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High levels of dietary vitamin E improve the reproductive performance of female *Oreochromis niloticus*

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ABSTRACT. This study aimed to evaluate the effect of vitamin E on reproductive responses of female tilapia (*Oreochromis niloticus*). To this, isonitrogenous and isocaloric diets with different levels of vitamin E supplementation (200, 300, 400, and 500 mg kg^{-1}) were provided to groups of females for 90 days. Supplementation positively influenced the volume of eggs produced, spawning rate, fecundity, hatching rate, average production of larvae, reproductive frequency and survival. The fertilization rate, weight, and diameter of the eggs were not affected by supplementation. This study showed that 400 mg kg^{-1} vitamin E in the diet during the reproductive period of female Nile tilapia are sufficient to ensure the best reproductive performance, providing efficient production of a larger number of larvae in the individuals of this species.

Keywords: α-tocopherol, broodstock nutrition, nutritional requirements, Nile tilapia.

Altos níveis de vitamina E na dieta melhoram o desempenho reprodutivo de fêmeas de *Oreochromis niloticus*

RESUMO. Este estudo teve como objetivo avaliar o efeito da vitamina E sobre as respostas reprodutivas de fêmea de tilápia do Nilo (*Oreochromis niloticus*). Para isso, foram fornecidas para grupos de fêmeas dietas isoproteicas e isoenergéticas com diferentes níveis de suplementação de vitamina E (200, 300, 400 e 500 mg kg^{-1}) durante 90 dias. A suplementação influenciou positivamente o volume de ovos produzidos, a taxa de desova, fecundidade, taxa de eclosão, a sobrevivência, a frequência reprodutiva e produção média de larvas. A taxa de fertilização, peso e diâmetro dos ovos não foi afetada pela suplementação. Este estudo mostrou que 400 mg kg^{-1} de vitamina E no período reprodutivo de fêmeas de tilápia são suficientes para assegurar o melhor desempenho reprodutivo, proporcionando a produção eficiente de um grande número de larvas em indivíduos dessa espécie.

Palavras-chave: α-tocoferol, nutrição de reprodutores, necessidades nutricionais, tilápia do Nilo.

Introduction

Knowledge of the nutritional requirements of animals enables to develop rations that maximize the productive performance and health of fish. However, there is no information allowing the balancing of specific diets for breeding, which have significant health and reproductive events, as well as for the fingerlings produced (BARROS et al., 2002). Improving nutrition and feeding of breeding fish not only directly affects the production of eggs and sperm quality, but also the production of larvae. The gonadal development and fecundity are affected by certain essential nutrients, especially in species with continuous spawning with short periods of vitellogenesis (IZQUIERDO et al., 2001).

The group of vitamin E consists of a mixture of vitamins in which the most important is the α-tocopherol that exists in nature accompanied by other compounds, β-, γ- and δ-tocopherols, and whose function is to inhibit or retard oxidation of animal tissue, especially unsaturated fatty acids and vitamin A. The lack or deficiency of this vitamin in animals can cause, depending on the species, muscular dystrophy, abnormalities in the vascular system and sterility (BOBBIO; BOBBIO, 1995). In fish, vitamin E is used for diet supplementation with the purpose of improving growth, resistance to stress and immune system. It is efficient in the conservation of fish during processing and storage, inhibiting the degradation of lipids by oxidation (WEBSTER; LIM, 2001) and also plays an important role in fish reproductive physiology, especially fertility and growth of crustaceans (CONKLIN, 2000). It is responsible for maintaining the permeability of capillaries and cardiac muscles, and

also acts on cellular respiration and in the biosynthesis of DNA (PEZZATO et al., 2004).

Few studies have been able to demonstrate the improvement of quality of larvae by improving the nutrition of breeding. It is known that the nutritional status of the embryo fish, necessary for the proper development of the animals, depends on the transfer of nutrients to the reproductive gametes during vitellogenesis, influencing the quality both in females as in males (DABROWSKI; BLOM, 1994).

