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# The effect of vitamin C on growth factors, survival, reproduction and sex ratio in guppy *(Poecilia reticulata)*


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**Abstract**. This study was conducted to examine the effects of dietary vitamin C (Ascorbic Acid, AA) on growth factors, survival, reproductive performance and sex ratio in guppy (*Poecilia reticulata* Peters,1859). Guppies were divided into 5 treatments with triplicate groups and fed with one of 5 diets for 20 weeks. The experimental vitamin C diets were formulated to contain 400, 800, 1200 and 2000 mg AA $kg^{-1}$ (treatment 1, 2, 3 and 4 respectively) with 1 control group. The data obtained from the trial were subjected to one-way analysis of variance (ANOVA) to test for effects of dietary treatments. In vitamin C 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 levels of vitamin C ($P<0.05$) and highest BWI, PBWI, SGR and DGR were observed in treatment 4. There were no significant differences in sex ratio observed between the treatments. In survival rate there was significant difference between treatment 2 with treatments 1, 3 and control ($P<0.05$). This study indicates that BWI, PBWI, SGR and DGR and reproductive performance can be improved by dietary vitamin C supplementation and also may be concluded that the vitamin C requirement of guppies fish for optimum growth and reproductive performance is 2000 mg/kg of dry diet.


**Key Words**: guppy, vitamin C, growth, reproductive performance, survival, sex ratio**.**



**چکیده.** این آزمایش به منظور تعیین اثرات ویتامین C جیره بر پارامترهای رشد (درصد افزایش وزن بدن، نرخ رشد ویژه . . . )، بقاء و زادآوری گوپی انجام گرفت. مقادیر مختلف ویتامین C (ال-آسکوربیک اسید) شامل دوزهای 400، 800، 1200 و 2000 (به‌ترتیب تیمارهای 1، 2، 3 و 4) (میلی‌گرم در کیلوگرم جیره) در سه تکرار به ماهیان داده شد، همچنین یک تیمار بدون ویتامین به‌عنوان تیمار شاهد برای آزمایش با سه تکرار در نظر گرفته شد. زیستسنجي ماهیان هر 4 هفته یکبار انجام مي-گرفت. پس از اتمام دوره پرورش، تعداد لاروهای متولدشده و نسبتجنسی هر تیمار محاسبه شد. آنالیز آماری به کمك آنالیز واریانس یکطرفه و برای مقایسه میانگین‌ها از آزمون دانکن استفاده شد. در تیمارهای ویتامین C، با افزایش سطح ویتامین، نرخ رشد ویژه، میزان افزایش وزن بدن، درصد افزایش وزن، درصد رشد روزانه و تعداد لاروهای متولدشده به‌صورت معنی‌داری افزایش یافتند ($P<0/05$) و بالاترین مقدار نرخ رشد ویژه، میزان افزایش وزن بدن، درصد افزایش وزن، درصد رشد روزانه و تعداد لاروهای متولدشده در تیمار 4 مشاهده شد. در میزان تلفات نیز اختلاف معنی‌داری بین تیمار 2 با تیمارهای 1، 3 و شاهد مشاهده شد ($P<0/05$)، سایر تیمارها فاقد اختلاف معنی‌دار با یکدیگر بودند. نسبت جنسی نیز اختلاف معنی‌داری را بین تیمارها نشان نداد. با توجه به نتایج مقدار پیشنهادی C برای تکثیر و پرورش ماهی گوپی برابر 2000 میلی‌گرم بر کیلوگرم می‌باشد.


