



The Effects of Vitamins C and E on the Growth Performance of Guppy (*Poecilia reticulata*) Fry^{1*}

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Abstract: This study was carried out to evaluate the effects of water-soluble vitamin C and fat-soluble vitamin E on the growth of guppy (*Poecilia reticulata*). The control feed did not contain vitamin C and E, while the other 15 experimental feeds were prepared by changing the amount of starch, without altering the chemical structure, and adding vitamin C (100, 500, 1000 mg/kg) and vitamin E (75, 150, 300 mg/kg) in various levels. At the end of the trial had an ascending amount of both vitamin C and E in the diet and resulted with an increase in the developmental parameters in the offspring of guppies ($P<0.05$). Among the experimental groups, the lowest feed conversion ratio was observed in group 16 (1000 mg vitamin C/kg+300 mg vitamin E/kg), and the highest was in group 1 (the control) ($P<0.05$). Differences between the groups, in which mortalities occurred, and the remaining groups, were found to be statistically significant ($P<0.05$). In addition, it was determined after a three month trial the group 16 of guppies had reached maturity, and juvenile. According to the results of this study, there is an increase in growth and survival of fry as the amount of vitamin C and E added in feed increases. The present study shows that a dosage of 1000 mg kg⁻¹ vitamin C+300 mg kg⁻¹ vitamin E has a beneficial effect on guppy growth parameters and survival rate.

Keywords: Feeding, growth, guppy, vitamin C, vitamin E.

C ve E Vitaminlerinin Lepistes (*Poecilia reticulata*) Yavrularında Büyüme Performansı Üzerindeki Etkileri

Öz: Bu çalışmada, suda eriyen C vitamini ile yağda eriyen E vitamininin lepistes (*Poecilia reticulata*) büyümesi üzerine olan etkileri araştırılmıştır. Kontrol yemi içerisinde Vitamin C ve E katkı maddeleri bulunmaz iken, diğer yem gruplarında nişasta miktarı değiştirilerek kimyasal yapı değiştirilmeksizin farklı miktarlarda Vitamin C (100, 500, 1000 mg/kg) ve vitamin E (75, 150, 300 mg/kg) ilavesi ile 15 adet deneme yemi hazırlanmıştır. Deneme yemindeki vitamin C ve E miktarları artış gösterdikçe lepistes yavrularının büyüme parametreleri de artış göstermiştir ($P<0.05$). Deneme grupları arasında en düşük yem değerlendirme oranının 16. grupta (1000 mg vitamin C/kg+300 mg vitamin E/kg), en yüksek yem değerlendirme oranının ise 1. (Kontrol Grubu) grupta olduğu tespit edilmiştir ($P<0.05$). Ölü balıkların tespit edildiği gruplarla diğer gruplar arasında istatistiki açıdan önemli farklılık olduğu bulunmuştur ($P<0.05$). Ayrıca 3 aylık deneme sonunda 16. gruptaki balıklar tamamen cinsi olgunluğa eriştiği ve yavru verdiği tespit edilmiştir. Bu çalışmada elde edilen sonuçlara göre, yemlere ilave edilen C ve E vitamini miktarları arttıkça yavruların büyümelerinde ve yaşama oranlarında artış olduğu tespit edilmiştir. Yapılan bu çalışma, 1000 mg kg⁻¹ C vitamini+300 mg kg⁻¹ E vitamini dozajının lepistes balıklarının büyüme parametreleri ve yaşama oranı üzerinde yararlı etkiye sahip olduğunu göstermektedir.

Anahtar kelimeler: Besleme, büyüme, lepistes, vitamin C, vitamin E.

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INTRODUCTION

Like every living being, fish need good living conditions and a balanced diet to sustain their vital activities (Hoşsu et al., 2003). The primary element comprising their nourishment is fish feed, containing essential nutrition compounds. The feed contains carbohydrate, protein, fat, minerals, and vitamins. Furthermore, various additives may be used in the making of fish feed (Arıman & Aras, 2002; Hoşsu et al., 2003).

