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# EFFECT OF DİETARY VITAMIN E SUPPLEMENTATION ON THE BLOOD PARAMETERS OF NILE TILAPIA (*OREOCHROMIS NILOTICUS)*

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## ABSTRACT

This study evaluated the influence of diets supplemented with 0, 80, 160, 240 mg $kg^{-1}$ ($E_0$, $E_{80}$, $E_{160}$, $E_{240}$) of vitamin E on the physiological responses of Nile tilapia (*Oreochromis niloticus*) fed for 3 months. Weight were not affected by dietary vitamin E concentrations. An increase ($p<0.05$) on the red blood cells count and on the hemoglobin concentration was obtained on treatments with 80 and 160 mg vitamin E $kg^{-1}$ relativeto control. Mean corpuscular volume presented a significant increase ($p<0.05$) on treatment with 240 mg vitamin E $kg^{-1}$ when compared to control. Mean corpuscular hemoglobin concentration was significantly decreased ($p<0.05$) on treatment with 240 mg vitamin E $kg^{-1}$. The vitamin E treatments, we noticed a significant increase ($p<0.05$) in the number of leucocytes relative to control. Our results suggest that 80 mg vitamin E $kg^{-1}$ is probably the most suitable concentrations for Tilapia diets, although high vitamin E diets are necessary for quantitative leucocyte increases for tilapia

**Keywords:** Nile Tilapia, *Oreochromis niloticus*, vitamin E, blood parameters.

## INTRODUCTİON

Tilapia culture is widely practiced in many tropical and subtropical regions of the world and constitutes the third largest group of farmed finfish, right after carp and salmonids, with an annual growth rate around 11.5%. Much of the rapid increase in aquaculture production has come from the increasing of existing systems (Bittencourt *et al,*. 2003)

Nutrient supplementation in fish diets has been an economically promising method for improving the performance of different intensive fish production systems. Vitamin E is among the most important a nutrient influencing the fish immune system, and the supply of vitamin E can reduce mortality and improve fish performance, while increasing specific and nonspecific immune responses (Wahli *et al.,* 1998, Ortuno *et al.,* 2001, Shiau and Hsu, 2002, Puangkaew *et al.,* 2004). In addition, vitamin E is potent antioxidant that offer protection against oxidative damage to various fish tissues (Adham *et al.,* 2000), enhance resistance of red blood cell membranes (Kiron *et al.,* 2004), and protect leukocyte functions (Sahoo and Mukherjee, 2002).

Since Tilapia are the most economically important farmed fish species, it is necessary to recognise their dietary requirements with respect physiological system. The aim of this study was to investigate the effect of dietary vitamin E levels on the growth and some haematological parameters of tilapia .

## MATERİALS AND METHODS

**Experimental design:** One hundred and forty-four juvenile tilapia, 12.8 ± 0.37 g mean body weight were obtained from fish reproduction unit of the Fisheries Faculty of Firat University, Elazig, Turkey. Fish were distributed in 12 groups of 12 fish each in 50 L glass aquaria. Fish were weighed individually at the beginning and at the end of the experimental period using a digital scale with precision of 0.1 g. The natural light cycle was close to 12h light/12h dark. Water quality was monitored weekly throughout the experiment. Dissolved oxygen concentration (DO), pH and temperature were determined with digital oxygen meter and pH-meter. The experiment was carried out with three replicates per treatment.

**Diets:** The experimental design consisted of four treatments: control, a control diet contain 32% crude protein was prepared (Table 1) and three experimental groups. Diet ingredients were contributed 18.2 mg $kg^{-1}$ vitamin E to the control diet. The vitamin E was added at the proportion 80 mg $kg^{-1}$, 160 mg $kg^{-1}$ and 240 mg $kg^{-1}$ ($E_{80}$, $E_{160}$, $E_{240}$) to the control diet for preparation of experimental diets. Tilapia were fed with 4 % of their total weight daily, three times in a day during 3 month.