Studies show that the requirement of vitamin E is directly related to the level of lipids in the diet. According to Satoh et al. (1987) levels of vitamin E for tilapia is 50-100 mg kg^{-1} for diets containing 5% lipid and up to 500 mg kg^{-1} in diets containing 10% lipid. Results challenged by Shiau and Shiau (2001) indicate 42-44 mg kg^{-1} for diets containing 5% lipid and 60-66 mg kg^{-1} for diets containing 12% lipid. But these studies evaluate the performance of tocopherol in relation to growth and not about the importance of this nutrient on the reproductive performance of this species. The aim of this study was to evaluate the inclusion of high levels of vitamin E as a nutrient in the diet on the reproductive responses of female Nile tilapia (*Oreochromis niloticus*).

Material and methods

Animal welfare statement

This research agrees with the Ethical Principles in Animal Research adopted by the National Council for the Control of Animal Experimentation - Brazil and was approved by the Ethical Committee for Animal Research from UNESP.

Animals and experimental conditions

The current experiment was carried out at the Laboratory of Tilapiculture, Aquaculture Center (UNESP, Jaboticabal, São Paulo State, Brazil) (21°15'S 48°18'W). One-hundred and four adult Nile tilapia, GIFT strain, were randomly selected, measured, marked with microchips and distributed in 7,000 L four circular flexible PVC laminate tanks connected to the water recirculation system with mechanical and biological filter, continuous aeration and 12h light 12h $dark^{-1}$ photoperiod. A ratio of females ($n = 19$, about 1 year old, average weight 659 ± 121g and total length of 35.90 ± 1.71 cm) to males ($n = 7$, about 1 year old, mean weight of 1004 ± 71.60 g and total length of 40.65 ± 0.96 cm) of 2.7:1 was maintained in each group. The acclimation period was approximately 30 days and 90 days composed the experimental period. The parameters of water quality assessed twice daily were dissolved oxygen (5.60 ± 0.97 mg L^{-1}) and temperature (27.0 ± 2.6°C), and weekly evaluated were pH (7.80 ± 0.27), ammonia (208.0 ± 30.1 μ L^{-1}) and nitrite (81.8 ± 20.2 μ L^{-1}).

Pilot experiment

The pilot experiment was conducted with the objective of establishing the minimum level of vitamin E in the diet sufficient to induce an improvement in reproductive performance of study animals, which were kept under the same experimental conditions of the main study for a period of 90 days. The composition of the basal diets were the same except for the different levels of vitamin E (E-50 adsorbate Rovimix, Roche®) per kilogram of diet, providing 0 (control diet), 50, 100 or 200 mg kg^{-1}. The best results were presented by the animals fed the diet containing 200 mg kg^{-1} (initial level of vitamin E included in the main experiment). The animals fed the control diet had low reproductive rates and high percentage of larvae with deformities and mortality, cardiac edema and hemorrhage.

Experimental diets

The experimental diets were cold processed into 6 mm pellets in the laboratory of Aquatic Organisms Nutrition of the Aquaculture Center (UNESP, Jaboticabal, São Paulo State, Brazil) and air dried for 24 hours. The composition of basal diets was the same except for the different levels of vitamin E per kilogram of diet, providing 200, 300, 400 or 500 mg kg^{-1}. Levels of dietary vitamin E were set in relation to levels reported in a variety of fish species taking in consideration the level of dietary lipid (SATOH et al., 1987). Diets were formulated to contain levels of 320 g kg^{-1} of digestible protein, a 13020 kj kg^{-1} digestible energy and 100 g kg^{-1} of fat in dry matter (Table 1). All diets were stored at -20°C. The fish were fed the equivalent of 2.5% body weight divided in four daily meals (800, 1100, 1400 and 1700).

