicals.

A dietary requirement of vitamin E has been demonstrated in a number of fish. Vitamin E was originally considered as a dietary factor of animal nutrition, which has an importance in reproduction. In aquaculture, vitamin E is used for the fortification of feed to improve the growth, resistance to stress and disease as well as for survival of fish and shrimp (Vismara et al., 2003). As in higher vertebrates, vitamin E deficiency affects reproductive performance, causing immature gonads and lower hatching rate and survival of offspring (Izquierdo et al., 2001).

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 (*Cyprinus 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).

The nutritional status of the broodstock can affect offspring quality. The accumulation of essential nutrients such as essential fatty acids and vitamins are dependent

*Corresponding author. E-mail: bahar.mehrad@yahoo.com.

on the nutrient reserves in the mother animal, and consequently on the dietary input of broodstock in the period preceding gonadogenesis (Blom and Dabrowski, 1996; Bell, et al., 1997). Food or vitamin shortages may have caused suspension of vitellogenesis, resorption of oocytes, and decreased fecundity in the goldfish, *Carassius auratus* (L.) (Bekker, 1958).

The commercial production of ornamental tropical fish is gaining momentum in many regions of the world. Zebrafish (*Danio rerio,* Cyprinidae) serves as an important vertebrate model for studying development, genetics, production and diseases (Kimmel, 1989; Briggs, 2002; Ingham, 1997; Ward and Lieschke, 2002). Zebrafish is a group-spawning, egg-scattering member of the Cyprinid family found throughout southern Asia (Laale, 1977). Its ease of maintenance and ability to produce large numbers of transparent eggs has made it an ideal species for studies (Grunwald and Eisen, 2002).

The main purpose of the present experiments was to investigate the influence of different vitamin E levels for Zebrafish. This was considered an important task because nutritional studies on fecundity and fertility in fish have been mainly concerned with only quantity of food.

MATERIALS AND METHODS

Aquaria

Fifteen aquaria (40 × 30 × 50 cm) were used in this experiment. The water temperature was kept at 26±2°C. The aquarium water was permanently aerated. Before starting the experiments water was dechlorinated. Once a week pH was checked and total hardness in water (dH) was examined titrimetrically. Oxygen content was recorded weekly.

Experimental diets

The basal experimental diets were formulated with the commonly available ingredients (Table 1). Five graded levels of vitamin E (á-tocopherol) at 0, 100, 300, 500 and 1000 mg Kg^{-1} diets were included in the basal diet (Vitamin E was supplemented separately to the basal diet at the expense of wheat flour). The ingredients were grinded, milled, weighed, mixed and pelleted with a meat mincer through a 0.8 mm die. After cold pelleting, the feeds were air dried and put in an air-tight container. All diets were stored at -20°C until fed.

Experimental fish and feeding regime

Zebrafish (*D. rerio*) were obtained from an Institute of Ornamental Fish Hatchery (Golestan), and were transferred to the place of experiment and acclimated for 2 weeks. Zebrafish were fed a vitamin E-free diet (a basal diet which finally served as the control diet) for 2 weeks while acclimating to experimental conditions. Twenty uniform fish were randomly selected and stocked into each of the 15 aquariums, which in turn were randomly assigned in triplicate to each treatment. Fish were fed approximately 5% of their body weight daily, and it was divided into four equal feedings (08:00, 12:00, 16:00 and 20:00 h) for 5 months. Feed preparation was carried out bi-weekly to prevent long storage. The all of fish from each aquarium were counted and weighed at 2-week intervals to monitor growth and adjust feed rations. Mortalities and general health were recorded. Any dead fish were removed and not replaced during the experiment.

Calculations and statistical analysis

Sampling was carried out fortnightly. 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)

Body weight gain (BWG) = $(W_t - W_0) \times N_t$ (De Silva and Anderson, 1995)

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

Gonadosomatic Index = $GW/BW_t \times 100$

W_t and W_0 were final and initial fish weights (g), respectively; N_t and N_0 were final and initial numbers of fish in each replicate, respectively, t is the experimental period in days, GW was gonad weight and BW was final body weight (g).