**Blood sample collection and analysis:** Blood samples were drawn from the caudal vessel from seven fish per tank using heparinized syringes for determining red blood cell indices at the end of experient. Hematocrit (Ht, %) was determined by microhematocrit centrifugation. Red blood cell (RBC, $x10^6$) and total leukocyte counts (WBC, $x10^4$) were determined optically with a Neubauer chamber using a Natt and Herrick solution (Konuk, 1981). Hemoglobin concentration (Hb, g/100 mL)) was determined with Drabkin's reagent and read at absorbance at 540 nm (Jain, 1993). Mean cellular volume (MCV), mean cellular haemoglobin (MCH) and mean cellular hemoglobin concentration (MCHC) were determined. The three parameters were calculated using the following formulas (Jain, 1993).

MCV=(packed cell volume as percentage/RBC in millions)x10 $\mu^3$

MCH=(Hb in g/RBC in millions)x10 pg

MCHC=(Hb in g/packed cell volume)x100 g per 100 mL

**Table 1: Composition of the reference and test diets (%)**

| Ingredients | Reference diet | Experimental Idiet | Experimental II diet | Experimental II diet |
|---|---|---|---|---|
| Anchovy meal | 12 | 12 | 12 | 12 |
| Soybean meal (solvent extracted) | 43 | 42.992 | 42.984 | 42.976 |
| Wheat flour | 42.7 | 42.7 | 42.7 | 42.7 |
| Sunflower oil | 1.9 | 1.9 | 1.9 | 1.9 |
| Antioxidant[a] | 0.1 | 0.1 | 0.1 | 0.1 |
| Vitamin premix[b] | 0.25 | 0.25 | 0.25 | 0.25 |
| Mineral premix[c] | 0.05 | 0.05 | 0.05 | 0.05 |
| Vitamin E | 0 | 0.008 | 0.016 | 0.024 |

a Antioxidant (mg/g): butylated hydroxytoluene, 12.5. b Vitamin premix contains (IU or mg/g of premix): retinol, 1600 IU; calciferol, 800 IU; menadione, 4; thiamine, 4; riboflavin, 6; niacin, 12; pantothenic acid, 20; pyridoxine, 4; cobalamin, 0.008; folic acid, 2; biotin, 0.4; choline chloride, 200; ascorbic acid, 80. c Mineral premix contains (mg/g of premix): manganese 25, iron 44, zinc 100, copper 3, iodine 10, selenium 0.3.

**Statistical analysis:** All replicates were used for calculation of mean values. Statistics were performed with the SPSS 10.1 computer program (SPSS Inc. Chicago, Illinois, USA). Differences in hematological parameters between different concentrations and between exposure times were processed statistically by means of the analysis of variance (One-way ANOVA). The hematological parameters were expressed as means ± standard deviation. Differences were considered significant at the 0.05 probability.

## RESULTS AND DİSCUSSİON

The physicochemical parameters of water were within the range for culture of tilapia (Table 2). The table shows that these values fall within the normal range for the warm water culture of *O. niloticus*. There were no effects of either levels of dietary vitamin E on fish growth ($p>0.05$) (Table II). Survival in the current experiment was 100%.

The effects on haematological parameters of vitamin E were showed in Table 3. A significant ($p<0.05$) increase of haematological parameters in fish fed $E_{80}$ and $E_{160}$ experimental diets. Fish fed diets $E_0$ and $E_{240}$ had lower haemoglobin and RBC than those fed the other diets ($p< 0.05$). Hb and RBC of fish fed $E_{240}$ was lower than that of fish fed $E_0$ diet ($p<0.05$). In addition, the levels of MCHC after feed $E_{240}$ was found to be lower than the control, but the level of MCV. MCV presented a significant increase ($p<0.05$) on treatment with 240 mg vitamin E $kg^{-1}$ when compared to control. The vitamin E treatments, we noticed a significant increase ($p<0.05$) in the number of leucocytes relative to control.

Adequate vitamin E supplementation in fish diets under intensive rearing is essential for survival and growth performance (Chagas *et al.,* 2003). However, in this study after the 3 month feeding trial, Tilapia showed no significant changes on their growth among the diets they were subjected to. Similar results have been found for species such as Atlantic salmon (Hardie *et al.,* 1990), coho salmon (*Oncorhynchus kisutch*) (Forster *et al.,* 1988), rainbow trout (Blazer and Wolke, 1984), catfish (Bai and Gatlin, 1993) or seabass (*Dicentrarchus labrax*) (Stephan *et al.,* 1993). However, other authors have demonstrated the effects of vitamin E deficient diets on growth for different species, such as Atlantic salmon (Hamre *et al.,* 1994), rainbow trout (Cowey *et al.,* 1984), chinook salmon (Thorarinsson *et al.,* 1994) and hybrid striped bass (*Morone chrysops* female *Morone saxatilis* male) (Kocabas and Gatlin, 1999). The apparent difference among studies could be due to differences among species, the size of the fish used (as those studies describing effects of dietary vitamin E on fish growth or survival used fry, whereas those studies describing no effects used juvenile fish) or the differences in experimental procedures and culture.

