Kafkas Univ Vet Fak Derg
16 (Suppl-B): S219-S222, 2010
DOI:10.9775/kvfd.2009.1364

RESEARCH ARTICLE

The Effect of Vitamin E on Black Sea Trout *(Salmo labrax)* Broodstock Performance

Ramazan SEREZLİ * Süleyman AKHAN * Fatma DELİHASAN SONAY *

* Rize University Faculty of Fisheries, TR-53100, Rize - TURKEY

Makale Kodu (Article Code): KVFD-2009-1364

Summary

This study was conducted to examine the effects of feeding diets, different dose of vitamin E contains, on female and male Black Sea trout's *(Salmo labrax)* reproductive performance. Broodfish were fed with dry feed contained 100 (control), 250, 500, 1000 mg kg^{-1} vitamin E. Milt volume, spermatozoid number, fecundity and egg size were determined. Milt volume and sperm number were positively affected at a dose of 250 and 500 mg kg^{-1} vitamin E in the diet. A similar positive trend was also observed at the dose of 1000 mg kg^{-1}. Egg size is not affected by dietary vitamin E dose, but there are positive relationship between total fecundity and dietary vitamin E dose especially at the first stripping (P<0.05).

Keywords: *Milt, α-tocopherol, Broodstock, Fecundity, Vitamin*

E Vitamininin Karadeniz Alabalığı *(Salmo labrax)* Damızlık Stoklarında Üreme Performansına Etkisi

Özet

Bu çalışma Karadeniz Alabalığı'nda *(Salmo labrax)* 6 hafta süreyle, yemlerine farklı dozlarda ilave edilen E vitamini ile beslemenin üremeye etkisini ortaya koymak amacıyla yapılmıştır. Anaç stoktaki balıklar 1 kg yemde 100 mg (kontrol grubu), 250 mg, 500 mg ve 1000 mg olacak şekilde E vitamini ilave edilen yemle beslenmişlerdir. Sperm hacmi, spermatazoa sayısı, fekondite ve yumurta çapı verileri incelenmiştir. Sperm hacmi ve spermatozoa sayısı 250 ve 500 mg E vitamin içeren yemlerle beslenen balıklarda pozitif yönde etkilenmiştir. Benzer ilişki 1000 mg kg^{-1} dozda da görülmüştür. Yumurta büyüklüğü yemdeki E vitamininden etkilenmezken, özellikle ilk sağımda total fekondite ve vitamin E miktarı arasında pozitif ilişki vardır (P<0.05).

Anahtar sözcükler: *Sperm, α-tokoferol, Anaç stok, Fekondite, Vitamin*

INTRODUCTION

Vitamin E is lipid soluble antioxidant, and it is also important for fish reproduction. The requirement for vitamin E as an essential dietary component in fish has long been recognized and minimum requirements for any fish species have already been established. The recommended dietary allowance for vitamin E is 15 mg day^{-1} with an adult upper limit of 1000 mg/day [1]. On the other hand, the Committee on Animal Nutrition of American National Research Council [2] suggested a general vitamin E supplement in the diet around 50 mg kg^{-1} fish. High concentrations of vitamin E content can result in yolk sac hypertrophy and decreased survival caused by oxidative stress. Unlike vitamin A and D, vitamin E is essentially nontoxic [3] and it was first shown to be involved in prevention of sterility and fetal resorption in rats. It may likewise be involved in embryo membrane permeability and hatchability of fish eggs [4]. There are no significant apparent benefits by using higher levels of vitamin E on growth and diet utilization [5].

Vitamins are organic substances that are necessary for health, growth, maintenance and also spermatogenesis and oogenesis in animals. Especially vitamin E is essential for fertility and reproduction in fish and fish

İletişim (Correspondence)

+90 464 2233385/1424 GSM: +90 535 7985077
rserezli@gmail.com

cannot synthesize vitamin E, so the maternal dietary content of each prior to oogenesis is an important determinant of reproductive fitness [2].

