AACL BIOFLUX

Aquaculture, Aquarium, Conservation & Legislation
International Journal of the Bioflux Society

Dietary vitamin E requirement, fish performance and reproduction of guppy (*Poecilia reticulata*)

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Abstract. The aim of this study was evaluate the effects of dietary vitamin E on growth factors, survival, reproductive performance and sex ratio in guppy. Guppies were divided into 5 treatments with triplicate groups and fed with one of 5 diets for 20 weeks. The experimental vitamin E diets were formulated to contain 100, 300, 500 and 1000 mg kg^{-1} vitamin (treatment 1, 2, 3 and 4 respectively) with 1 control group (Treatment 5). The data obtained from the trial were subjected to one-way analysis of variance (ANOVA) to test for effects of dietary treatments. 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 ($P<0.05$) and highest BWI, PBWI, SGR and DGR were observed in treatment 4. There were no significant differences observed in survival rate and sex ratio between the treatments. This study indicates 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.

Key Words: Guppy, vitamin E, growth, reproductive performance, survival, sex ratio.

چکیده. هدف از انجام این آزمایش تعیین نیاز ویتامین E و اثرات آن بر پارامترهای رشد (مانند درصد افزایش وزن بدن، نرخ رشد ویژه . . .)، بقاء و زادآوری گوپی بود. مقادیر مختلف ویتامین E (آلفاتوکوفرول) شامل دوزهای 100، 300، 500 و 1000 (به‌ترتیب تیمارهای 1، 2، 3 و 4) (میلی‌گرم در کیلوگرم جیره) در سه تکرار به ماهیان داده شد، همچنین یک تیمار بدون ویتامین نیز به‌عنوان تیمار شاهد برای آزمایش با سه تکرار در نظر گرفته شد. زیست‌سنجي ماهیان هر 4 هفته یك‌بار انجام مي‌گرفت. پس از اتمام دوره پرورش، تعداد لاروهای متولدشده و نسبت‌جنسی هر تیمار محاسبه شد. آنالیز آماری به کمك آنالیز واریانس یك‌طرفه و برای مقایسه میانگین‌ها از آزمون دانکن استفاده شد. نرخ رشد ویژه، افزایش وزن بدن، درصد افزایش وزن بدن، درصد رشد روزانه و تعداد لاروهای متولدشده با افزایش سطح ویتامین افزایش یافتند که این افزایش‌ها بین تیمار 4 با سایر تیمارها معنی‌دار بود (P<0/05). در میزان درصد بقاء و نسبت جنسی نیز اختلاف معنی‌داری بین تیمارها مشاهده نشد. ویتامین E سبب بهبود فاکتورهای تغذیه‌ای شده اما، تاثیر معنی‌داری بر بقای مولدین نداشت. با توجه به نتایج به‌دست آمده از این بررسی مقدار پیشنهادی ویتامین E موردنیاز برای تولیدمثل و رشد بهتر ماهی گوپی برابر 1000 میلی‌گرم بر کیلوگرم می‌باشد.

کلمات کلیدی: گوپی، ویتامین C، رشد، تولیدمثل، بقاء، نسبت جنسی

Introduction. 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, which includes 120 mg kg^{-1} diet (Hamre & Lie 1995) for Atlantic salmon, 30 to 50 mg kg^{-1} diet for channel catfish (Murai & Andrews 1974; Wilson et al 1984) and 200 to 300 mg kg^{-1} diet for common carp (Watanabe et al 1977).

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 commercial production of ornamental tropical fish is gaining momentum in many regions of the world. The live bearer guppy fish (*Poecilia reticulata*) are the most popular among hobbyists because of their vibrant colours and the fact that they are easy to breed and keep (Dahlgren 1980).

Many authors have studied the impact of vitamin E on growth and immune response in various organisms but studies related to Dietary vitamin E requirement, fish performance and reproduction in ornamental fishes are scanty. So, this study was conducted to study the effect of different levels of dietary vitamin E on growth factors, survival, reproduction and sex ratio in the guppy, *P. reticulata.*