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



**Rezumat**. Studiul de față a fost demarat pentru a se observa efectele administrării vitaminei C (acid ascorbic, AA) asupra factorilor de creștere, supraviețuirii, performanțelor de creștere și raportului între sexe la guppy (*Poecilia reticulata* Peters, 1859). Peștii au fost împărțiți în 5 grupuri experimentale (trei repetiții) și au fost hrăniți astfel timp de 20 săptămâni. Vitamina C a fost repartizată de așa natură încât dietele să conțină 400, 800, 1200 și 2000 mg AA $kg^{-1}$ (loturile 1, 2, 3 și respectiv 4), având și un lot martor. Datele obținute astfel au fost incluse în programul statistic ANOVA (cu o singură variabilă) pentru testarea efectelor tratamentelor efectuate. În cazul tratamentelor cu vitamina C, masa corporală (BWI), procentajul masei corporale (PBWI), rata de creștere specifică (SGR), rata de creștere zilnică (DGR) și performanțele reproductive au crescut semnificativ cu creșterea dozelor de vitamină C ($P<0.05$), iar cele mai ridicate BWI, PBWI, SGR și DGR au fost observate în lotul 4 experimental. Nu au existat diferențe semnificative între loturile experimentale în ceea ce privește raporturile dintre sexe. Privitor la rata de supraviețuire, aceasta a fost semnificativ diferită între cazurile lotului 2 comparativ cu loturile 1, 3 și martor ($P<0.05$). Studiul nostru arată că BWI, PBWI, SGR, DGR și performanțele reproductive pot fi îmbunătățite prin administrarea suplimentară de vitamină C, iar o altă concluzie ar putea fi aceea că necesarul de vitamină C la peștii guppy pentru creșterea și reproducerea în condiții optime este de 2000 mg/kg furaj uscat.


**Key Words**: guppy, vitamină C, creștere, performanțe reproductive, supraviețuire, sex-ratio.

**Introduction**. Vitamin C is an essential vitamin for normal physiological functions in animals including fish (Wilson & Poe 1973; Lim & Lovell 1978). Most teleosts are unable to synthesize ascorbic acid due to the lack of L-gulonolactone oxidase that is responsible for synthesis of vitamin C de novo (Wilson 1973; Fracalossi et al 2001). Therefore, an exogenous source of vitamin C is required in fish diets. Inadequate supply of dietary vitamin C usually results in a number of deficiency signs such as spinal deformation, impaired collagen formation, internal haemorrhaging and retarded growth (Halver et al 1969; Al-Amoudi et al 1992; Gouillou-Coustans et al 1998). The requirement of vitamin C varies, to some degree, with fish species, size, diet and experimental conditions.

In female brood stock, the fast growth of oocytes, embryonic development, and early performance of fry are critically affected by the supply of nutrients during vitellogenesis. Therefore, sequestration and packaging of substances from the maternal bloodstream into the yolk will probably increase the maternal demand for ascorbic acid and require enhanced ascorbic acid levels in brood stock diets (Blom & Dabrowski 1995).

L-Ascorbic acid is the source of vitamin C traditionally used to feed fish and shrimp, but it is a water soluble, thermolabile vitamin and is easily oxidized to an inactive form, diketogulonic acid, during processing and storage (Lovell & Lim 1978; Hilton et al 1979). One method of physically protecting ascorbic acid from oxidation is encapsulation. Ethylcellulose, a water-soluble "coating" typically used as a compressible tableting compound, has been somewhat effective in providing increased stability of pure crystalline ascorbic acid during storage. However, when blended with other ingredients and subjected to all of the processes involved in feed manufacturing, ascorbic acid with this type of coating is only slightly protected.

The commercial production of ornamental tropical fish is gaining momentum in many regions of the world. The live bearer guppy fish (*Poecilia reticulata* Peters, 1859) are the most popular among hobbyists because of their vibrant colours and the fact that they are easy to breed and keep. The guppy is considered omnivorous and requires around 40% dietary protein (Dahlgren 1980). Guppy fish have served as a subject for numerous behavioural studies related to predator avoidance mechanisms and evolution-related studies (Reznick 1982; Reznick et al 1990; Godin & Briggs 1996; Reznick et al 2001); genetic models (Reznick 1982; Breden et al 1987; Shikano et al 2000); and different factors affecting their reproductive behaviour and reproductive performance (Liley 1968; Endler 1980; Dzikowski et al 2001).