Broodstock diet has been considered as one of the factors affecting fecundity, egg, larval and sperm quality in fish [6,7]. The concentration of sperm in the seminal fluid has been traditionally used for the assessment of sperm quality in fish [8]. Low concentration of anti-oxidants such as vitamin E and vitamin C reduce sperm quality, the rate of egg fertility and affects gamete and fry quality [9]. During the last phase of gonad development, vitamin E is transferred from the muscular tissue to the reproductive apparatus. This process happens during physiological fasting before the final sexual maturation and for this reason and the amount of vitamin E accumulated in the previous months is very important. In some experiments on Salmonid diets supplemented with 60 or 270 mg of vitamin E demonstrated that high levels of fatty acids (almost 60 g kg^{-1}) coupled with high concentration of vitamin E had the best reproductive performances in terms of fry survival rate. Furthermore, the trials showed that the requirement of this vitamin is also related to the level of fatty acids in the diet [10]. According to Wen [1], the recommended dietary allowance for vitamin E is 15 mg day^{-1} with an adult upper limit of 1000 mg/day.

There is no data available regarding the effects of Vitamin E on semen and egg quality in Black Sea trout *(Salmo labrax)*. The aim of the study affects of six weeks (42 days) treatment of broodfish with vitamin E added feed on sperm and egg quality in Black Sea trout.

MATERIAL and METHODS

Fish

Two hundred and fifty, four years old, Black Sea Trout *(Salmo labrax)*, born and farmed in the experimental commercial farm (in Findikli, Rize, Turkey) were used in the experiment. The fish were randomly divided into four groups. Each group consisted of twenty female (687.5±43.6 g 35.6±0.59 cm) and ten male (555±21.5 g 34.3±0.35 cm) broodfish, and stocked in 8 x 2000 L ponds. Held under a constant natural water flow (8±2°C; 0.5 l sec^{-1}; total hardness 20 mg l^{-1}) and photoperiod (8 h light and 16 h dark) were maintained.

Commercial trout feed was prepared as mentioned from Canyurt and Akhan [11]. Feed was weighed and vitamin E (purity: 50% D-α-tocopherol acetate; BASF Fine Chemicals, Ludwigshafen, Germany) was added and thoroughly mixed at ratios of 150 mg, 400 mg and 900 mg kg $feed^{-1}$ mixed. Sunflower oil was then sprayed on those feeds in order to facilitate absorption of fat soluble vitamin E. Fish were fed *ad libitum* with 250 mg (E250), 500 mg (E500), 1000 mg (E1000) vitamin-E supplemented feeds and no vitamin-added groups (Named as E100 as a control) commercial pellets (6 mm) two times a day for six weeks (from 26 October 2008 - 4 December 2008; 42 days).

Milt and Egg Sampling

After 6 weeks vitamin treatment, a first batch of fish ripened (first spawn) and the others ten days later (second spawn). Milt was taken from all males first and second spawning without anesthetic manipulation, but females were anesthesia with 40 mg l^{-1} benzocaine. The stripped milt was kept in dry glass vials. Freshly stripped milt was stored on ice in 15 ml glass containers until used. Any samples that were obviously contaminated (e.g. urine, faces) were discarded. Eggs taken from females were fertilized with milt from males of the same group. Eggs data were examined after water hardening.

Sperm concentration was determined using a Thoma hemocytometer under the microscope (40x=400 magnification). Immediately after collection of semen, the volume was determined using a graded level collection tube. The mean of the three counts was calculated for each of the two dilutions, and then the mean of these two values was used to calculate the actual sperm density. The mean number of eggs per spawning was evaluated.

Statistic Analysis

All data were subjected to one-way analysis of variance [12]. The significance of differences between groups were determined using Duncan's multiple range test (P<0.05) using SPSS for WINDOWS (Version 13.0). Values are expressed as means ±SE.

RESULTS

Mean body weights (initial and final) of the broodfish showed no significant differences (P>0.05) among groups (E100, E250, E500, E1000); initial (pre-spawning) weights of female: 683±40.1 g and male: 548±15.6 g were determined. Post stripped weight of female was determined as 587±36.7 g. The dietary treatments did not influence the final body weight among the broodfish groups statistically.