Material and Method

Experimental diets. The basal experimental diets were formulated with the commonly available ingredients (Table 1). The formula and analyzed proximate composition of the basal diet is shown in Table 1. Five graded levels of vitamin E (α-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 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. Guppy fish (*P. reticulata*) (initial weight, 0.01 g) were obtained from an Institute of Ornamental Fish Hatchery in gorgan, and were transferred to the place of experiment and acclimated for 2 weeks. Guppies were fed an Vitamin E-free diet (Means the basal diet which finally served as the control diet) for 2 weeks while acclimating to experimental conditions. Thirty uniform fish were randomly selected and stocked into each of 15 aquarium (30×40×50 cm), which in turn were randomly assigned to each treatment. Controlled temperature was (28±2 °C) and three replicate aquarium were assigned to each dietary treatment.
Aquaria were using dechlorinated municipal water with a hardness of 10–30 mg L^{-1} as calcium carbonate. Water quality was maintained by continuous aeration, and water temperature was 27±2 ^{o}C (mean±S.D.). A diurnal light:dark cycle of 12:12 h was maintained during the feeding trial. Fish were fed approximately 5% of their body weight daily, and it is divided into four equal feedings (08:00, 11:00, 14:00 and 17:00 h) for 5 months. Feed preparation was carried out bi-weekly to prevent long storage. 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.

Measurements and sample analysis. Sampling was carried out fortnightly. The water quality parameters were monitored during the trial by the staffs of limnology division in gorgan university of agricultural science and natural resources, and average value for temperature, dissolved oxygen, hydrogen ion concentration (pH) and conductivity were 28±2 °C, 6.15±0.45 mg l^{-1}, 8.3±0.3 units and 655±100 µmhos cm^{-3} respectively. Proximate composition of diets was carried out using the Association of Analytical Chemists (AOAC 2000) methods. Sample of diet were dried to a constant weight at 105 °C to determine moisture. Protein was determined by measuring nitrogen (N×6.25) using the Kjeldahl method; Crude fat was determined using petroleum ether (40–60 Bp) extraction method with Soxhlet apparatus and ash by combustion at 550 °C.

Calculations and statistical analysis. The following variables were calculated:
Body weight increase (BWI) = $W_t - W_0$ (Tacon 1990)
Percent body weight increase (PBWI) = $[(W_t - W_0)/ W_t]\times 100$ (Bekcan et al 2006)
Specific growth rate (SGR) = $(\ln W_t - \ln W_0)\times 100\ t^{-1}$ (Hevroy et al 2005)
Condition factor (CF) = $(W / L^3) \times 100$ (Ai et al 2006)
Body weight gain (BWG) = $(W_t - W_0) \times N_t$ (De Silva & Anderson 1995)
Daily growth rate (DGR) = $[(W_t - W_0)/ t]\times 100$ (De Silva & Anderson 1995)
Survival = $N_t \times 100\ N_0^{-1}$ (Ai et al 2006)

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; L (cm) was final length; and t is the experimental period in days. The number of newborn fish in each aquarium in each day was counted, and they transferred into related larval aquaria; and finally the sex ratio of newborn fish after they displayed the morphological characteristics of male or female were calculated.

The data obtained from the trial were subjected to one-way analysis of variance (ANOVA) (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% ($P<0.05$).

Table 1

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

Ingredients	*(%)*
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

[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.

Results. The factors related to the growth in guppy fed with diet containing different levels of vitamin E are presented in Table 2.

Specific growth rate (SGR) was increase significantly ($P<0.05$) with the increase the level of vitamin E, and highest SGR was observed in treatment 4 (29.28) and lowest SGR was observed in treatment 1 (25.54). Body weight increase (BWI), percent body weight increase (PBWI) and daily growth rate (DGR) were also increased with the level of vitamin E, and they were significant between treatment 4 with other treatments ($P<0.05$). In body weight gain (BWG) significant differences were observed between guppies fed the supplemented diet using 1000 mg kg^{-1} vitamin E (treatment 4) with other groups, and between guppies fed the supplemented diet using 5000 (treatment 3) with treatment 1 and control group ($P<0.05$). The highest BWG was observed in treatment 4 (21.83) and lowest BWG was observed in treatment 1 and control group (13.53 and 13.84 respectively). Condition factor (CF) was also significant between treatment 1 and control group with treatment 4 ($P<0.05$), significant differences was not observed between other treatments.

