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Effects of Different Levels of Vitamin E on Some Growth Parameters and Gonadal Histology of Tilapia *Oreochromis niloticus* L., 1758 Fingerlings

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Abstract: The effects of diets including different levels of Vitamin E (110.25 mg kg^{-1} feed (Control Group), 175 mg kg^{-1} feed (Group 1), 200 mg kg^{-1} feed (Group 2) and 250 mg kg^{-1} feed (Group 3)) on growth parameters, survival rate and gonadal histology of *Oreochromis niloticus* L., 1758 were examined in this study. Fish (20.62±1.19 g and 10.33 ±0.20 cm) was fed with Vitamin E enriched feed for 60 days. At the end of the experiment, the highest body weight, total length, daily growth rate, specific growth rate, condition factor and gonadosomatic index were in Group 1. Whereas, the best survival rate (100%) was taken form Group 2 and 3, lower rates (78 and 85%) were seen in the other groups. As to histological view, it was also observed that as Vitamin E levels increased growth stages of the ovarian and testis tissues. It was concluded from all results in this study that the most proper ratio of Vitamin E in the feed is 250 mg Vitamin E kg^{-1} feed for growth of *O. niloticus* of such size.

Key words: *Oreochromis niloticus*, Vitamin e, growth parameters, survival rate, gonad development

INTRODUCTION

Vitamin E (α-tocopherol) is one of the indispensable substances for growth, reproduction and health of fishes (Sealey and Gatlin, 2002; Hunt *et al*., 2004). Vitamin E requirements vary depending on such factors as species and stages of growth or maturation of fishes.

Various ratios were reported for the requirement of Vitamin E even for the same species in previous studies. For example, this ratio was reported to be 30 mg of Vitamin E kg^{-1} feed (Lowel, 1988), but 60 mg kg^{-1} feed (Hamre and Lie, 1995) for salmonids and 200-300 mg kg^{-1} feed (Watanabe and Takashima, 1977) and 80-100 mg kg^{-1} feed (Canyurt, 1986) for common carp. As to Turkey, its level in carp larvae and growout feeds was 73.5 and 110.25 mg (100-150 IU) kg^{-1} (Pinar Feed Company), respectively. The Vitamin E requirement of tilapia also suffers this uncertainty (Shiau and Shiau, 2001) due to varied and dissimilar numbers given on Vitamin E requirement 20-100 mg Vitamin E kg^{-1} feed (Eleraky *et al*., 1995); 50-100 mg Vitamin E kg^{-1} feed (De Silva and Anderson, 1995); 48.51 mg Vitamin E kg^{-1} feed (Fitzsimmons, 2005); 200 mg Vitamin E kg^{-1} feed (Ridha, 2005).

In addition, it is not generally given exactly in the previous studies that informed quantities of Vitamin E are needed for which growth period of the fish.

Therefore, determining the required quantities of Vitamin E, which may vary depending on the species and growth period of fishes, is essential. For these reasons, in this study, it was aimed to determine the effects of different ratios of Vitamin E on growth performance, survival rate and gonad histology of tilapia during growout and suggest its optimal inclusion in feeds for tilapias.

MATERIALS AND METHODS

Fish (*Oreochromis niloticus* L., 1758) obtained from Fisheries Department, Regional Directorate of State Hydraulic Works; Adana-Turkey was transported to the Culture Unit of Fisheries Faculty of University of Cukurova (YBUAL), stocked in 1 ton tank of water with a continuous water flow of 11 min) for acclimatization that lasted for 2 weeks during which fish was fed once a day until the experiment was started.

The experiment was carried out for 60 days with 4 groups in triplicate, including a control group. Artificial feed (including 110.25 mg Vitamin E (150 IU) kg^{-1}, from Pinar Feed Anonymous Company, Turkey) was used to prepare the feed materials in this study (Table 1).