In contrast to the vast number of studies on these subjects, research aimed at better understanding the nutritional needs of these fish is scarce. The aim of the present study, therefore, was to establish the ascorbic acid requirements in guppy necessary to maintain optimal growth, survival and reproduction, with L-Ascorbic acid as the dietary source of this vitamin.

**Material and Method**. Experimental diets: The basal experimental diets were formulated with the commonly available ingredients (see Table 1). The formula and analyzed proximate composition of the basal diet is shown in Table 1. Five graded levels of vitamin C (L-ascorbic acid, AA) at 0, 400, 800, 1200 and 2000 mg $Kg^{-1}$ diets were included in the basal diet (AA 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 (*Poecilia 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 C-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 10–30mg $L^{-1}$ as calcium carbonate. Water quality was maintained by continuous aeration, and water

temperature was 27±2 °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, 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 liminology 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) = $[(W_t - W_0)/ W_t]\times100$ (Bekcan et al 2006)

Specific growth rate (SGR) = $(\ln W_t - \ln W_0)\times100\ t^{-1}$ (Hevroy et al 2005)

Condition factor (CF) = $(W / L^3) \times100$ (Ai et al 2006)

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

Daily growth rate (DGR) = $[(W_t - W_0)/ t]\times100$ (De Silva & Anderson 1995)

Survival = $N_t\times100\ N_0^{-1}$ (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 transfered into related larval aquaria; and finally the sex ratio of newborn fish after they displayed the morphological charactristics 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 contains (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; Vitamin E, 100 mg.

**Results**. The factors related to the growth in guppy fed with diet containing different levels of vitamin C (Ascorbic Acid, AA) are presented in Table 3. A significantly higher ($P<0.05$) increase in specific growth rate (SGR), body weight increase (BWI), percent body weight increase (PBWI) and daily growth rate (DGR) was recorded in diets supplemented with vitamin C levels of ≥800 mg $kg^{-1}$ AA diet when compared to the control diet and 400 mg $kg^{-1}$ AA diet. SGR, BWI, PBWI and DGR was highest for guppies fed the supplemented diet using 2000 mg $kg^{-1}$ AA diet (2.98, 0.819, 8190 and 0.546 respectively) followed by 1200 mg $kg^{-1}$ AA diet (2.90, 0.7645, 7645 and 0.509 respectively) and 800 mg $kg^{-1}$ AA diet (2.76, 0.573, 5730 and 0.382 respectively). Lowest SGR, BWI, PBWI and DGR was observed for 400 mg $kg^{-1}$ AA diet and the diet devoid of vitamin C.

In body weight gain (BWG) a significant difference was observed between guppies fed the supplemented diet using 1200 and 2000 mg $kg^{-1}$ AA diet with other groups ($P<0.05$) and highest BWG was observed in fish fed 1200 mg $kg^{-1}$ AA diet. Condition factor (CF) was lowest in 2000 mg $kg^{-1}$ AA diet and had significant difference with other three groups and control group ($P<0.05$).

The survival rate of guppies fed with diets containing graded levels of vitamin C are shown in Figure 1. The survival rate of guppies fed the control diet and the diet containing 400 and 1200 mg $kg^{-1}$ AA was higher than other groups and the highest survival rate was observed in 1200 mg $kg^{-1}$ AA and lowest survival rate was observed in 800 mg $kg^{-1}$ AA (100% and 85% respectively).

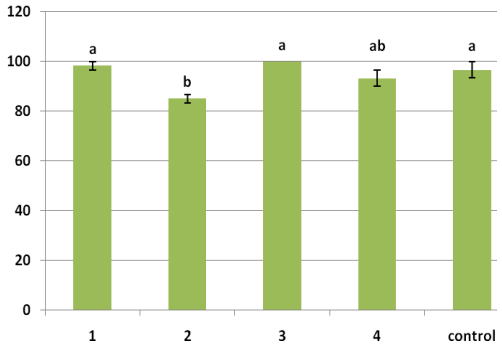


Figure 1. Effects of vitamin C on survival rate.