Vitamins are organic compounds that are not particularly required in high levels but are essential for healthy growth, reproduction, and other bodily functions (Akyurt, 2004). Vitamins, apart from growth and reproduction activities, are also among the indispensable substances for the healthy development of fish. The principal vitamins which ensures the reproduction process and constitutes the fundamentals of fish farming, are vitamin A, B1, B6, C, and E (Hunt et al., 2004; Sealey & III Gatlin, 2002).

Natural foods are rich in vitamins, however, that is not the case with artificial fish feed. Vitamin requirement of fish varies depending on the species (Shiau & Hsu, 2002). The feed consumed during the spawning and growing periods of fish must contain various vitamins (especially vitamin E and C). Vitamin E is effective in the oogenesis and the formation of the extraembryonic membrane. It is known that vitamin C also affects reproduction (Emata et al., 2000; Lee & Dabrowski 2004; Santiago & Gonzal, 2000). Among vitamins, C and E have a great effect both on fish metabolism and the fish feed. Vitamin E and C have a significant interaction (Frischknecht et al., 1994). Other vitamins in the fish body also interact with each other. Vitamin E and C, especially, work as intracellular antioxidants. Vitamin C reduces the usage of vitamin E by decreasing lipid peroxidation in the cell membrane. Moreover, adding vitamin E and C combined to the fish feed during the spawning period increases the reproductive performance, spawn efficiency, and growth rate of the fish (El-Gamal et al., 2007; Emata et al., 2000; Hamre et al., 1997; James & Vasudhevan, 2011; Santiago & Gonzal, 2000).

The supply of aquarium fish is carried out mostly from nature. Moreover, the decline in fish populations and the constraint on natural resources have led certain ornamental fish trading countries, like Singapore, to industrialization by establishing new fish farms cultivating especially tropical livebearing fish (guppy, swordtail, molly, etc.). Thus, this situation paved the way to determine the nutrition requirements of ornamental fish. The prioritized matter was the maximum growth rate in commercial farms. Subsequent goals at hand were coloration, the development

of gonad, and ensuring the fish reach their market size as fast as possible (Sales & Janssens, 2003).

Guppy (*Poecilia reticulata* Peters, 1860) is amongst the multi-colored fish species in the Poeciliidae family. Guppies are economically important ovoviviparous fish that constitute a large portion of the ornamental fish culture industry. The species is omnivores and *Poecilia reticulata* feeds on zooplankton, detritus and larvae of small insects in nature (Harpaz et al., 2005; Lawal et al., 2012). In this study, the effects of vitamins C and E on the growth performance of guppy fry were investigated and the requirement of the mentioned vitamins was evaluated.

MATERIAL AND METHOD

Fish and experimental design: The study was conducted in Sinop University Fisheries Faculty. Guppy fry were obtained from Akdeniz Fisheries Research, Production, and Education Institute (Kepez, Antalya, Turkey). Fish were acclimated to treatment conditions for 2 weeks prior to the beginning of the feeding trial. A total of 720 fish were obtained and placed into 48 aquaria (dimensions: 20x35x15 cm, volume: 10 L) in triplicate (16 groups in total). Groups are formed according to the amount of vitamins they contain:

- Group1: 0 mg vitamin C/kg+0 mg vitamin E/kg,
- Group2: 0 mg vitamin C/kg+75 mg vitamin E/kg,
- Group3: 0 mg vitamin C/kg+150 mg vitamin E/kg,
- Group4: 0 mg vitamin C/kg+300 mg vitamin E/kg,
- Group5: 100 mg vitamin C/kg+0 mg vitamin E/kg,
- Group6: 500 mg vitamin C/kg+0 mg vitamin E/kg,
- Group7: 1000 mg vitamin C/kg+0 mg vitamin E/kg,
- Group8: 100 mg vitamin C/kg+75 mg vitamin E/kg,
- Group9: 500 mg vitamin C/kg+75 mg vitamin E/kg,
- Group10: 1000 mg vitamin C/kg+75 mg vitamin E/kg,
- Group11: 100 mg vitamin C/kg+150 mg vitamin E/kg,
- Group12: 500 mg vitamin C/kg+150 mg vitamin E/kg,
- Group13: 1000 mg vitamin C/kg+150 mg vitamin E/kg,
- Group14: 100 mg vitamin C/kg+300 mg vitamin E/kg,
- Group15: 500 mg vitamin C/kg+300 mg vitamin E/kg,
- Group16: 1000 mg vitamin C/kg+300 mg vitamin E/kg.