Since, the feed had already included Vitamin E before, 0, 64.75, 89.75 and 139.25 mg of Vitamin E (DL-α-tocopherol, from Sigma) was added to per kg of the feed

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Table 1: Chemical composition of artificial feed

Chemical composition	Ratio
Moisture	Maximum 12%
Crude protein	Minimum 28%
Crude cellulose	Maximum 5%
Crude ash	Maximum 14%
Vitamin A and D_3	1500 IU kg^{-1}
Vitamin E	150 IU kg^{-1}
Vitamin C	70 mg kg^{-1}
Vitamin B_2-B_{12}	20-20 mg kg^{-1}
Vitamin K	10 mg kg^{-1}
Inositole	100 mg kg^{-1}
Coline	1000 mg kg^{-1}

to reach the total ratios of 175, 200 and 250 mg kg^{-1} feed (for Control, Group 1-3), respectively. After Vitamin E was solved in liquid lipid (25 mL sunflower lipid kg^{-1} feed), sprayed onto the feed by pulverization (Ortuno *et al*., 2000). A control diet was prepared by spraying the same amount of sunflower lipid without Vitamin E.

Then thirty fish (20.62±1.19 g, 10.33± 0.20 cm and 0.92±0.02% body weight (W), total length (L) and Gonadosomatic Index (GSI), respectively) were stocked into each part of the fiberglass tank (150×35×40 cm in size, 210 L) divided into 2 with a net. Fish was fed *ad libitum* twice in a day (10 am and 14 pm). There was no water exchange (0 1 min) in the experimental tanks. Tank water was aired continuously. After bottoms of tank were siphoned every 2 days, the same amount of clean water was added to each tank. The tanks were covered with a net to prevent the fish from escaping. Water temperature and dissolved oxygen level in each tank were measured (oxygenmeter, Mettler Toledo mark) daily and maintained at 27.25±0.18°C and 7.28±0.26 mg L^{-1}, respectively.

Ten individuals were sampled randomly every 20 days and measured for W and L (by scale in 0.01 g sensitivity and milimetric ruler). Then the fish was immersed in ice to anaesthetize, their gonads removed and weighted for GSI (by scale in 0.01 g sensitivity).

In all cases, tissues were fixed in formaldehyde solution (4%) for 24 h or longer, dehydrated in a progressive series of ethanol and embedded in paraffin. Sections of 3-5 μm were stained with hematoxylin-eosin. Histological studies were carried out according to Rothbard *et al.* (1987) and Crocker *et al.* (1989) in Pathological Laboratory of Medical Faculty of the same university.

Means of W, L and GSI were calculated for each sampling period and the means of Daily Growth Rate (DGR), Specific Growth Rate (SGR), Condition Factor (C) and GSI were calculated at the end of the experiment to determine performances. The growth responses and survival rates of the fish in each treatment group were calculated according to the following formulas:

$$\text{DGR g day}^{-1} = \frac{W_t - W_0}{t} \text{ (Wootton, 1990)}$$

$$\text{SGR \%/day} = \frac{\ln W_t - \ln W_0}{t - t_0} \times 100$$

(De Silva and Anderson, 1995)

$$C = \frac{W}{L^3} \times 100 \text{ (Ricker, 1975);}$$

$$\text{GSI \%} = \frac{GA}{W} \times 100 \text{ (Avsar, 1998)}$$

$$S(\%) = \frac{N_e}{N_b} \times 100 \text{ (Pechsiri and Yakupitiyage, 2005)}$$

where:

W_t = In final weight (g)
W_0 = In initial weight
t-t_0 = Day of experiment (day)
W = Fish weight
L = Fish length
GA = Gonad weight
N_e = Fish number at the end of the experiment
N_b = Fish number at beginnig of the experiment

Statistical analyses were performed at 0.05 significance level with Duncan's Multiple Range Test in SPSS 10.0 Packet Program (1999).

RESULTS AND DISCUSSION

Growth performance and survival rates: Mean W and L values of the fish in Control Group in all periods were lower than those of the others ($p>0.05$) (Table 2).

At the end of the experiment, the lowest means of DGR and SGR were seen in Control Group, but the lowest mean of C was found in Group 1. However, the highest DGR and SGR values were observed in Group 1, whereas the highest C value was seen in Group Control at the same period. While, the lowest mean of S was in Group Control, the highest mean was in Group 2 and 3 (Table 3 and Fig. 1).