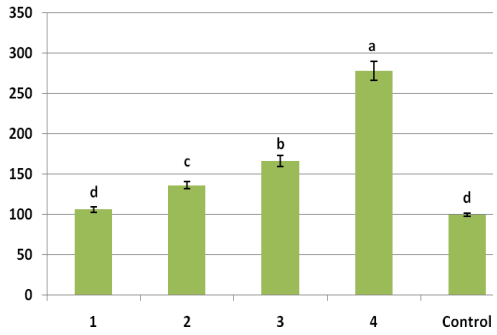


Figure 2. The reproductive performance of guppies (the number of newborn fish).

The reproductive performance of guppies (the number of newborn fish) fed with different levels of dietary ascorbic acid is presented in Figure 2. Reproductive performance of guppies were increased significantly with increasing the levels of vitamin C ($P<0.05$), but these increase was not significant between guppies using 400 mg $kg^{-1}$ AA diet and the diet devoid of vitamin C and the highest number of newborn fish was observed in guppies fed the 2000 mg $kg^{-1}$ AA diet (278). There were no significant differences in sex ratio observed between the treatments (see Table 2).

**Discussion**. Majority of the fish species require a dietary supply of vitamin C to maintain normal growth because they lack the ability to convert L-gulonolactone to 2-keto-L-gulonolactone (NRC 1993).

Table 2

Sex ratio in newborn fish

| | Diet 1 (400 mg $kg^{-1}$ AA) | Diet 2 (800 mg $kg^{-1}$ AA) | Diet 3 (1200 mg $kg^{-1}$ AA) | Diet 4 (2000 mg $kg^{-1}$ AA) | Diet 5 (Control) (0 mg $kg^{-1}$ AA) |
|---|---|---|---|---|---|
| Sex ratio (Female/Male) | 2.57±0.537[a] | 3.07±0.076[a] | 2.6±0.634[a] | 2.48±0.661[a] | 2.49±0.77[a] |

Table 3

Effects of vitamin C on growth parameters

| Parameters | Diet 1 (400 mg $kg^{-1}$ AA) | Diet 2 (800 mg $kg^{-1}$ AA) | Diet 3 (1200 mg $kg^{-1}$ AA) | Diet 4 (2000 mg $kg^{-1}$ AA) | Diet 5 (Control) (0 mg $kg^{-1}$ AA) |
|---|---|---|---|---|---|
| Initial fish length | 8±0.6 | 8.1±0.1 | 7.4±0.3 | 8.35±0.75 | 8.25±0.25 |
| Final fish length | 35.8±0.2 | 38.875±0.125 | 42.35±0.15 | 46±0.9 | 34.8±0.2 |
| Initial fish weight | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| Final fish weight | 0.487±0.0055 | 0.628±0.032 | 0.7745±0.0205 | 0.874±0.026 | 0.487±0.009 |
| Specific growth rate (SGR) | $2.59±0.007^{d}$ | $2.76±0.03^{c}$ | $2.90±0.018^{b}$ | $2.98±0.02^{a}$ | $2.590±0.012^{d}$ |
| Body weight increase (BWI) | $0.4765±0.0055^{d}$ | $0.573±0.013^{c}$ | $0.7645±0.0205^{b}$ | $0.819±0.019^{a}$ | $0.477±0.009^{d}$ |
| Percent body weight increase (PBWI) | $4765±55^{d}$ | $5730±130^{c}$ | $7645±205^{b}$ | $8190±190^{a}$ | $4770±90^{d}$ |
| Body weight gain (BWG) | $14.054±0.076^{b}$ | $14.618±0.62^{b}$ | $22.935±0.61^{a}$ | $0.509±0.014^{b}$ | $0.00102±1.62×10^{-5b}$ |
| Daily growth rate (DGR) | $0.318±0.0037^{d}$ | $0.382±0.0087^{c}$ | $22.913±0.29^{a}$ | $0.546±0.013^{a}$ | $0.0009±2.6×10^{-5c}$ |
| Condition factor (CF) | $0.00106±2.98×10^{-5ab}$ | $0.00107±4.42×10^{-5ab}$ | $13.84±0.74^{b}$ | $0.318±0.006^{d}$ | $0.001156±4.13×10^{-5a}$ |