Pregnant females were recognized by the convex abdomen and other signs. At the estimated time for parturition, 3 females from each experiment were killed by tricaine methanesulphonate. Fixation was achieved in 4% formalin. Total body length and total body weight (somatic weight plus gonad weight) (Lagler et al., 1962) were recorded. The number of fish oocyte (fecundity) of each female was counted and finally the egg diameters were measured (15 oocytes per fecundity).

The data obtained from the trial were subjected to one-way analysis of variance (ANOVA) and T-test (using SPSS 16.0 programme) to test for effects of dietary treatments. When ANOVA identified significant difference among groups, multiple comparison tests among means were performed using Duncan's new multiple range test. For each comparison, statistically significant differences were determined by setting the aggregate type I error at 5% ($P < 0.05$).

RESULTS

A significantly greater ($P < 0.05$) increase in specific growth rate (SGR), and body weight increase (BWI) was recorded in diets supplemented with vitamin E levels of ≥ 500 mg kg^{-1} diet when compared to the control diet, 100 and 300 mg kg^{-1} vitamin E diet (Figures 1 and 2). SGR and BWI were the highest for zebrafish fed the supplemented diet using 500 mg kg^{-1} vitamin E diet (2.83 and 0.684 respectively) followed by 1000 mg kg^{-1} vitamin E diet (2.77 and 0.667 respectively) and 300 mg kg^{-1} vitamin E diet (2.57 and 0.617 respectively).

For body weight gain (BWG), a significant difference was observed between zebrafish fed the supplemented diet using 500 and 1000 mg kg^{-1} vitamin E diet compared to the other groups ($P < 0.05$). The highest BWG was observed in fish fed the 1000 mg kg^{-1} vitamin E diet (Figure 3).

The survival rate of zebrafish fed with diets containing graded levels of vitamin E are shown in Figure 4. The

Table 1. Formulation and proximate composition of the basal diets (dry weight).

Ingredient	(%)
Fish meal	60
Barley meal	7.5
Wheat flour	7.5
Corn meal	7.5
Soybean meal	7.5
Mineral mixture [a]	5
Olive oil	2
Fish oil	3
Proximate composition	
Moisture	13.4
Ash	11.5
Crude protein	38.7
Crude lipid	13
Crude fiber	3.3
Carbohydrate	19

[a] Mineral mixture contain (mg/g mixture): Ca, 180000; P, 90000; Cu, 600; Zn, 300; Co, 300; I, 100; Co_3^{-2}, 100; Mg, 190000; Se, 1; Na, 60000; Mn; 200; Fe, 3000. Vitamin A, 500000 IU; vitamin D_3, 100000.

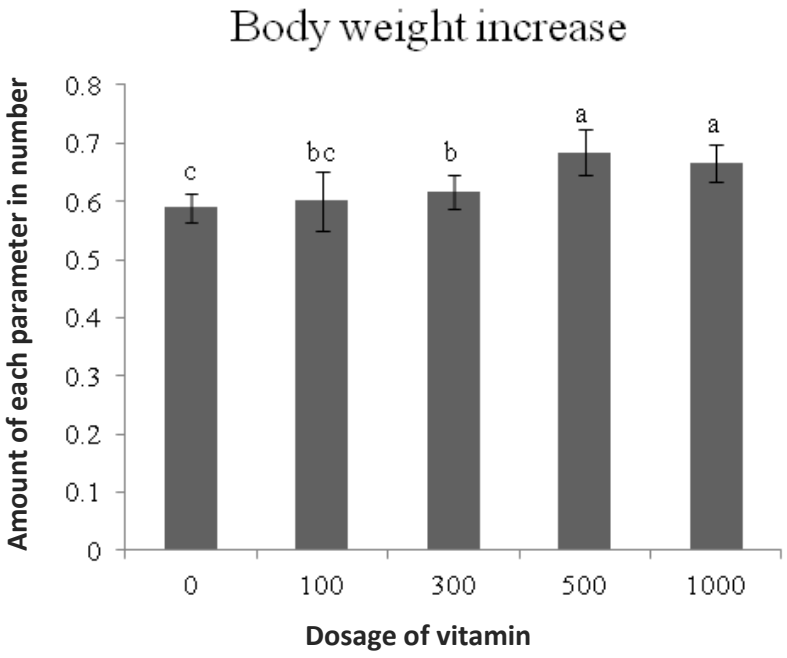

Figure 1. Effects of vitamin E on body weight increase of zebrafish.

survival rate of zebrafish fed the diets containing 500 and 1000 mg kg^{-1} vitamin E was higher than other groups and the highest survival rate was observed in 500 mg kg^{-1} vitamin E and lowest survival rate was observed in 0 mg kg^{-1} vitamin E (100 and 80% respectively).