The highest GSI mean was found in Group 3 in the first period, but it was in Group 1 in the 2nd and 3rd periods (Table 4).

Table 2: Mean W and L of the groups in sampling periods

Sampling periods	1st period		2nd period		3rd period	
	W	L	W	L	W	L
Groups						
Control	22.28±0.52^{a}	10.90±0.03^{a}	25.42±1.66^{a}	11.57±0.23^{a}	28.46±1.90^{a}	11.93±0.21^{a}
Group 1	26.09±1.14^{b}	11.63±0.23^{a,b}	31.73±1.63^{d}	13.06±0.23^{b}	36.91±0.81^{c}	13.36±0.12^{c}
Group 2	25.79±0.77^{b}	11.94±0.29^{b}	29.51±1.16^{c}	12.25±0.17^{a,b}	33.23±1.02^{b}	12.76±0.18^{b}
Group 3	25.43±0.70^{b}	11.64±0.13^{a,b}	28.58±1.32^{b}	12.20±0.17^{a,b}	35.20±1.92^{b,c}	13.28±0.09^{c}

a,b,c and d show statistical differences among groups ($p<0.05$)

Table 3: Mean values of DGR, SGR, C and S of the groups

Groups	DGR (g day^{-1})	SGR (% day^{-1})	C	S (%)
Control	0.13±0.01^{a}	0.53±0.05^{a}	1.68±0.05^{a}	78±1.86^{a}
1	0.27±0.07^{c}	0.96±0.02^{c}	1.50±0.04^{b}	85±1.45^{b}
2	0.22±0.02^{b,c}	0.75±0.02^{b}	1.58±0.02^{a,b}	100±0.00^{c}
3	0.24±0.02^{b}	0.88±0.06^{c}	1.50±0.05^{b}	100±0.00^{c}

a,b,c and d show statistical differences among groups ($p<0.05$)

Table 4: Mean values of GSI of the groups

Groups	Control	Group 1	Group 2	Group 3
Sampling periods				
1st	0.80±0.10^{a}	0.67±0.05^{a}	1.57±0.49^{a}	2.79±0.53^{b}
2nd	2.99±0.15^{a}	3.82±0.95^{a}	3.58±0.41^{a}	3.15±0.58^{a}
3rd	2.88±0.20^{a}	5.96±0.77^{b}	5.16±0.21^{b}	5.52±0.41^{b}

a,b,c and d show statistical differences among groups ($p<0.05$)

Table 5: Ovarian development stages of the fish

Groups	Control	Group 1	Group 2	Group 3
Sampling periods				
1st	CN	CN	P-CA	CA
2nd	CN	CN	CA	V
3rd	CN	P	V	O

CN: Cromatine Nucleolus, P: Peri-nucleolar CA: Cortikal Alveolar, V: Vitellogenesis, O: Maturing

Histological results

Ovarian: During oogenesis, 5 of 6 stages in ovarian development were determined: chromatin nucleolus, perinucleolar, cortical alveolar, vitellogenesis and maturation except ovulation (Table 5 and Fig. 2).

Testis: Related to testes, three stages of development were determined; immaturate, maturing and maturated during spermatogenesis (Table 6 and Fig. 3).

The highest DGR and thus W, L and SGR means were provided by Group 1 (175 mg of Vitamin E kg^{-1} of feed). In other words, the fish development was not parallel to Vitamin E increase. Moreover, in the other two experimental groups, its development was affected adversely.