The red blood cell indices (Ht, Hb and RBC) can be an indicator of oxidative status, because erythrocytes are one of the major production sites of free radical and some of them can trigger peroxidation of sutured fatty acids in their membrane phospholipids, therefore altering their quality (integrity, size) and quantity (Pearce *et al.,* 2003, Kiron *et al.,* 2004). To date, little is known about the haematology of the tilapia, which is one of the most important species in freshwater aquaculture worldwide; the results presented above have revealed an interesting pattern of response on the haematological variables in fish.

**Table 2. Biological and water quality parameters studied during experiment**

| | Initial weight (g) | Final weight(g) | Temperature (°C) | Dissolved oxygen (mg/L) | pH |
|---|---|---|---|---|---|
| $E_{80}$ | 12.75 ± 0.06 | 39.30 ± 1.20 | 26.0 ± 1.0 | 7.6 ± 0.9 | 7.2 ± 0.4 |
| $E_{160}$ | 12.79 ± 0.08 | 39.00 ± 1.19 | 26.3 ±1.1 | 7.3 ± 1.1 | 7.4 ± 0.3 |
| $E_{240}$ | 12.76 ± 0.07 | 38.60 ± 1.18 | 27.0 ± 0.8 | 7.5 ± 1.2 | 7.4 ± 0.4 |
| $E_0$ (Control) | 12.78 ± 0.08 | 38.50 ± 1.17 | 26.6 ± 0.6 | 7.5 ± 1.0 | 7.5 ± 0.4 |

**Table 3. Haematological parameters of fish after 3 month of feeding the experimental diets**

| | Ht (%) | Hb (g/dl) | RBC ($x10^6$ /$m^3$) | WBC ($x10^4$) | MCV ($\mu^3$) | MCH (pg) | MCHC (g/dl) |
|---|---|---|---|---|---|---|---|
| $E_{80}$ | 19.58 ± 0.99 | $5.84 \pm 0.98^d$ | $1.12 \pm 0.08^d$ | 4.050 ± 5.73 | 175.47 ± 12.58 | 52.39 ± 9.64 | 29.85 ± 4.77 |
| $E_{160}$ | 19.00 ± 0.85 | $6.08 \pm 0.57^{c,d}$ | $1.09 \pm 0.03^d$ | $5.208 \pm 9.85^{c,b}$ | 174.75 ± 9.97 | 55.91 ± 5.71 | $31.69 \pm 3.33^d$ |
| $E_{240}$ | 19.00 ± 1.04 | 5.03 ± 0.66 | 0.99 ± 0.08 | $5.192 \pm 11.05^{c,b}$ | $188.94 \pm 18.42^{c,b}$ | 51.02 ± 8.16 | 26.64 ± 4.64 |
| $E_0$ (Control) | $17.91 \pm 1.31^c$ | 5.32 ± 0.46 | 1.05 ± 0.09 | 3.600 ± 8.47 | 170.40 ± 14.82 | 50.84 ± 7.54 | 29.74 ± 3.50 |

*n:*36
*c: control; a: exp. I(*$E_{80}$*); b: exp. II (*$E_{160}$*); d: exp.III(*$E_{240}$*)*

Poston and Livingston (1969), reported that lower haematocrit were observed in brook trout fry fed a diet containing a high level of vitamin E (5000 mg/kg). Baker and Davies (1996), also reported that African catfish fed high α-tocopheryl acetate dose (500 mg/kg dry feed) were observed to have significantly lower hematocrit than fish fed the basal diet. However, Bai and Lee (1998), were showed in *Sebastes schlegeli*, hematocrit of fish fed control group was lower than that of fish fed high level of vitamin E. Our results for this parameter showed similarity to the results of Bai and Lee (1998).