Mean values of GSI increased with increasing the level of vitamin E, and the results were significant ($P < 0.05$). The results are shown in Figure 5. In egg diameter there were no significant differences between groups, (Figure 6). A significantly higher fecundity existed in the group of

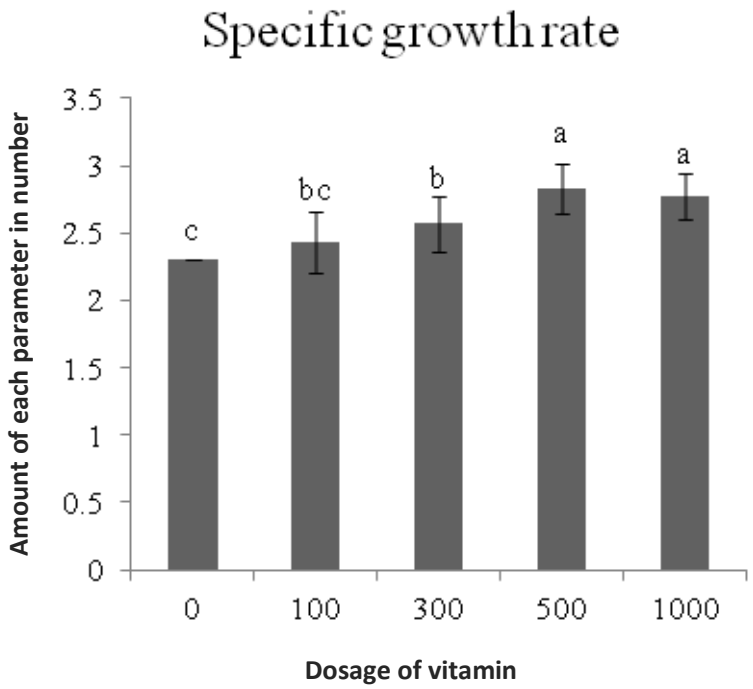

Figure 2. Effects of vitamin E on specific growth rate of zebrafish.

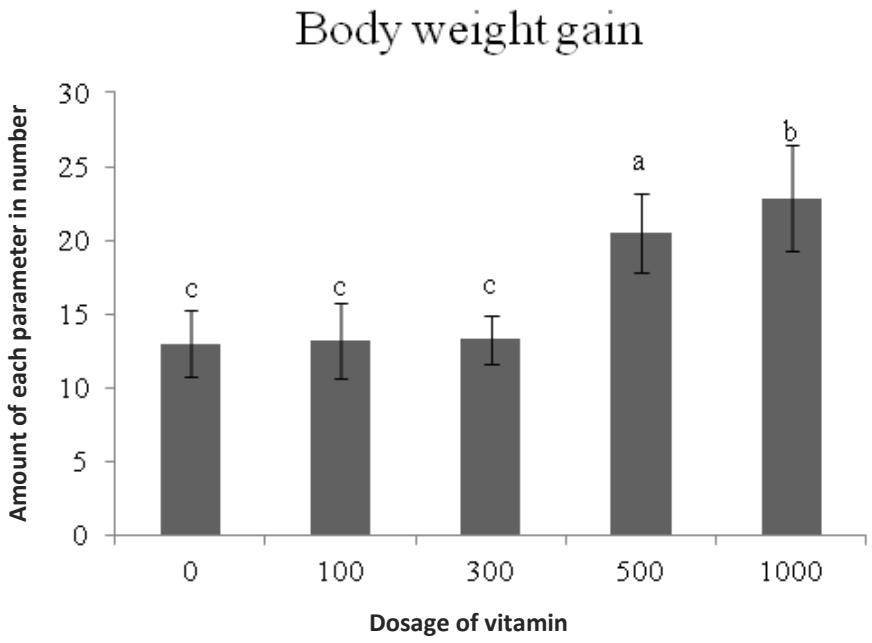

Figure 3. Effects of vitamin E on body weight gain of zebrafish.

fish fed 500 mg kg^{-1} vitamin E diet compared with groups fed 0, 100 and 300 mg kg^{-1} vitamin E diets. There was no significant difference between fish fecundity in fish fed 500 and 1000 mg kg^{-1} AA diet and between fish fed 0 and 100 mg kg^{-1} AA diet (Figure 7).