Experimental protocol and procedures

During the experimental period, at four day-intervals, all females were monitored and subjected to oral examination to detect the occurrence of spawning. Eggs were removed and females were identified by a microchip reader, anesthetized in 0.5 g L^{-1} saturated benzocaine and measured. Unviable eggs were discarded and a sample was identified and taken to Ultrafreezer (-80°C) for further analysis. Then eggs were placed individually in incubator connected to a recirculating water system with a mechanical filter under controlled temperature (28.0 ± 2.0°C). At the end of the experiment, all animals were measured and the gonads, liver and viscera were collected from five fish per treatment for reproductive rate analysis.

Table 1. Composition of the experimental diet.

Ingredients	Composition (g kg^{-1} diet)			
Poultry by-product meal	100			
Soybean meal	350			
Wheat meal	100			
Rice bran	100			
Fish meal	140			
Yellow corn	150			
Soybean oil	30			
NaCl	5			
Dicalcium phosphate	15			
Vitamin and mineral premix (vitamin E free)[1]	10			
Total	1000			
Approximate composition				
Digestible energy [2] (kj kg^{-1})	13020			
Crude protein (g kg^{-1})	360			
Digestible Protein [2] (g kg^{-1})	320			
Lipid (g kg^{-1})	100			
Vitamin E (g kg^{-1} diet)	200 (201.40)	300 (301.04)	400 (401.56)	500 (501.02)

[1]Mineral and vitamin mix (% unless otherwise noted IU. which is given per kg): antioxidant. 0.06; vitamin C.35 000; vitamin A. 1000 000 IU; vitamin D3. 500 000 IU; vitamin K3. 0.05; thiamine. 0.125; riboflavin. 0.25; pyridoxine. 0.25; pantothenic acid. 0.5; niacin. 0.5; biotin. 0.0125; folic acid. 0.025; vitamin B12. 0.375; ascorbic acid. 2.8; Co. 0.0025; Cu. 0.2; I. 0.01; Fe. 1.382; Mn. 0.375; Zn. 0.015; Se. 0.0075; 2Calculated values of digestible energy and protein presented in the NRC (1993).

Analysis

The productive parameters evaluated were weight gain, feed efficiency, specific growth rate, absolute growth rate and survival. Reproductive parameters were evaluated by the methods used by Coward and Bromage (1999a and 1999b) and Godinho (2007). Protein and lipid of the diets were analyzed by the Kjeldahl method and ether extraction method, respectively. A sample of 0.5 g was used to determine the energy content using a calorimeter bomb. The morphometric analysis was performed by spawning in a total of 70 samples of eggs and larvae of each treatment using a Leica MZ8 stereomicroscope connected to the instant messaging program LEICA 50. The concentration of vitamin E was determined by high performance liquid chromatography (HPLC) on samples of the diet and eggs.

Statistical analysis

Females were assigned to four treatments and 19 repetitions (corresponding to the number of females marked with microchip in each treatment) in a completely randomized design. The data was analyzed using analysis of variance (ANOVA) using the GLM procedure for the effects of vitamin E levels in the SAS 9.1 software. Polynomial linear, quadratic and cubic contrasts were used to evaluate the effect of different levels of vitamin E. Statistical significance was assumed at $p < 0.05$.

Results and discussion

Production parameters

The productive performance of females fed different levels of vitamin E is listed in Table 2.

Survival was 100% in all groups tested, without significant difference. Animals fed diets containing 400 mg kg^{-1} vitamin E showed lower productivity results ($p < 0.05$) compared with other treatments. This result may be related to feed intake that was nearly 50% lower in this treatment compared to the others. That is, the higher reproductive frequency, the longer the incubation period, and consequently the longer the period of food restriction. However, the treatment with 500 mg kg^{-1} vitamin E showed a higher reproductive frequency, but without affecting the productive performance. Therefore, the results presented by treatment with 400 mg kg^{-1} vitamin E suggest that the reproductive performance of tilapia is not influenced by the decrease in feed intake.