When we compare *O. aureus* normal blood values with those obtained previously for *O. niloticus*, we find that Hussein *et al.,* (1996), reported approximate values haematocrit (20%) and RBC count ($1.31x10^6$ cells/µl) and haemoglobin concentration (6 g/dL). However, Tavares-Dias and Faustino (1998), also observed that hemoglobin and hematocrit values in tilapia ranged, respectively, from 5.4 g/dl and 23% to 12.7 g/dl and 41%. The results in the study are in agreement with the results in previously investigations.

Leucocytes or white blood cells (WBC) are vitamins efficiency as wll as defence mechanisms indicator in fish, once it has been reported that vitamin E are potential antioxidant that provide protection to the leucocyte function (Wahli *et al.,* 1998; Sahoo and Mukherjee, 2002). The some studies were also reported for other fish species when fed with of vitamin E suplement diet and an not in the number of circulating leucocytes was reported (Blazer and Wolke, 1984, de Andrade *et al.,* 2007). But, in the present study, the WBC values of the control individual as well as the those supplemented with vitamin E were significantly increased ($p<0.05$) (Table 3). The other some studies suggest that high vitamin E doses (1000 mg/kg) result in undesirable immunological effects, as a immunoglobulin reduction, or from the leucocyte phagocytosis (Kiron *et al.,* 2004, Puangkaew *et al.,* 2004) and leucocyte number (Puangkaew *et al.,* 2004). Fish in the high-density production systems are exposed constantly to high bacterial loads in the water and less than optimal water quality, factors which may influence WBC counts.

According to the results in this study, to add vitamin E level (18.2 mg/kg) in the control diet sufficiently for *O. niloticus*. But, the supplemented of vitamin E in control diet for tilapia fish the percent weight gain and feed conversion ratio were not affected by dietary vitamin E concentrations. However, the results confirm that vitamin E alter Tilapia physiological profile and will probably protect fish under stress and disease. Based on the hematological response obtained, recommended high vitamin E suplemented for tilapia.

## REFERENCES

Adham, K. G., H. O. Hashem, M. B. Abu-Shabana, and A. H. Kame (2000). Vitamin C deficiency in the catfish (*Clarias gariepinus*). Aquacult Nut. 6: 129–139.

Bai, S. C., and D. M. Gatlin (1993). Dietary vitamin E concentration and duration of feeding affect tissue alpha-tocopherol concentrations of channel catfish (*Ictalurus punctatus*). Aquaculture. 113: 129–135.

Bai, S. C., and K. J. Lee (1998). Different levels of dietary DL-alpha-tocopheryl acetate affect the vitamin E status of juvenile Korean rockfish, *Sebastes schlegeli*. Aquaculture. 161: 405–414.

Baker, R. T. M., and S. J. Davies (1996). Oxidative nutritional stree associated with feeding rancid oils to African cat fish, *Clarias gariepinus* (Burchell) and the protective role of a-tocopherol. Aquac Res. 27: 795–803.

Bittencourt N. L. D., L. M. Molinari, Scoaris D. de O., R. B. Pedroso, C. V. Nakamura, T. Ueda-Nakamura, B. A. de A. Filho, and B.P. D. Filho (2003). Haematological and biochemical values for Nile tilapia *Oreochromis niloticus* cultured in semi-intensive system, Acta Scintiarum. Biological Sci. Maringá. 25: 385-389.

Blazer, V. S., and R. E. Wolke (1984). The effects of alpha-tocopherol on the immune responses and non-specific resistance factors of rainbow trout (*Salmo gairdneri* Richardson). Aquaculture. 37: 1–9.

Jain, N. C (1993).Essentials of Veterinary Hematology, Lea and Febiger, Philadelphia. 417 p..

Chagas, E. C., and A. L. Val (2003). Efeito da vitamina C no ganho de peso e em parâmetros hematológicos de tambaqui. Pesquisa Agropecuária Brasileira. 38: 397–402.

Cowey, C. B., E. Degener, A. G. Tacon, A. Youngson, and J. G. Bell (1984). The effect of vitamin E and oxidized fish oil on the nutrition of rainbow trout (*Salmo gairdneri*) growth at natural, arying water temperatures. Brit J Nut. 51: 443–451.

de Andrade, J. I., E. A. Ono, G. C. de Menezes, E. M. Brasil, R. Roubach , E. C Urbinati, M. Tavares-Dias, J. L Marcon , E. G. Affonso (2007). Influence of diets supplemented with vitamins C and E on pirarucu (Arapaima gigas) blood parameters. Comp Biochem Physiol A Mol Integr Physiol. 146 (4): 576-580

Forster I, D. A. Higgs, G. R. Bell, B. S. Dosanjh, and B. E. March (1988). Effect of diets containing herring oil oxidized to different degrees on growth and immunocompetence of juvenile coho salmon (*Oncorhynchus kisutch*). Can J Fish Aquat Sci. 45: 2187–2194.