In the present study, the growth of guppy improved significantly with increasing supplementation of dietary ascorbic acid. These results further confirm that guppies need adequate exogenous vitamin C to maintain normal growth and physiological functions. The significant differences also revealed that the supplemented groups would have performed better than the control group. These results agree with previous studies on some other fish (Eya & Mgbenka 1990; Al-Amoudi et al 1992; Gouillou-Coustans et al 1998; Shiau & Hsu 1999; Sealey & Gatlin 1999; Wang et al 2003; Ai et al 2004; Ibiyo et al 2007). The reduction in growth performance of fish fed the control diet in the present study seems to indicate that AA has a specific effect on growth as first suggested by Ram (1966). Vitamin C is an essential coenzyme in certain oxidative processes, including the oxidation of tyrosine and phenylalanine (Brander & Pugh 1977). This probably explains the differences that occur in the percent body weight increase (PBWI) with respect to the vitamin C free and enriched groups (Ibiyo et al 2007). 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 2000 mg of ascorbic acid $kg^{-1}$ diet was found to be the optimal dietary requirement for guppies.

The ascorbic acid requirement value by guppies attained in this experiment was higher than those reported for *Oreochromis aureus* between 10 and 25 mg ascorbic acid who also examined juvenile hybrid tilapia, *Oreochromis niloticus x O. aureus* (Shiau & Hsu 1999). Also, the requirement based on growth performance in this study was higher than that for *Oreochromis spilurus* (100-200 mg ascorbic acid kg-1 diet) (Al-Amoudi et al 1992).

The difference is probably related to fish species, size, the form of vitamin C and experimental conditions in different studies (Lovell 1989). The diet without ascorbic acid supplementation decreased the specific growth rate (2.590) of guppies and this is in accordance with studies conducted by Ai et al (2004) who also observed declining specific growth rate with ascorbic acid deficient diet for seabass (*Scophthalmus maximus*).

No significant difference in survival rate was observed between the treatments. This result is not conform with study conducted by Lee & Dabrowski (2004) on yellow perch, they suggested survival of yellow perch fed with vitamin C diets were significantly higher than the fish fed with diet without vitamin C.

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.*

Vitamin C plays important roles in fish reproduction, affecting hormone synthesis, vitellogenesis, and results in variable oocyte AA retention and gamete quality (Dabrowski 2001). Studies by Waagbo et al (1989) demonstrated a relationship between AA intake and circulating levels of 17-β-estradiol and vitellogenin, confirming the role of AA in hormone synthesis in endocrine tissues. In these trial the number of newborn fish was increased with increasing the level of AA. These results agree with the result of Blom & Dabrowski (1995, in Dabrowski 2001) which demonstrated that fecundity increased in female rainbow trout with increased dietary vitamin C levels. Vitamin C was necessary for successful reproduction in rainbow trout, apparently in protecting oxidation sensitive genetic material in gametes, and probably has the same function in warmwater fish (Dabrowski 2001) like *P. reticulata*.

**Conclusions**. From the present results of this study we conclude that 2000 mg ascorbic acid $kg^{-1}$ in the diet is the optimal dietary requirement for *P. reticulata* and must be fortified to pelleted diets in order to maximize ascorbic acid bioavailability to *P. reticulata.*

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