Milt Volume and Sperm Number

It is observed that high vitamin E level increased milt volume. In the second milt striping, milt volumes were diminishing. As milt volume of control group (E100) was not different from first and second stripping, treatment groups' milt volumes were higher than control. Sperm concentration was high at the first stripping but very diminish was observed second stripping *(Fig. 1, Fig. 2)*.

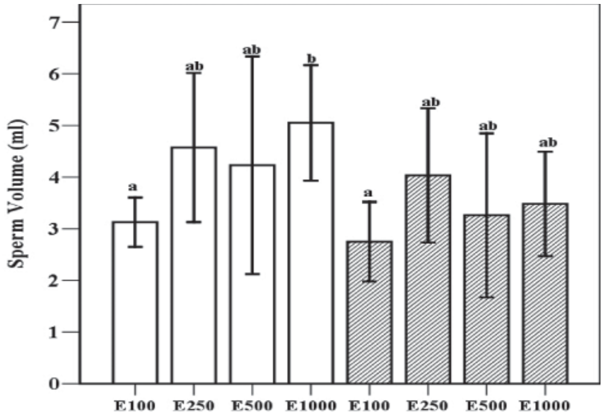

Fig 1. Sperm volume first and second stripping (mean ml per group, n=10 per group). Means with different superscript differs (P<0.05). Error bars: ±SE. Grey bars represent second spawning

Şekil 1. Birinci ve ikinci sağımdaki sperm hacmi (ml, her grup için n=10). Farklı gruplar farklı harflendirilmiştir (P<0.05). Hata çubukları: ±SE. Gri sütun ikinci yumurtlamayı göstermektedir

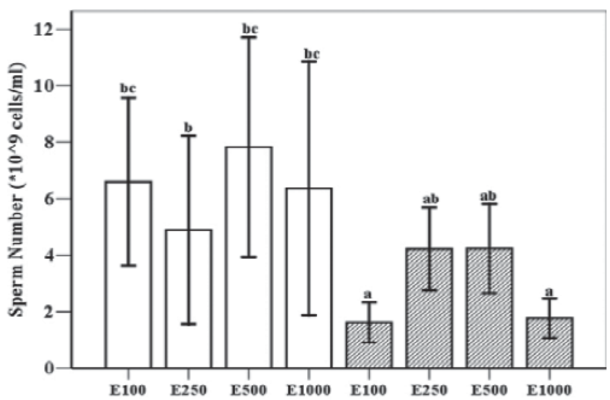

Fig 2. Sperm concentration (number ^109 ml-1) in groups (n=10 per group). Means with different superscript differs (P<0.05). Error bars: ±SE. Grey bars represent second spawning

Şekil 2. Sperm yoğunluğu ortalaması (adet*109 ml-1, n=10). Farklı gruplar farklı harflendirilmiştir (P<0.05). Hata çubukları: ±SE. Gri sütun ikinci yumurtlamayı göstermektedir

Fecundity

No abnormalities were observed between the groups in egg development. The total and relative fecundity per kg body weight was determined. And egg number was determined gravimetrically *(Fig. 3 and Fig. 4)*.

Each group (E100, E250, E500, E1000) first and second striping total fecundities were observed as 943±69, 1165±140, 1227±154, 929±104; 1036±138, 1002±104, 951±68, 1115±193 respectively. Relative fecundities were determined as 1909±212, 2110±232, 1657±198,

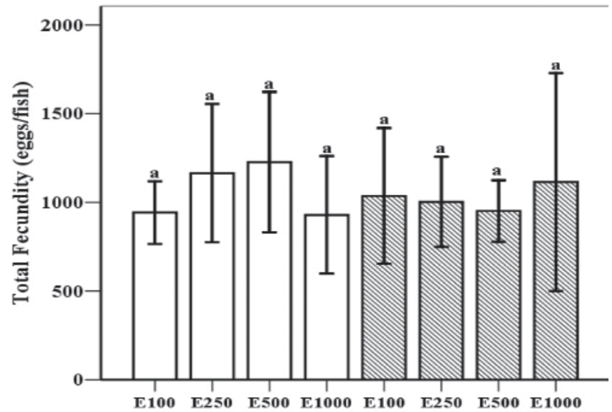

Fig 3. Total Fecundity first (Week 1) and second (Week 2) stripping (eggs per female) in groups. Means with different superscript differs (P<0.05). Error bars: ±SE. Grey bars represent second spawning