DISCUSSION

The requirement for vitamin E as an essential dietary component in fish has long been recognized, and minimum requirements for many fish species have

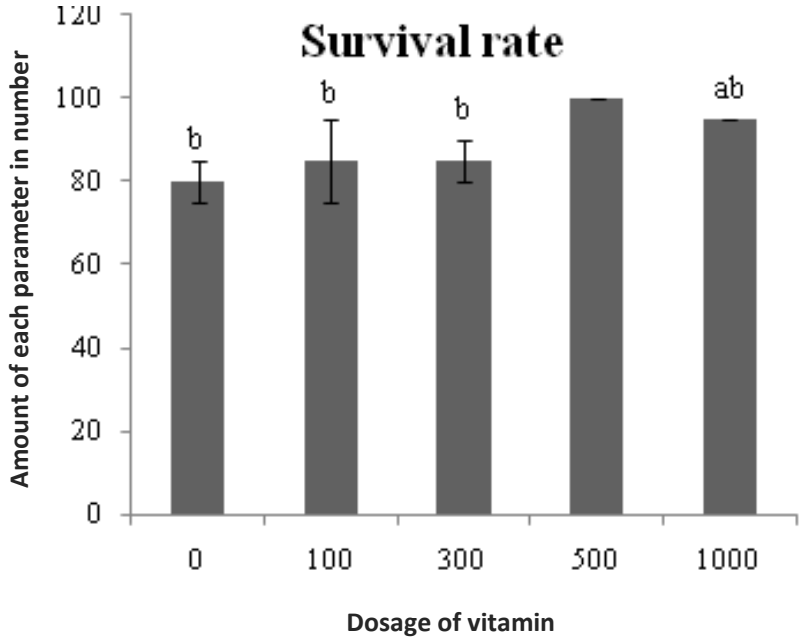

Figure 4. Effects of vitamin E on survival rate of zebrafish.

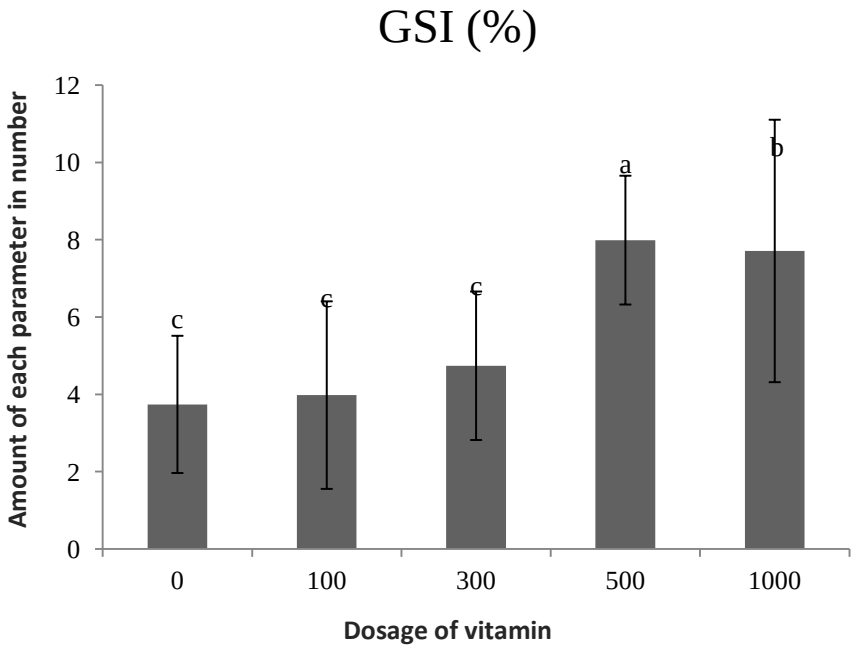

Figure 5. Effects of vitamin E on gonadosomatic index of zebrafish.

already been established. Naziroglu et al. (2003) mentioned that vitamin E especially á-tocopherol form, has very effective role on immune system response, and it is one the few nutrients for which supplementation with higher than recommended levels enhance certain aspects of immune function in fish.