Table 2

Effects of vitamin E on growth parameters

Parameters	*Diet 1 (100 mg kg^{-1} Vit E)*	*Diet 2 (300 mg kg^{-1} Vit E)*	*Diet 3 (500 mg kg^{-1} Vit E)*	*Diet 4 (1000 mg kg^{-1} Vit E)*	*Diet 5 (Control) (0 mg kg^{-1} Vit E)*
Initial fish length	8.6±0.4	7.65±0.35	8.25±0.05	8.05±0.25	8.25±0.25
Final fish length	34.365±0.465	37.5±0.5	40.675±0.925	43.9±0.7	34.8±0.2
Initial fish weight	0.01	0.01	0.01	0.01	0.01
Final fish weight	0.461±0.002	0.5895±0.0025	0.6675±0.0075	0.8075±0.0025	0.487±0.009
Specific growth rate (SGR)	2.554±0.003[e]	2.718±0.003[c]	2.801±0.0075[b]	2.928±0.002[a]	2.590±0.012[d]
Body weight increase (BWI)	0.451±0.002[d]	0.5795±0.0025[bc]	0.6125±0.0525[b]	0.7525±0.0425[a]	0.477±0.009[cd]
Percent body weight increase (PBWI)	4510±20[d]	5795±25[bc]	6125±525[b]	7525±425[a]	4770±90[cd]
Body weight gain (BWG)	13.53±0.06[c]	16.51±0.79[bc]	17.77±1.53[b]	21.83±1.23[a]	13.84±0.74[c]
Daily growth rate (DGR)	0.301±0.001[d]	0.386±0.002[bc]	0.408±0.035[b]	0.502±0.028[a]	0.318±0.006[cd]
Condition factor (CF)	$0.00114 \pm 5.11 \times 10^{-5}$[a]	$0.00112 \pm \times 10^{-5}$[ab]	$0.00099 \pm 5.66 \times 10^{-5}$[ab]	$0.00095 \pm 4.27 \times 10^{-5}$[b]	$0.001156 \pm 4.13 \times 10^{-5}$[a]

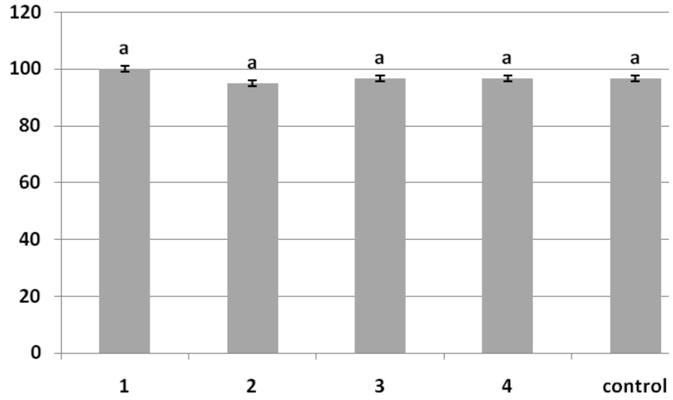

Figure 1. Effects of vitamin E on survival rate.

The survival rate of guppies fed with diets containing graded levels of vitamin E are shown in Figure 1. No significant differences in survival rate observed between the treatments and the highest survival rate was observed in 100 mg kg^{-1} vitamin E and lowest survival rate was observed in 300 mg kg^{-1} vitamin E (100% and 95% respectively).

The reproductive performance of guppies (the number of newborn fish) fed with different levels of dietary vitamin E is presented in Figure 2. Reproductive performance of guppies were increased significantly with increasing the levels of vitamin E ($P<0.05$), but these increase was not significant between guppies using 100 and 300 mg kg^{-1} vitamin E diets and the diet devoid of vitamin E, and between guppies using 300 with 500 mg kg^{-1} vitamin E diet, and the highest number of newborn fish was observed in guppies fed the 1000 mg kg^{-1} vitamin E diet (178).

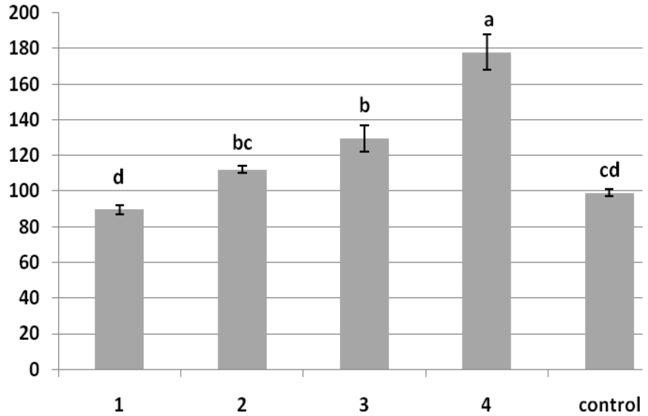

Figure 2. The reproductive performance of guppies (the number of newborn fish).

There were no significant differences in sex ratio observed between the treatments (Table 3).

Table 3

Sex ratio in newborn fish

	Diet 1 (100 mg kg^{-1} Vit E)	Diet 2 (300 mg kg^{-1} Vit E)	Diet 3 (500 mg kg^{-1} Vit E)	Diet 4 (1000 mg kg^{-1} Vit E)	Diet 5 (Control) (0 mg kg^{-1} Vit E)
Sex ratio (female/male)	3.43 ± 0.445^{a}	3.05 ± 0.079^{a}	3.14 ± 0.44^{a}	3.44 ± 0.61^{a}	2.49 ± 0.77^{a}

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 already been established. Naziroglu et al (2003) mentioned that vitamin E especially α-tocopherol form, have 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 *P. reticulata*. 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 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).