Corroborating these results, Miranova (1977) observed that egg production of *Oreochromis mossambicus* was higher under 25-50% food restriction than with abundant food, despite the fact that the abundant feeding resulted in better growth of females. In another study, a 50% reduction in the supply of food for breeding *Brycon siebenthalae*, during three months prior to the breeding season, demonstrated that feeding management, compared with fish fed 3% biomass daily, showed similarities in the rates of fertilization and hatching.

Some studies found no significant effect of the vitamin E on the performance of various species. Gatta et al. (2000) showed that the addition of vitamin E in the diet had no influence on the specific growth rate and feed conversion of European sea bass (*Dicentrarchus labrax*). Scaife et al. (2000) found similar results for Atlantic salmon (*Salmo salar*). Chen et al. (2004) observed no difference in the performance of golden shiner (*Notemigonus crysoleucas*) fed diets supplemented with vitamin E after 14 weeks.

Table 2. Means of the productive parameters of female Nile tilapia fed diets containing different levels of vitamin E (n = 19 per treatment).

Productive parameters	Vitamin E (mg kg^{-1})				Probability			CV
	200	300	400	500	L.EF.	Q.EF.	C.EF.	
Weight gain (g)	257	251	153	234	0.18^{NS}	0.03*	0.13^{NS}	54.27
Feed efficiency	0.27	0.26	0.15	0.23	0.16^{NS}	0.02*	0.10^{NS}	54.00
Feed intake (%)	40.5	38	21.4	37.1	0.13^{NS}	0.03*	0.18^{NS}	54.28
Survival (%)	100	100	100	100	-	-	-	

[1]Linear, quadratic and cubic polynomial contrasts were used to evaluate the effect of different levels of vitamin E; [2]L. EF.: linear effect; Q. EF.: quadratic effect; C. EF.: cubic effect; NS: Non-significant; ⋆: Significant at 5%; ⋆⋆: Significant at 1%.

Reproductive parameters

Number, weight and diameter of eggs

Data on the number, weight and diameter of the eggs are shown in Table 3.

The number of eggs of animals fed diets with 500 mg vitamin E increased 84% on average compared to eggs of animals fed diets with 200 mg. As a consequence, there was an increase in the spawning volume ($p < 0.05$) (Figure 1). This result may be related to the antioxidant effect of the vitamin, which promoted the protection of the cell membrane against lipid peroxidation, preserving the viability of oocytes. Studies have shown that inclusion of high levels of vitamins E and C in diets (899 and 957 mg kg^{-1}, respectively) increases the volume and weight of eggs of Malaysian prawn (*Macrobrachium rosenbergii*) (CAVALLI et al., 2003). Harlioğlu and Barim (2004) reported an increase in egg production of crayfish (*Astacus leptodactylus*) when added 100 mg kg^{-1} of vitamin E in the diet.

Table 3. Means of the reproductive parameters of female Nile tilapia fed diets containing different levels of vitamin E (*n = 19 per treatment*).