Each group consisted of 15 individuals. At the beginning of the experiment, the fish were weighed individually with a KERN brand EW420-3NM model scales (initial mean body weight: 0.011±0.02 g). Fish were fed with powder feeds (suitable for mouth size) *ad libitum* twice a day during the experiment. Temperature of the aquarium unit was set to 24±1°C throughout the experiments using an air conditioner. The aquariums were siphoned twice a week to remove fish feces and feed residues and water level in aquariums was completed with the addition of water at the same temperature. Aeration was carried out using an air stone.

During the following three months (90 days) experimental period, water temperature, dissolved oxygen, and pH were measured by using a YSI instrument

(Professional Plus Multiparameter, USA) and maintained at 24 ± 1 °C, 8.81 ± 0.00 mg/L, and 6.81 ± 0.00 , respectively. Daylight photoperiod as 12:12 h (light:dark) was applied in the experiment.

Fish were weighed individually to determine their average weight in the beginning and in the end of the experiment. Dead fish were recorded daily.

Experimental diets: The raw materials in the experimental feed were obtained from SİBAL Inc. and vitamins were obtained from DSM – Nutritional Products. The experimental feed contained fishmeal, soybean meal, sunflower seed meal, starch, maize protein, fish oil, a mixture of minerals and vitamins (A, B1-Thiamin, B2-Riboflavin, B3-Niacin, B6-Pyridoxine, B12-Cobalamin, C-Ascorbic acid, D, E, K) as raw materials. The control feed did not contain vitamin C and E, while the other 15 experimental feeds were prepared by changing the amount of starch, without altering the chemical structure, and adding vitamin C (100, 500, 1000 mg/kg) and vitamin E (75, 150, 300 mg/kg) in various levels. The vitamin levels used in the trial were determined based on the literature data (Blom & Dabrowski, 2000; Emata et al., 2000; Gouillou-Coustans et al., 1998; Hamre et al., 1997; Izquierdo et al., 2001; Lim et al., 2002; Wang et al., 2003). The raw materials added to cover the targeted nutritional values for the experiment were combined and mixed in dry form. Then, after adding fish oil and mixing again, 25-35% water was added to the mixture, after the vitamins were added to the feeds, the wet trial feeds were dried in a drying cabinet at 50°C for about 6 hours. The reason for keeping the temperature and time low is to minimize the losses that may occur in vitamins. Feeds were used in powder form in accordance with the mouth size. Composition of the basal diet used in the study is given in Table 1. Analyses regarding the amount of nutrients were conducted by SİBAL Inc., and determination of vitamin C and E levels were conducted by TÜBİTAK Marmara Research Center.

Table 1. Composition of the basal diet (g/100g).

Ingredient	Amount
Fishmeal	28
Soybean Meal	21
Sunflower Seed Meal	22
Maize Protein	4.5
Starch	18.5
Fish Oil	4
Mineral	1
Vitamin*	1
Chemical Composition (% dry matter basis)	
Dry Matter	83.90
Crude Protein	40.10
Crude Fat	6.71
Crude Ash	6.52
Crude Fiber	1.82
Nitrogen-Free Extract**	28.75

* 2200 IU of vitamin A, 85 mg of B1-Thiamin, 77 mg of B2-Riboflavin, 350 mg of B3-Niacin, 88 mg of B6-Pyridoxine, 0.2 mg of B12-Cobalamin, 200 mg of D, 20 mg of K)

**NFE (%) = 100 - (% moisture + % crude protein + % crude fat + % ash + % crude fiber).

To delay the spoiling of vitamins used in the trial, they were packed properly for protection from sunlight and heat (in black vacuum bags) and stored in a refrigerator.