The study showed that vitamin E significantly influences growth and reproductive performance in zebrafish. These results agree with the result of James et al. (2008) which demonstrated that growth and fecundity increased in female goldfish (*Carassius auratus*) with increased dietary vitamin E levels, but these results was not confirm

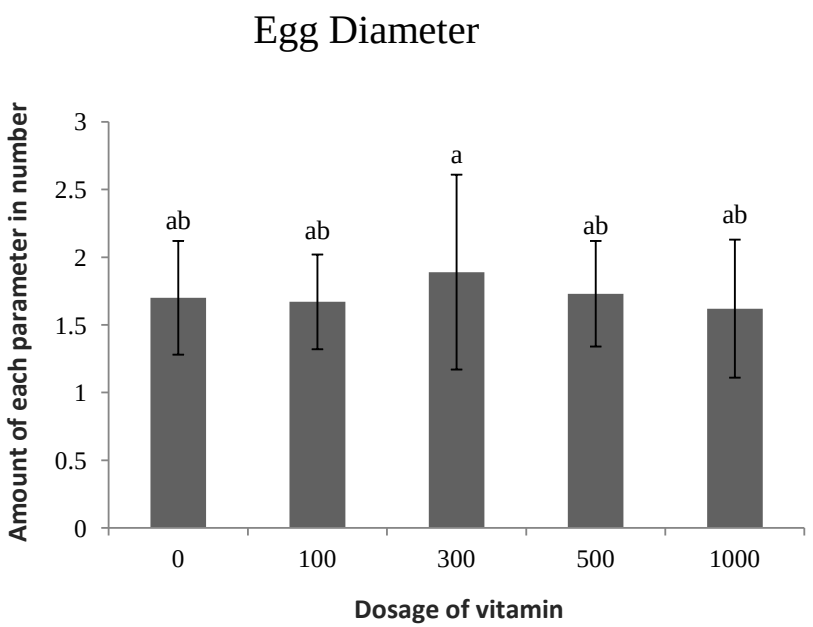

Figure 6. Effects of vitamin E on egg diameter of zebrafish.

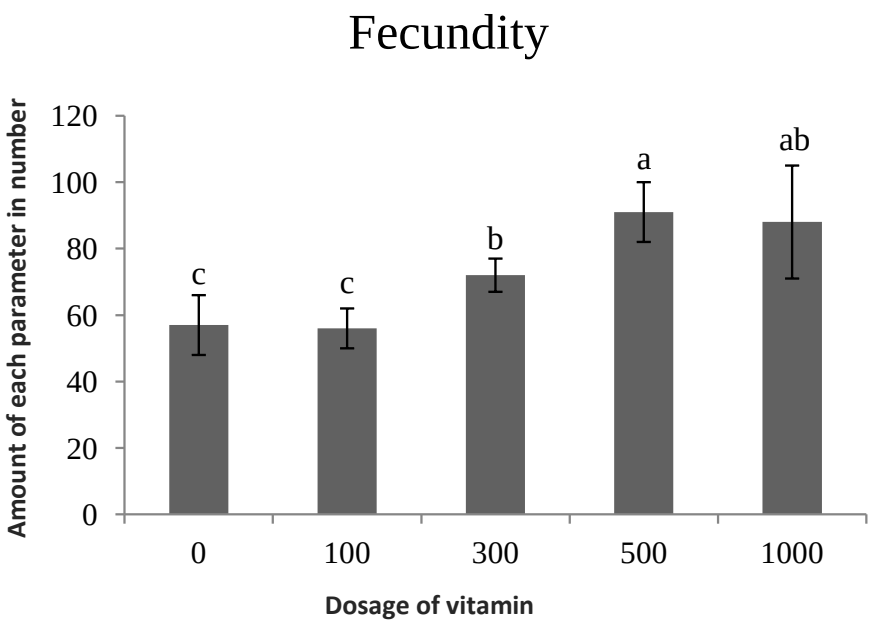

Figure 7. Effects of vitamin E on fecundity of zebrafish.

the findings of Boggio et al. (1985) and Kiron et al. (2004), that no difference occurred in the growth factors like weight gain of fish fed diets containing either lower (100 mg kg^{-1}) or higher level of vitamin E (1000 mg kg^{-1} diet).