Şekil 3. İlk (birinci hafta) ve ikinci sağımda toplam fekondite (dişi başına den yumurta sayısı). Hata çubukları: ±SE. Farklı gruplar farklı harflendirilmiştir (P<0.05). Hata çubukları: ±SE. Gri sütun ikinci yumurtlamayı göstermektedir

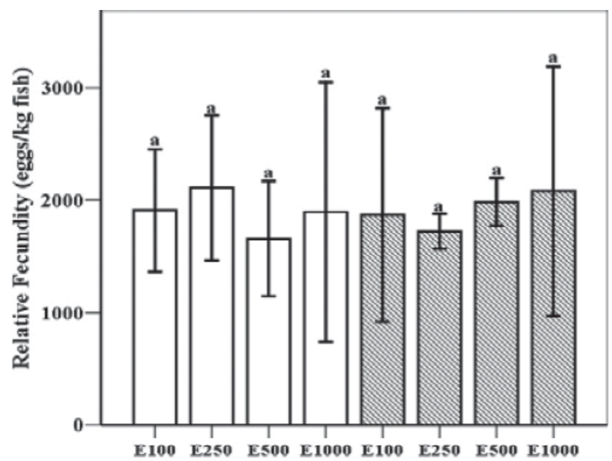

Fig 4. Relative Fecundity (eggs per kg post stripped female weight) between groups. Means with different superscript differs (P<0.05). Error bars: ±SE. Grey bars represent second spawning

Şekil 4. Relatif fekondite (adet yumurta/sağım sonrası balık ağırlığı). Farklı gruplar farklı harflendirilmiştir (P<0.05). Hata çubukları: ±SE. Gri sütun ikinci yumurtlamayı göstermektedir

1893±362 and 1867±341, 1723±63, 1983±82, 2081±349 egg kg^{-1} broodfish respectively

Egg Size

Egg size was determined as egg diameter which was determined 30 eggs length in a Von Bayer trough. There were no differences of the egg size among the groups and egg sizes in the first spawning are presented in *Fig. 5*. First and second striping time, egg size was observed as 5.4±0.1, 5.3±0.16, 5.3±0.17, 5.1±0.05; 5.2±0.07, 5.3±0.03, 5.2±0.15.2±0.09 respectively.

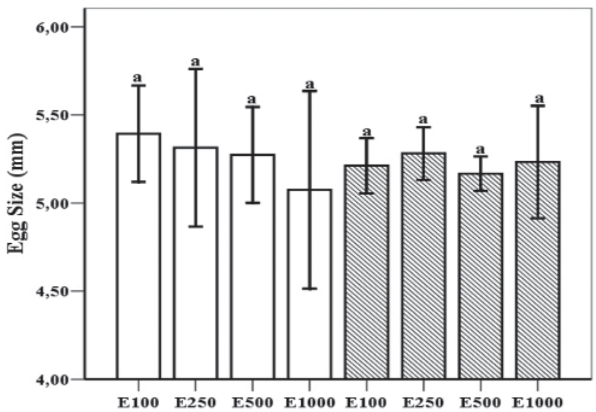

Fig 5. Egg size (mm as diameter) in groups (n=10 per group). Means with different superscript differs (P<0.05). Error bars: ±SE. Grey bars represent second spawning

Şekil 5. Grupların yumurta büyüklüğü (mm olarak çap). Farklı gruplar farklı harflendirilmiştir (P<0.05). Hata çubukları: ±SE. Gri sütun ikinci yumurtlamayı göstermektedir

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. For instance, salmonid fish require for normal growth 30-60 mg of vitamin E per kg of diet [1,2]. But this dose adequate or not for broodstock nutrition is dispute. Commercial feed producers maintained that there is 100-200 mg kg^{-1} vitamin E in their feed in Turkey. But vitamins must be added in the diet freshly. So, the vitamin dosage in the diet diminished slowly dependent on stock conditions.