As a result of different amount of Vitamin E feeding on perch (*Dicentrarchus labrax*) (139, 254, 493 and 942 mg kg^{-1} of feeds), Gatta *et al.* (2000) found that SGR means (0.63, 0.54, 0.57, 0.61, respectively) were statistically similar ($p>0.05$). Tocher *et al.* (2002) found that weight gain and SGR of the juveniles of turbot (*Scophthalmus maximus*) and Atlantic halibut (*Hippoglossus hippoglossus*) was affected through Vitamin E increase

Table 6: Testis development stages of the fish

Groups	Control	Group 1	Group 2	Group 3
Sampling periods				
1st	I	I	II	II
2nd	I	II	II	III
3rd	I	II	II	III

I: Immature, II: Maturing III: Maturated

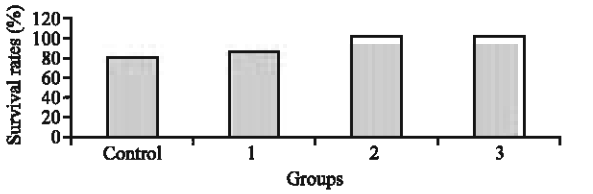

Fig. 1: Mean L and W of the groups in sampling periods

(0, 100, 1000 mg Vitamin E kg^{-1} feed). Additionally, Fernandez and Fenucci (1998) declared various Vitamin E supplements into feed (0, 100, 600, 1500 mg kg^{-1} feed) did not have any effect on the growth rates of Argentine red shrimp (*Pleoticus muelleri*). However, Fernandez *et al.* (2004) stated that feeding with various Vitamin E (0, 1250, 1500, 1750 ve 2000 mg Vitamin E kg^{-1} feed) during 40 days increased the weight of Argentine red shrimp (*P. muelleri).* In our study, however, Vitamin E supplement was observed to have some effect only at the rate of 175 mg kg^{-1} considering DGR, W, L and SGR means. Since, the amount of Vitamin E varies depending on the species (Hung *et al.*, 1980 and 1981), it can be said that the differences among the results may have realized due to differences of this species.

Tocher *et al.* (2002) recorded that there was an increase in weight and thus SGR means of sea bream through Vitamin E (3.8±0.3, 4.1±0.5, 4.0±0.6 of SGR means with 0, 100, 1000 mg kg^{-1} feed, respectively). However, this increase did not take place through the highest Vitamin E supplement (1000 mg kg^{-1} feed) but by means of lower, just as it was seen in our study. Therefore, there seems to be a consistence between the results of 2 studies, in which the high doses were observed to prevent fish growth, either in tilapia or in sea bream.

In the present study the condition factor ranged between 1.50±0.04 and 1.68±0.05. The condition factor

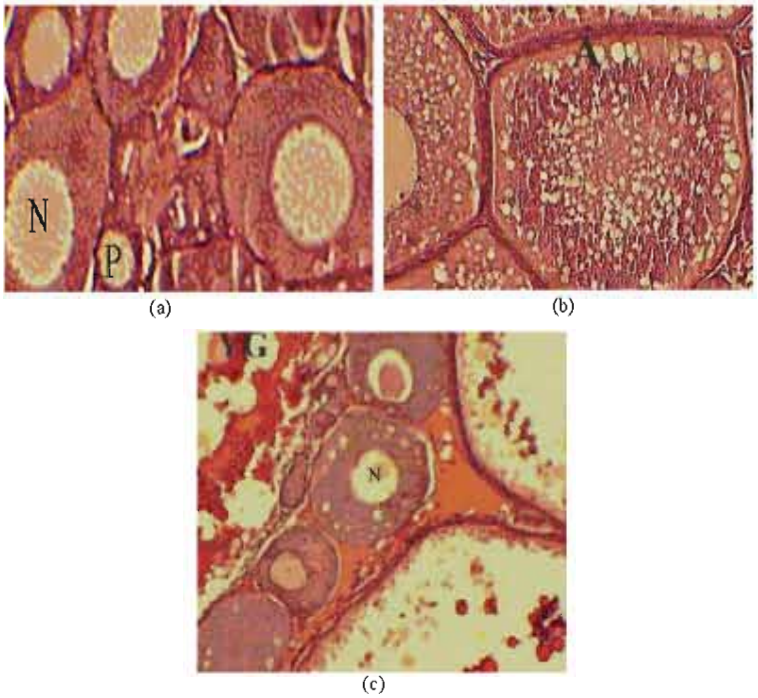

Fig. 2: Ovarian development stages (HE, x40), a): Cromatine nucleolus stages, b): Cortical alveolar stage, c): Nucleus in maturation stage) N: Nucleus, P: Primer oosit, B: Connecting tissue; A: Alveolar; N: Nucleus, YG: Yolk Granule