In our study, the growth of zebrafish improved significantly with increasing supplementation of dietary vitamin E. These results further confirm that zebrafish need adequate exogenous vitamin E to maintain normal growth and physiological functions. Growth is a function of both the nutritional quality and the rate of consumption, among other things (Stickney, 2000). In this research trial, a diet containing 500 mg of vitamin E kg^{-1} diet was found to be the more optimal dietary requirement for zebrafish than other groups.

A major function of vitamin E is to prevent peroxidation of polyunsaturated fatty acids of phospholipid and cholesterol in cellular and subcellular membranes. In

general, because of peroxidative damage to cellular membranes nutritional muscular dystrophy, fatty liver degeneration, anemia, exudative diathesis, erythrocyte hemolysis, hemorrhages, depigmentation and reduction of fertility are observed in fish in the deficiency of vitamin E (He and Lawrence, 1993).

The diet without vitamin E supplementation decreased the specific growth rate of zebrafish in compared to other groups that contain vitamin E, and this is in accordance with studies conducted by Mehrad and Sudagar who also observed declining specific growth rate with vitamin E deficient diet for guppy (*Poecillia reticulate*). A significant difference in survival rate was observed between the treatments containing vitamin E. This result did not conform to a study conducted by Mourente et al. (2002) in gilthead sea bream, they found that addition of vitamin E in diets containing oxidized oil did not improve the survival of sea bream, and also not conform to a study conducted by Mehrad and Sudagar (2010) on *P. reticulate*.

In this study a significantly higher fecundity existed in group of fish fed 500 mg kg^{-1} vitamin E diet compared with other groups. The importance of vitamin E in fish reproduction has been reported. For example, vitamin E caused higher gonadosomatic index, larger ova, and more eggs than a control in a study on the effect of vitamin E and growth hormone on the gonadal maturity of freshwater fish (*Cyprinus carpio*) (Gupta et al., 1987). In addition, complete spawning occurred in fish fed a diet containing vitamin E, but only partial spawning occurred in the fish fed diets without vitamin E (Gupta et al., 1987). In a different study, Sutjaritvongsanon (1987) found better gonad development and spawning for goldfish (*C. auratus*) fed with added vitamin E.

Conclusions

The significance of the results herein obtained underlined the importance of diet in the reproductive process, supporting the hypothesis that feed additives can improve fecundity. Considering that the zebrafish has been clearly established as a vertebrate model for biomedical research, these results support the potentiality of feed additives such as vitamins, frequently used in the human diet, as a new technology to improve reproduction in all vertebrates, including humans.

REFRENCES

Ai Q, Mai K, Tan B, Xu W, Duan Q, Ma H, Zhang L (2006). Replacement of fish meal by meat and bone meal in diets for large Yellow croaker (*Pseudosciaena crocea*). Aquaculture, 260: 255-263.

Bekker VE (1958). On the influence of environmental conditions on gonad development and spawning intensity in the goldfish (*Carassius auratus* L.). DokI. Akad. Nauk SSSR Biol. Sci. Sect. (Engl. Trans. Ser.), 117: 960-963.

Bell JG, Famdale BM, Bruce MP, Navas JM, Carrillo M (1997). Effects of broodstock dietary lipid on fatty acid composition of eggs from sea bass (*Dicentrarchus labrax*). Aquaculture, 149: 107-119.

Blom JH, Dabrowski K (1996). Ascorbic acid metabolism in fish: Is there a maternal effect on the progeny? Aquaculture, 147: 215-224.

Boggio RW, Hardy J, Babbitt K, Brannon EL (1985). The influence of dietary lipid source and alpha-tocopheryl acetate level on product quality of rainbow trout (*Salmo gairdneri*). Aquaculture, 51: 13-24.