Reproductive parameters	Vitamin E (mg kg^{-1})				Probability			CV (%)
	200	300	400	500	[2]L.EF.	Q.EF.	C.EF.	
Spawning volume (mL)	6.64	10.79	10.57	11.08	0.01*	0.09^{NS}	0.27^{NS}	20.99
Egg weight (mg)	6.7	6.5	6.6	7.1	0.15^{NS}	0.06^{NS}	0.97^{NS}	11.15
Egg diameter (mm)	2.54	2.48	2.52	2.54	0.70^{NS}	0.08^{NS}	0.22^{NS}	3.49
Number of eggs	499	828	877	919	0.03*	0.29^{NS}	0.65^{NS}	47.10
Fertilization rate (%)	99.01	95.01	97.70	96.18	0.41^{NS}	0.42^{NS}	0.12^{NS}	6.26
Hatching rate (%)	45.97	72.83	74.30	40.20	0.65^{NS}	0.002**	0.76^{NS}	52.48
Tocopherol in eggs (mg kg^{-1})	11.94	17.91	23.88	29.85	0.03*	0.21^{NS}	0.10^{NS}	18.81
Spawning index (%)	0.64	0.90	0.90	1.04	0.01*	0.51^{NS}	0.33^{NS}	45.18
Gonadosomatic index (%)	4.05	4.07	4.17	3.96	0.86^{NS}	0.77^{NS}	0.48^{NS}	56.31
Hepatosomatic index (%)	2.76	2.36	1.94	1.92	0.01*	0.82^{NS}	0.56^{NS}	45.12
Visceral fat index (%)	5.52	4.73	3.87	3.84	0.01*	0.75^{NS}	0.12^{NS}	37.33
Relative fecundity	0.94	1.42	1.35	1.49	0.02*	0.26^{NS}	0.26^{NS}	24.03
Total fecundity	621	1069	945	1018	0.03*	0.08^{NS}	0.10^{NS}	7.32
Reproductive frequency (%)	5.3	9.6	11.7	12.7	0.01*	0.98^{NS}	0.56^{NS}	78.75
Larval survival (%)	52.5	69.82	89.87	52.51	0.60^{NS}	0.002**	0.11^{NS}	50.93
Number of larvae	120	421	586	194	0.41^{NS}	0.01*	0.39^{NS}	64.73

[1]Linear, quadratic and cubic polynomial contrasts were used to evaluate the effect of different levels of vitamin E; [2]L. EF.: linear effect; Q. EF.: quadratic effect; C. EF.: cubic effect; ^{NS}: No significant; *: Significant at 5%; **: Significant at 1%.