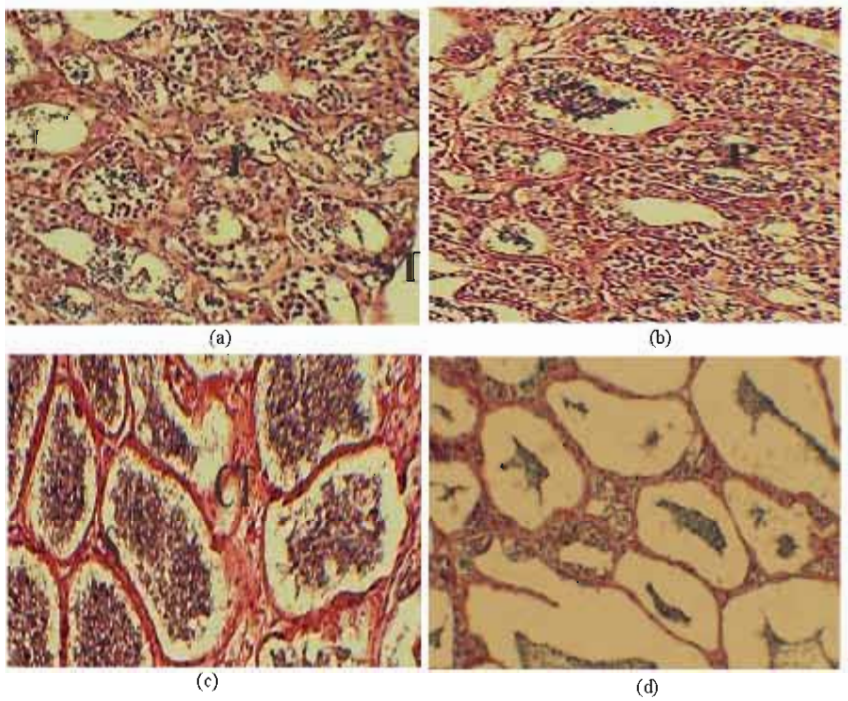

Fig. 3: Testis development stages (HE, X40), a): Immature stage, b): Immature testis stage, c): Maturing testis stage, d): Maturated testis stage). P: Primer spermataogoniums, ST: Sertoli cell; CT: Connected tissue, S: Spermatitis

is one of the criteria for feeding and growth (Olurin and Aderibigbe, 2006). These researches found C value as 1.11 for the *O. niloticus* juvenile reared in pond. Huang and Chiu (1997) found in their study that C value

of tilapia fry (*Oreochromis niloticus* X *Oreochromis mossambicus*) kept in aquaria was higher than 3 on mean at the end of the experiment.

Condition factor values of present study were better than those found in Olurin and Aderibigbe (2006), while it was lower than those found in Huang and Chiu (1997). Disharmony may be explained with the differences in the environmental conditions; fish size which enable better growth when the fish is fry and also differences in species.

As was seen in Fig. 1, survival rates increased in relation to increased levels of Vitamin E in the diet.

Previous researchers (Blazer and Wolke,1984a, b; Verhlac *et al*., 1991; Sealey and Gatlin, 2002) reported that some improvements were observed in immune responses of rainbow trout after dietary supplementation with vitamin E. Tocher *et al*. (2002) showed that the survival rates of juvenile turbot, Atlantic halibut and sea bream (*Sparus auratus*) increase with an increase in Vitamin E in the food. However, Fernandez and Fenucci (1998) stated that the mentioned amount of Vitamin E had no effect on the survival rate of Argentine red shrimp. Kanazawa (1985) said that the increase in the survival rate of the larvae of *Marsupenaeus japonicus* was due to the increase of Vitamin E in the diet.