Briggs JP (2002). The zebrafish: a new model organism for integrative physiology. Am. J. Physiol. Regul. Integr. Comp. Physiol., 282: R3-R9.

De Silva SS, Anderson TA (1995). Fish nutrition in Aquaculture, Chapman & Hall. Press London., p. 319

Grunwald DJ, Eisen JS (2002). Headwaters of the zebrafish emergence of a new model vertebrate. Nat. Rev. Genet., 3: 717-724.

Gupta SD, Khan HA, Bhowmick RM (1987). Observations on the effect of vitamin E and growth hormone on the gonadal maturity of carps. J. Inland Fish. Soc. India, 19(2): 26-31.

He H, Lawrence AL (1993). Vitamin E requirement of *Penaeus vannemei*. Aquaculture, 118: 245-255.

Hevroy EM, Espe M, Waagbo R, Sandness K, Rund M, Hemre G (2005). Nutrition utilization in Atlantic salmon (*Salmo salar*) fed increased level of fish protein hydrolyses during a period of fast growth. Aquacul. Nutr., 11: 301-313.

Ingham PW (1997). Zebrafish genetics and its implications for understanding vertebrate development. Hum Mol Genet 6:1755–1760.

Izquierdo MS, Fernández-Palacios H, Tacon AGJ (2001). Efffect of broodstock nutrition on reproductive performance of fish. Aquaculture, 197: 25-42.

James R, Vasudhevan I, Sampath K (2008). Effect of Dietary Vitamin E on Growth, Fecundity, and Leukocyte Count in Goldfish (*Carassius auratus*). Israeli J. Aquac. – Bamidgeh, 60(2): 121-127.

Kimmel CB (1989). Genetics and early development of zebrafish. Trends Genet., 5: 283-288.

Kiron V, Puangkaew J, Ishizaka K, Satoh S, Watanabe T (2004). Antioxidant status and nonspecific immune responses in rainbow trout (*Oncorhynchus mykiss*) fed two levels of vitamin E along with, three lipid sources. Aquaculture, 234: 361-379.

Laale HW (1977). The biology and use of zebrafish, *Brachydanio rerio*, in fisheries research. A literature review. J. Fish Biol., 10: 121-173.

Lagler KF, Bardach JE, Miller RR (1962). Ichthyology: The Study of Fishes. John Wiley and Sons, Inc., New York, London; Toppan Co. Ltd., Tokyo, p. 545.

Mehrad B, Sudagar M (2010). Dietary vitamin E requirement, fish performance and reproduction of guppy (*Poecilia reticulata*). AACL Bioflux, 3(3): 239-246.

Mourente G, Dı´az-Salvago E, Bell JG, Tocher DR (2002). Increased activities of hepatic antioxidant defence enzymes in juvenile gilthead sea bream (*Sparus aurata L.*) fed dietary oxidized oil: attenuation by dietary vitamin E. Aquaculture, 214: 343-361.

Naziroglu M, Ispir U, Yonar ME (2003). Effects of vitamin E on immune response in fish. Kafkas Univ Vet Fak Derg, 9(1): 101-106.

NRC (National Research Council) (1993). Nutrient requirements of Fish. National Academy Press, Washington, DC, p. 114.

Stickney RR (2000). Encyclopedia of aquaculture. John Wiley and Sons, Inc: New York, 960 p.

Sutjaritvongsanon S (1987). Level of vitamin E content suitable for gonad developing and spawning of goldfish, *Carassius auratus* (Linn.). Abstracts of Master of Science Theses Fisheries Science, Notes Fac. Fish. Kasetsart Univ., Bangkok, 12: 2.

Tacon AGJ (1990). Standard method for nutritional and feeding of farmed fish and shrimp. Argent librations press. Redmond, Wash, 1: 117

Vismara R, Vestri S, Kusmic C, Brarsanti L, Gualtieri P (2003). Natural vitamin enrichment of *Artemia salina* fed freshwater and marine microalgae. J. Appl. Phycol., 15: 75-80.

Ward AC, Lieschke GJ (2002). The zebrafish as a model system for human disease. Front Biosci., 7: 827-833.