Hamre, K., R. Waagbo, R. K. Berg, and O. Lie (1997). Vitamins C and E interact in juvenile Atlantic salmon (*Salmo salar*, L.). Free Radical Biology and Medicine. 22: 137–149.

Hardie, L. J., T. C. Fletcher, and J. C. Secombes (1990). The effect of vitamin E on the immune responses of the Atlantic salmon (*Salmo salar* L.). Aquaculture. 87: 1–13.

Hussein, S. Y., M. A. El–Nasser, and S. M. Ahmed (1996). Comparative studies on the effects of the herbicide atrazine on freshwater fish *Oreochromis niloticus* and *Chrysichthyes auratus at Assiut*. Egypt. Bulletin of Environmental Contamination and Toxicology. 57: 503– 510.

Kiron V., J. Puangkaew, K. Ishizaka, S. Satoh, and T. Watanabe (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

Kocabas, A. M., and D. M. Gatlin (1999). Dietary vitamin E requirement of hybrid striped bass (*Morone chrysops* female *M. saxatili* male). Aquacult Nut. 5: 3–7.

Ortuno, J., A. Cuesta, M. A Esteban, J. Meseguer (2001). Effect of oral administration of high vitamin C and E dosages on the gilthead seabream (*Sparus aurata* L.) innate immune system. Vet Immun Immunopathol. 79: 167–180.

Pearce, J., J. E. Harris, and S. J. Davies (2003). The effect of vitamin E on the serum complement activity of the rainbow trout, *Oncorhynchus mykiss* (Walbaum). Aquacul Nut. 9: 337–340.

Poston, H. A., and D. L. Livingston (1969). Effects of massive doses dietary vitamin E on fingerling brook trout. Fish Res Bull. 33: 9-12.

Puangkaew, J., V. Kiron, T. Somamoto, N. Okamoto, S. Satoh, T. Takeuchi, and T. Watanabe (2004) Non-specific immune response of rainbow trout (*Onchorhynchus mykiss*, Walbaum) in relation to different status of vitamin E and highly unsaturated fatty acids. Fish Shellfish Immun 16: 25–39

Sahoo, P. K., and S. C. Mukherjee (2002). Influence of fish dietary α-tocopherol intakes on specific immune response, non-specific immune resistance factors and disease resistance of healthy and aflatoxin B1-induced immunocompromised Indian major carp, *Labeo rohita* (Hamilton). Aquacul Nut. 8 159–167.

Shiau, S. Y., and C. Y. Hsu (2002). Vitamin E sparing effect by dietary vitamin C in juvenile hybrid tilapia, *Oreochromis niloticus×O. aureus*. Aquaculture. 210: 335–342.

Stephan, G., J. L. Messager, L. Lamour and F. B. Laurencin (1993). Interactions between dietary alpha-tocopherol and oxidized oil on sea bass *Dicentrarchus labrax*. In: (INRA, ed.) Fish Nutrition in Practice (Les Colloques, No. 61), pp. 215–218

Tavares-Dias, M., and C. D. Faustino (1998). Hematological parameters of Nile tilapia *Oreochromis niloticus* (Cichlidae) in extensive culture. Ars Vet. 14: 254–263.

Thorarinsson, R., M. L. Landolt, D. G. Elliot, R. J. Pascho, and R. W. Hardy (1994). Effect of dietary vitamin E and selenium on growth, survival and the prevalence of *Renibacterium salmoninarum* infection in chinook salmon (*Oncorhynchus tshawytscha*). Aquaculture. 121: 343–358.

Wahli T, V. Verlhac, J. Gabaudan, W. Schüep, and W. Meier (1998). Influence of combined vitamins C and E on non-specific immunity and disease resistance of rainbow trout, *Oncorhynchus mykiss* (Walbaum). J Fish Dis. 21: 127–137.