No significant difference in survival rate was observed between the treatments. This result is conform with 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.

Also no clear differences in fry sex ratio were detected among the treatments, which is analogy with the result obtained by Garcia & Garcia (2004) and Kavumpurath & Pandian (1993) for *P. reticulata.*

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 & Lawrence 1993).

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.

In these trials the number of newborn fish was increased with increasing the level of vitamin E. These results agree with the result of Harlioglu & Barim (2004) which demonstrated that the number of pleopodal eggs increased in freshwater crayfish (*Astacus leptodactylus*) with increased dietary vitamin E levels.

Conclusion. Our research results showed supplemented dietary vitamin E up to 1000 mg kg^{-1} can be useful for improving the growth factors and reproductive performance of *P. reticulata.*

References

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.

Association of Official Analytical Chemists (AOAC), 2000 17th Edition, A.O.A.C., Washington DC. **21**:447.

Bekcan S., Dogankaya L., Cakirogullari G. C., 2006 Growth and body composition of European catfish (*Silurus glanis L.*) fed diets containing different percentages of protein. The Israeli Journal of Aquaculture Bamidgeh **58**:137-142.

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

Dahlgren B. T., 1980 The effects of three different dietary protein levels on fecundity in the guppy, *Poecilia reticulata* (Peters). Journal of Fish Biology **16**:83-97.
De Silva S. S., Anderson T. A., 1995 Fish nutrition in Aquaculture, Chapman & Hall. Press London, 319p.
Garcia U., Garcia O., 2004 Reproductive performance of guppy, *Poecilia reticulata,*fed with live artemia franciscana cultured with inert and live diets. Avances en investigacion agropeciaria **8**:1-7.
Gupta S. D., Khan H. A., Bhowmick R. M., 1987 Observations on the effect of vitamin E and growth hormone on the gonadal maturity of carps. Journal of the Inland Fisheries Society of India **19**(2):26–31.
Hamre K., Lie Ø., 1995 Minimum requirement of vitamin E for Atlantic salmon, *Salmo salar L.*, at first feeding. Aquacult Res **26**:175–184.
Harlioglu M., Barim O., 2004 The effect of dietary vitamin E on the pleopodal egg and stage-1 juvenile numbers of freshwater crayfish *(Astacus leptodactylus).* Aquaculture **236**:267–276.
He H., Lawrence A. L., 1993 Vitamin E requirement of *Penaeus vannemei*. Aquaculture **118**:245–255.
Hevroy E. M., 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.
Izquierdo M. S., Fernández-Palacios H., Tacon A. G. J., 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*). The Israeli Journal of Aquaculture - Bamidgeh **60**(2):121-127.
Kavumpurath S., Pandian T. J., 1993 Masculinization of *Poecilia reticulata* by dietary administration of synthetic or natural androgen to gravid females. Aquaculture, **116**(1):83-89.
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.
Mourente G., Dı´az-Salvago E., Bell J. G., Tocher D. R., 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.
Murai T., Andrews J. W., 1974 Interactions of dietary a-tocopherol, oxidized menhaden oil and ethoxyquin on channel catfish (*Ictalurus punctatus*). J Nutr **104**:1416–1431.
Naziroglu M., Ispir U., Yonar M. E., 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. 114.
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, no. **12**, p. 2.
Tacon A., Albert G.J., 1990 Standard method for nutritional and feeding of farmed fish and shrimp. Argent librations press. Vol 1,117pp.
Vismara R., Vestri S., Kusmic C., Brarsanti L., Gualtieri P., 2003 Natural vitamin E enrichment of *Artemia salina* fed freshwater and marine microalgae. J Appl Phycol **15**:75–80.
Watanabe T., Takashima F., 1977 Effect of α-tocopherol defficiency on carp. IV. Deficiency symptoms and changes in fatty acid and triglyceride distribution in adult carp. Bulletin of the Japanese Society of Scientific Fisheries **43**:819-830.
Wilson R. P., Bowser R. P., Poe W. E., 1984 Dietary vitamin E requirement of fingerling channel catfish. J Nutr **114**:2053–2058.

Received: 12 August 2010. Accepted: 07 September 2010. Published online: 07 September 2010.
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How to cite this article:
Mehrad B., Sudagar M., 2010 Dietary vitamin E requirement, fish performance and reproduction of guppy (*Poecilia reticulata*). AACL Bioflux **3**(3):239-246.