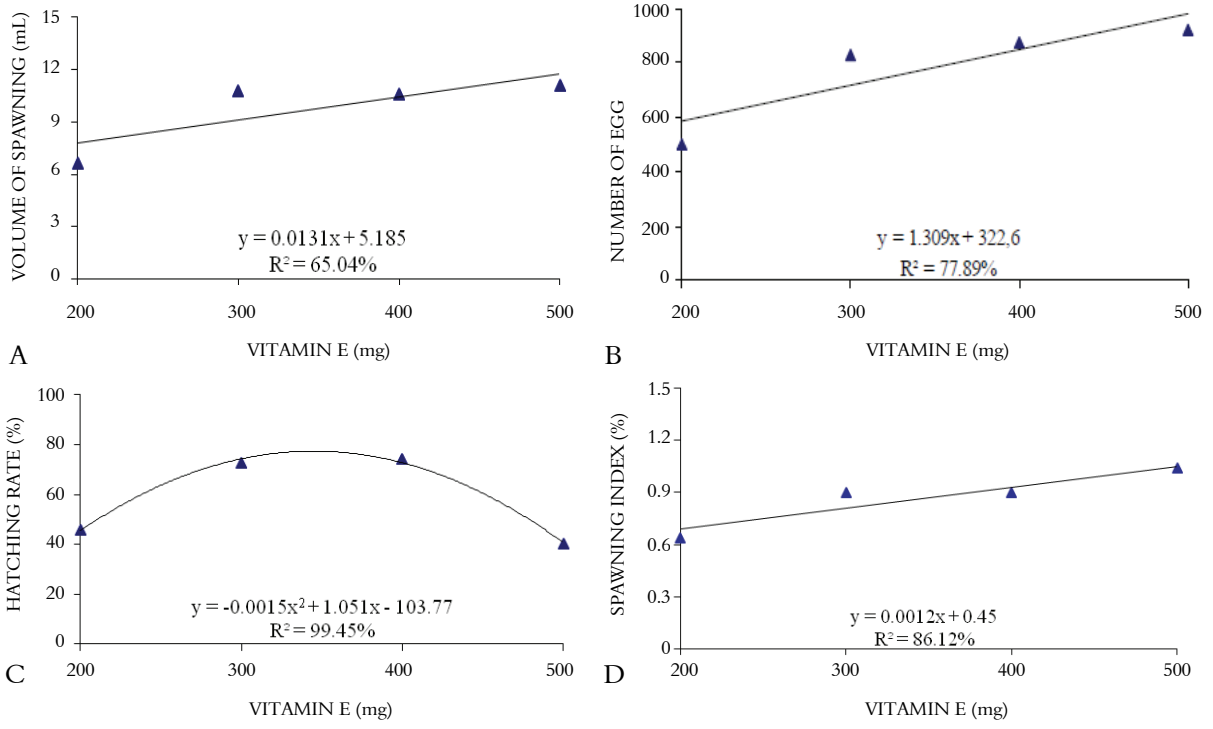

Figure 1. A-Spawning volume, B-number of eggs, C-hatching rate and D-spawning index of female Nile tilapia fed diets containing different levels of vitamin E.

The different levels of vitamin E had no significant effect on weight and diameter of eggs ($p > 0.05$). Differences in egg size is due to vitellogenesis duration, when occurs the isolation of vitellogenin (derived from the liver and responsible for the transport of α-tocopherol) which accumulates in the oocyte cytoplasm in the form of yolk granules, thus influencing the quality of larvae and not the size of eggs, which explains the lack of interference from supplementation on egg weight and diameter. Similar results were obtained by Emata et al. (2000) who found that the mean diameter of eggs was not affected by dietary supplementation of vitamins C and E in milkfish (*Chanos chanos*). However, in a study on the effect of vitamin E and growth hormone on gonad maturity of common carp (*Cyprinus carpio*), the dietary vitamin E led to an increase in diameter and number of eggs compared to control. Furthermore, the complete spawning occurred in fish fed a diet containing vitamin E, but only partial spawning was found in most fish fed diets without vitamin E (GUPTA et al., 1987).

Reproductive frequency, fertilization rate and hatching

Significant differences were detected in reproductive frequency among different levels of supplementation, and the results of reproductive frequencies were directly proportional to the levels of supplementation ($p < 0.05$; Table 3). Different from the results found in this study, Cavalli et al. (2003) found no significant effect on the reproductive frequency of Malaysian prawn (*Macrobrachium rosenbergii*) fed dietary supplementation of vitamins C and E. Likewise, the fertilization of eggs of Ross hens (HOSSAIN et al., 1998) and the fertilizing capacity of sperm in rainbow trout (*Oncorhynchus mykiss*) (CANYURT; AKHAN, 2008) were not affected. However, supplementation improved the fertilizing capacity of sperm of yellow perch (LEE; DABROWSKI, 2004) and fertilization rates of Japanese quail (*Coturnix japonica*) (BISWAS et al., 2006).

Herein, the inclusion level of 400 mg kg^{-1} of vitamin E increased the hatching rate by 62% ($p < 0.01$) compared to the lowest level of supplementation (200 mg), suggesting the role of vitamin E in the diet of Nile tilapia (Table 3, Figure 1). The cellular homeostasis, the key for the production of viable gametes and embryos, is only achieved when there is balance between pro-oxidants and neutralization by antioxidants, by preventing lipid peroxidation of membranes, mitochondrial changes, embryonic cell blocking, cell injury and apoptosis; assisting in the process of oocyte maturation, fertilization and embryonic development (AGARWAL et al., 2005, 2008), increasing hatching rates.

Results of this study were similar to those obtained elsewhere. Hatching rate in ayu (*Plecoglossus altivelis*) was lower given the lack of α-tocopherol in the diet (TAKEUCHI et al., 1981). Cahu et al. (1991) found a linear correlation between the egg hatching rate and concentration of α-tocopherol in the diet of Indian prawn (*Penaeus indicus*). Fernandez-Palacios et al. (1998) observed that increasing the concentration of α-tocopherol in the diet from 22 to 207 mg kg^{-1} reduced the percentage of abnormal eggs in gilthead seabream (*Sparus aurata*), with a consequent increase in hatching rate.