All vitamins used in the trial, particularly pure Rovimix Vitamin C (L-ascorbic acid), and Vitamin E (α -tocopherol acetate), were added to the feed according to loss rates determined by the manufacturer (DSM Nutritional Products). Vitamins were stored at ± 4 °C in a dry, dark place until use.

Calculations and statistical analysis: The following variables were calculated according to Salem & Abdel-Ghany (2018) as follows:

Weight Gain (g) = Final weight (g) – Initial weight (g)

Specific growth rate (SGR, % day^{-1}) = $\frac{\ln W_2 - \ln W_1}{\text{Number of experiment days}} \times 100$

W2: Final weight (g)

W1: Initial weight (g)

Feed conversion ratio (FCR) = $\frac{\text{Total dry feed given (g)}}{\text{Total weight gain (g)}}$

Survival rate (SR, %) = $\frac{\text{Final number of fish}}{\text{Initial number of fish}} \times 100$

Statistical analyses were performed using “Minitab Release 10 for Windows” software at a significance level of 0.05. All data on growth, FCR and survival are expressed as mean \pm standard error (SE). Data from different experimental groups for each sampling period were analyzed by one-way analysis of variance (ANOVA) to test for effects of dietary treatments. Significant means were compared with Tukey test.

Ethical note: This research that produced from doctorate thesis was approved by the Ethics Committee of Ondokuz Mayıs University with Reference No. 2007/12. All the procedures applied in this study considered the importance of preventing, or at least minimizing, any kind of animal discomfort or suffering.

RESULTS AND DISCUSSION

Mean water temperature, pH, dissolved oxygen level and NH_4 content of water at the beginning of the experiments were 24.00 ± 0.01 °C, 6.81 ± 0.00 , 8.81 ± 0.00 mg/l and 0.4 ± 0.01 mg/l, respectively. When the water parameters of each group were analyzed at the beginning and the end of trials, the differences were statistically insignificant ($P > 0.05$).

The highest weight gain among the trial groups was observed in group 16, followed by groups 15 and 13, respectively. The lowest weight gain was observed in groups 1 and 6, and the differences between these groups and others were statistically significant ($P < 0.05$) (Table 2).

Table 2. Growth parameters, feed conversion ratio (FCR) and survival rate of guppy (*Poecilia reticulata*) fed different amounts of vitamin for 90 days (mean± standard error).

Experimental Groups*	Final Weight (g)	Weight gain (g)	Specific Growth Rate (%)	Feed Conversion Ratio	Survival Rate (%)
1	0.079±0.03	0.069±0.07 ^a	2.19±0.01 ^a	2.52±0.05 ^h	84.44±0.70 ^a
2	0.107±0.07	0.097±0.09 ^c	2.53±0.01 ^c	1.56±0.01 ^f	91.10±0.70 ^b
3	0.106±0.07	0.095±0.08 ^c	2.51±0.00 ^c	1.96±0.11 ^g	93.33±1.21 ^c
4	0.110±0.01	0.098±0.02 ^c	2.55±0.01 ^c	1.37±0.00 ^e	100.0±0.00 ^d
5	0.110±0.01	0.098±0.02 ^c	2.55±0.01 ^c	1.24±0.04 ^{cd}	91.10±0.70 ^b
6	0.100±0.01	0.089±0.02 ^b	2.45±0.00 ^b	1.24±0.02 ^{cd}	93.33±0.00 ^c
7	0.140±0.12	0.103±0.10 ^{cd}	2.59±0.03 ^{cd}	1.38±0.04 ^e	100.0±0.00 ^d
8	0.124±0.08	0.112±0.04 ^d	2.68±0.01 ^d	1.31±0.08 ^e	93.33±1.21 ^c
9	0.129±0.00	0.117±0.00 ^d	2.73±0.00 ^e	1.22±0.02 ^c	100.0±0.00 ^d
10	0.130±0.01	0.119±0.11 ^{de}	2.74±0.00 ^e	1.27±0.00 ^d	100.0±0.00 ^d
11	0.146±0.02	0.135±0.06 ^f	2.86±0.03 ^f	1.21±0.04 ^c	93.33±0.00 ^c
12	0.163±0.01	0.152±0.02 ^g	2.99±0.01 ^g	1.05±0.04 ^{bc}	100.0±0.00 ^d
13	0.185±0.01	0.174±0.03 ^h	3.13±0.00 ^h	0.73±0.02 ^{ab}	100.0±0.00 ^d
14	0.167±0.01	0.156±0.00 ^g	3.02±0.01 ^g	0.68±0.01 ^{ab}	100.0±0.00 ^d
15	0.202±0.01	0.191±0.00 ⁱ	3.23±0.01 ^h	0.61±0.01 ^a	100.0±0.00 ^d
16	0.256±0.01	0.245±0.00 ⁱ	3.49±0.01 ⁱ	0.47±0.01 ^a	100.0±0.00 ^d