This result is quite compatible with the findings reached by Tocher *et al*. (2002). Moreover, although it was made for a different group, that is invertebrate, the results of Kanazawa (1985) indicated an increasing effect of Vitamin E on the survival rate, just as it was seen in our study.

Survival rate of the fish is a of great importance factor in aquaculture. Although, the highest daily means of DGR, W, L, SGR were taken from Group 1 (175 mg Vitamin E kg^{-1} feed), which had the lowest survival rate after the control, the ratio of Vitamin E applied in Group 3 (250 mg Vitamin E kg^{-1} feed), which showed the second highest growth rate, can be accepted as the best ratio since, the survival rate understandably affects the total biomass at the end of the production.

According to Lee and Dabrowski (2004), Testis Somatic Index values (TSI) of perch which were fed on Vitamin C and/or Vitamin E supplement were -C-E, 1.56±0.06; -C+E, 1.15±0.38; +C-E, 2.15±0.42; +C+E, 1.54±0.21 ($p>0.05$) at the end of week 20 respectively; -C-E, 1.64±0.40; -C+E, 2.57±0.28; +C-E 2.61±0.35; +C+E 2.51±0.27 ($p<0.05$) at the end of week 32, respectively, in which C means Vitamin C and E means Vitamin E, whereas '-' shows 'without' and '+' shows 'with'.

In our study TSI and Ovarian Somatic Index (OSI) were not determined separately. However, as can be seen from Table 5, GSI mean of each group showed an increase depending on fish growth just as Lee and Dabrowski (2004) mentioned for TSI of perch. The highest GSI was taken from Group 3, given the highest Vitamin E, at the first measurement, but it began to be taken from Group 1 by the second measurement: The last two measurements were 3.82±0.95 and 5.96±0.77 on mean, respectively.

In the study of Lee and Dabrowski (2004), it was reported for the end of week 32 that TSI was seen to increase in group fed on diets supplemented only with Vitamin E, but it was higher in group fed on diets supplemented with Vitamin C instead of Vitamin E. However, TSI in the group fed on both Vitamin C and E supplement were lower than the mean values of the former 2 groups.

Gonadal development: Ovarian development stages observed in this study was in agreement with the results of Wilson *et al*. (1984) and He *et al*. (1992), who said that Vitamin E enhances gonadal development of fish and thus affect the reproduction positively.

Testis development stages observed in this study was also consistent with the reports of Wilson *et al*. (1984) and He *et al*. (1992), who said that Vitamin E hastens testis development of fish and thus affect the reproduction positively.

One of the results reached via this study was that the fish given the highest Vitamin E achieved the gonadal development which can produce gamets at least 20-40 days before others do.

This is especially, valuable for the tilapia farming especially in subtropical regions in which early reproduction is of great importance in order to extent the growout period. Moreover, since the mean body weight of the individuals of this group was not very much lower than that of Group 1 ($p>0.05$), which showed the highest growth, the highest Vitamin E application was not a loss regarding broodstock development.

CONCLUSION

The results show that the minimum Vitamin E rate for the development of *O. niloticus* should be 175 mg Vitamin E kg^{-1} feed. However, this and lower application rates of Vitamin E resulted in lower survival rates compared to the other two groups. Although, the mentioned rate yielded the highest individual body weight on mean, the survival rate will invariably affect the total biomass at the end of the production period, which made the Vitamin E rate applied to Group 3 (250 mg Vitamin E kg^{-1} feed) the best rate, in which only the second growth rate was achieved.

Moreover, the mean body weight and total length of Group 3 was not much lower than Group 1, which attained the highest body weight and total length ($p>0.05$). Therefore, the fact that this rate (250 mg Vitamin E kg^{-1} feed) doesn't have any negative effect on *O. niloticus*, especially on body weight and total length increases its acceptability. This rate was also seen to cause the highest gonadal development.

Histological results of gonads in this study showed that the fish taken the highest Vitamin E reached the gonadal maturity to produce gamets at least 20-40 days earlier than others did. This situation is especially important for tilapia farming demanding earlier offspring in subtropical regions, where the growout period is relatively shorter.

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