There was no significant benefit of using the vitamin E in a diet about weight gain. This result confirmed the findings of Boggio et al.[13] and Kiron et al.[5], that no difference occurred in the weight gain of fish fed diets containing either lower (100 mg kg^{-1}) or higher level of vitamin E (1000 mg kg^{-1} diet). Naziroglu et al.[14] mentioned that vitamin E especially alpha 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.

Relative and total fecundity and egg size were not affected vitamin E content either positively nor negatively. There were no differences between groups (P<0.05). Our finding was similar to Tabak et al.[15], which found that relative fecundity as 1747±70 (1162-2494) and egg size 5.48±1.1 in Black Sea trout.

Groups E250, E500 and E1000 sperm volumes and sperm numbers differ from E100 statistically (P<0.05), especially 250 mg kg^{-1} (E250) and 500 mg kg^{-1} (E500) vitamin E affected positively. Total amount of spermatozoa and the morphology of the sperm were significantly lowered in cocks with increasing amounts of supplemented vitamin E. As mentioned Canyurt and Akhan [11], our research results did not show any negative effect of vitamin E on male rainbow trout sperm volume. These results showed that dietary vitamin E is useful for improving the sperm quality of male rainbow trout.

In conclusion, our research results did not show any negative effect of additional vitamin E in the diet on neither male nor female sea trout. Supplemented dietary vitamin E up to 250 mg kg^{-1} can be useful for improving the sperm and egg quality of Black Sea trout.

REFERENCES

1. Wen CJ: The role of vitamin E in the treatment of male infertility. *Nutrition Bytes,* 11, 1, 2006.

2. NRC (Committee on Animal Nutrition): Nutrient Requirement of Fish. National Academy Press, Washington, DC, USA, 1993.

3. Ball GFM: Vitamins in Foods, Vitamins in Foods: Analysis, Bioavailability, and Stability. CRC Press, Boca Raton, Florida, 2006.

4. Halver JE: The Vitamins. **In,** Halver JE, Hardy RW (Eds): Fish Nutrition. 3rd ed. pp. 61-141. Academic Press, San Diego CA, USA, 2002.

5. Kiron V , Puangkaew J, Ishizaka K, Satoh S, Watanabe T: 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, 2004.

6. Luquet P, Watanabe T: Interaction "nutrition-reproduction" in fish. *Fish Physiol Biochem,* 2, 121-129, 1986.

7. Brooks S, Tyler CR, Sumpter JP: Egg quality in fish: What makes a good egg? *Rev Fish Biol Fisher,* 7, 387-416, 1997.

8. Rurangwa E, Kime DE, Ollevier F, Nash JP: The measurement of sperm motility and factors affecting sperm quality in cultured fish. *Aquaculture,* 234, 1-28, 2004.

9. Canyurt MA, Akhan S: Effect of ascorbic acid supplementation on sperm quality of Rainbow Trout *(Onchorynchus mykiss). Turk J Fish Aquat Sci,* 8, 171-175, 2008.

10. Rainis S, Gasco L, Ballestrazzi R, Zoccarato I: Effects of vitamin E and phosphatidylcholine on qualitative and quantitative parameters of rainbow trout *(Oncorhynchus mykiss)* milt. *Ital J Anim Sci,* 6, 339-350, 2007.

11. Canyurt MA, Akhan S: Effect of dietary vitamin E on the sperm quality of rainbow trout *(Onchorynchus mykiss). Aquac Res,* 39, 1014-1018, 2008.

12. Ergün G, Aktas S: Comparisons of Sum of Squares Methods in ANOVA Models. *Kafkas Univ Vet Fak Derg,* 15 (3): 481-484, 2009.

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

14. Naziroglu M, Ispir U, Yonar ME: Effects of vitamin E on immune response in fish. *Kafkas Univ Vet Fak Derg,* 9 (1): 101-106, 2003.

15. Tabak I, Aksungur M, Zengin M, Yılmaz C, Aksungur N, Alkan A, Zengin B, Mısır S: The bio-ecological characteristics of the Black Sea Trout (*Salmo trutta labrax,* PALLAS, 1811) and its culture possibility. Final Project Report, (TAGEM/ HAYSUD /98/12/01/007), Central Fisheries Research Institute, Trabzon, 2002, (In Turkish).