Different letters in the same column indicate significant differences between the experimental groups (P<0.05).

When specific growth rates of the fish were evaluated, it was observed that guppy fry in group 16, which were fed with 300 mg vitamin E/kg+1000 mg vitamin C/kg supplemented feed, had the highest rate (3.50±0.01) whereas, guppy fry in group 1, which were fed with vitamins C and E deficient control feed, showed the lowest rate (2.19±0.01). The difference between each group was found to be statistically significant (P<0.05).

Among the experimental groups, the lowest feed conversion ratio was observed in group 16, and the highest was in group 1 (the control). Regarding the feed conversion ratio, differences between group 16, which had the lowest rate, and the remaining groups were observed to be statistically significant (P<0.05).

When the survival rate of each group was examined, it was found that mortalities were only in groups 1, 2, 3, 5, 6, 8 and 11. There were no mortalities in the other 9 groups. Differences between the groups, in which mortalities occurred, and the remaining groups, were found to be statistically significant (P<0.05).

At the end of the experiment, it was observed that the fish in groups 12, 13, 14, 15, and 16 reached sexual maturity. Furthermore, in group 16, a total of 18 fish fry were collected from aquaria 16-1 and 16-2 on the 87th day. When sexually mature fish in groups 12, 13, 14, 15, and 16 were counted separately as female (F) and male (M), the numbers were as follows: 26F-19M, 26F-19M, 27F-18M, 27F-18M, and 30F-15M, respectively.

In the study conducted by NRC (1993), it was reported that the required level of vitamin C and vitamin E for fish to grow, reproduce and sustain vital functions varies between 40-500 mg vitamin C/kg, and between 50-250 mg vitamin E/kg. Levels of vitamins C and E added to the feed in the present study were parallel to the levels reported by NRC (1993).

Vitamin C and E are very essentials for most fish species like as ornamental fish. It has also been recognized that these vitamins are among the most important nutrients that affect various aspects of fish including growth and

survival (Khara et al., 2016; Liu et al., 2016; Liu et al., 2018). When each group was statistically analyzed, the fish in group 16 had the highest mean individual weight gain, total weight gain, daily weight gain, specific growth rate, and the lowest feed conversion ratio. When each group was analyzed regarding growth parameters, there were statistically significant differences between fish in group 16 and the others (P<0.05). Moreover, the lowest values belonged to fish in group 1 (the control group), which were fed with vitamins C and E deficient feed. Groups that were fed with feed containing merely vitamin C, or E additives were statistically similar (P>0.05).

As ascorbic acid reserves are used out during the larval development of fish (Dabrowski et al., 1988, 1989; Dabrowski, 1990), it has been suggested that vitamin C requirements of fish during the early stages of their lives are might be higher than fingerling and maturity stages (NRC, 1993). Since fish are unable to synthesize vitamin C (ascorbic acid), they depend on dietary intake. The required level of vitamin C intake varies according to species, age, and physiological state (Hoşsu et al., 2003). In the study conducted by Shiau & Jan, (1992) it was reported that the ascorbic acid requirement of catfish, trout, and carp decreases as their size increases. Concordantly, considering the mean initial weight of the fish and the fact that they are fry, weight gain at the end of the trial increased depending on the level of vitamin addition. In the studies conducted by Dabrowski et al., (1988, 1989) it was suggested that the vitamin C requirement of fish during the early stages of life might be greater than fingerling and maturity stages since ascorbic acid reserves are depleted during the larval development.

Chen et al., (2004) conducted a study in which they fed *Notemigonus crysoleucas* with α -tocopherol (0 and 38 mg of α -tocopherol/kg feed) and ascorbic acid (23, 43, 98, and 222 mg ascorbic acid/kg feed) supplemented feed. In the study, they evaluated the growth, survival, immunity, and stress response of the fish (with 0.75 g initial weight) to determine vitamin E deficiency. At the

end of 14 weeks, they reported that α -tocopherol supplementation did not affect the weight gain, but increased the survival rate of the fish. In the present study, the lowest survival rate was observed in Group I, 100% survival rate was observed in group 4 (maximum vitamin E level), group 7 (maximum vitamin C level), groups 9, 10, 12, 13, 14, 15, and 16 (increasing levels of vitamins C and E combined).

In the study conducted by Gouillou-Coustans et al., (1998) on *Cyprinus carpio* larvae, it was reported that although the vitamin requirement of fish increased gradually as they grew from the larval stage, it was greater during the first stage of growth and reproduction, compared to other stages of life. Similarly, we observed that the vitamin requirement of guppy fry continued to increase as they grew, as suggested by Gouillou-Coustans et al., (1998).

Blom & Dabrowski, (2000) added 0, 30, 120, 360, 720, and 1440 mg vitamin C/kg to the feed, to determine the vitamin C requirement of angelfish. At the end of the experiment, they determined the weight gain and the feed conversion ratio values of the fish (with 1.12 ± 0.08 g initial mean weight). After 14 weeks, they reported that the differences between the groups fed with 0, 30, and 120 mg vitamin C/kg and the groups fed with 360, 720, and 1440 mg vitamin C/kg, were statistically significant ($P < 0.05$). However, the differences among the latter groups were statistically insignificant ($P > 0.05$). In the present study conducted on fry, group 16 showed the highest weight gain. The experimental feed received by this group contained 1000 mg vitamin C/kg, with proximity to 1440 mg vitamin C/kg, as specified in the literature.

Galaz et al., (2010) investigated the effects of vitamin E supplementation in various levels (0, 25, 50, 75, 100, and 500 mg/kg) on the growth performance of *Oplegnathus fasciatus* (barred knifejaw) juveniles and its efficacy against *Vibrio anguillarum*. After 12 weeks, they analyzed the weight gain, feed conversion ratio, and survival rate values. At the end of the experiment, they reported a 100% survival rate in all experimental groups. They also stated that the highest weight gain (60.2 ± 0.80) and the lowest feed conversion ratio values (1.39 ± 0.01) were observed in the group fed with 25 mg vitamin E/kg supplementation. In our study, vitamin E was added into the feed in various levels (0, 75, 150, and 300 mg/kg). We observed that as vitamin E level increased, the weight gain values of fish also increased, and the feed conversion ratio decreased. This result differs from the study of Galaz et al., (2010).

Mehrad & Sudagar (2010a, b) have done two studies on the effect of vitamin in guppies. In the first one (2010a), they investigated the effect of Vitamin C on growth, survival, reproduction and sex ratios in guppy, and

in the other study (2010b), the effects of Vitamin E on growth and reproduction in guppy fish. In two studies, the body weight increase (BWI), percent body weight increase (PBWI), specific growth rate (SGR) and daily growth rate (DGR) of guppies were increased significantly with increasing the levels of vitamin C and vitamin E ($P < 0.05$).

In conclusion, in the light of the findings of this study, it was determined that as the levels of vitamins C and E supplementation increased, the growth and survival rates of guppy fry also increased.

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