Reproductive index and fertility

The results of the indices of spawning, gonadosomatic index, hepatosomatic index, visceral fat index, relative and total fecundity are presented in Table 3. Note that the spawning index followed a linear trend, showing better results at higher levels of supplementation ($p < 0.05$; Figure 1). Differences were not observed between treatments for egg weight, the values obtained for the spawning index in this work are directly related to weight of females.

The relative and total fecundity ($p < 0.05$; Figure 2) followed a trend similar to the spawning index showing percentage increases of 58.5 and 64%, respectively, in females supplemented with 500 mg compared to 200 mg vitamin E. The fecundity of gilthead seabream was higher when fed diets supplemented with vitamin E (IZQUIERDO et al., 2001). Moreover, Cavalli et al. (2003) found no difference in fertility of Malaysian prawn fed diets supplemented with vitamins E and C. Increased levels of α-tocopherol up to 127% resulted in an improvement in fecundity of gilthead seabream (FERNANDEZ-PALACIOS et al., 1998).

There was no significant effect ($p > 0.05$) on the gonadosomatic index (Table 3). However, common carp, goldfish (*Carassius auratus*), and yellow perch had higher gonadosomatic index when fed diets supplemented with vitamin E (GUPTA et al., 1987; LEE; DABROWSKI, 2004). The values of hepatosomatic and visceral fat indices are inversely related to the amount of vitamin E in the food and with fecundity (Figure 2). Assuming that the liver is the transfer channel of fat from mesenteric deposits

to the gonads (WHITE et al., 1968), the variation in the values of hepatosomatic and visceral fat indices, between treatments, was probably associated with the mobilization of lipids to gonads, which was higher in animals with higher relative fecundity rates.

Survival and number of larvae

Table 3 shows data for larval survival and the number of larvae produced per female. There was a 71% increase in larval survival ($p < 0.01$) and more than 100% in the number of larvae ($p < 0.05$) in treatments in which females received dietary supplementation of 400 mg kg^{-1} vitamin E compared to the lower level of supplementation (Figures 2). The accumulation of tocopherol in eggs showed a linear trend, i.e., was greater among the highest levels of supplementation (Table 3). Similar results were reported by Tokuda et al. (2000) who evaluated the affinity of tocopherol for lipoproteins before, during and after the reproductive period of Japanese flounder (*Paralichthys olivaceus*) and noticed that the eggs of animals fed dietary vitamin E supplementation (1000 mg kg^{-1}) showed content of 70.4 ± 15 mg kg^{-1} vitamin E, while the unsupplemented group (16 mg kg^{-1}), only 11.6 ± 1.4 mg kg^{-1}.

The relationship between the survival rate and the dietary supplementation of vitamin E was showed in some studies and can be associated with the immunostimulatory function of this vitamin. Ciarcia et al. (2000) observed a drastic decrease in the concentrations of α-tocopherol in samples of viable eggs of European sea bass, which was not evident in non-viable eggs collected at the same time. The authors attributed the loss of vitamin E in viable eggs to its use as an antioxidant in the period when the enzymatic defenses are under development in the embryo.

Pacific white shrimp (*Penaeus vannamei*) showed an increased larval survival rate when fed Artemia enriched on a mixture of α-tocopherol, ascorbyl palmitate and astaxanthin (WUOTERS et al., 1999). A combination of vitamin C and E increased the survival rates of milkfish (EMATA et al., 2000) and yellow perch (LEE; DABROWSKI, 2004). Some studies have shown the influence of vitamin E on the larval production. Watanabe et al. (1985) and Harlioğlu et al. (2002) recommend 100 mg kg^{-1} of α-tocopherol to increase the number of larvae produced by red sea bream snapper (*Pagrus major*) and crayfish, respectively.

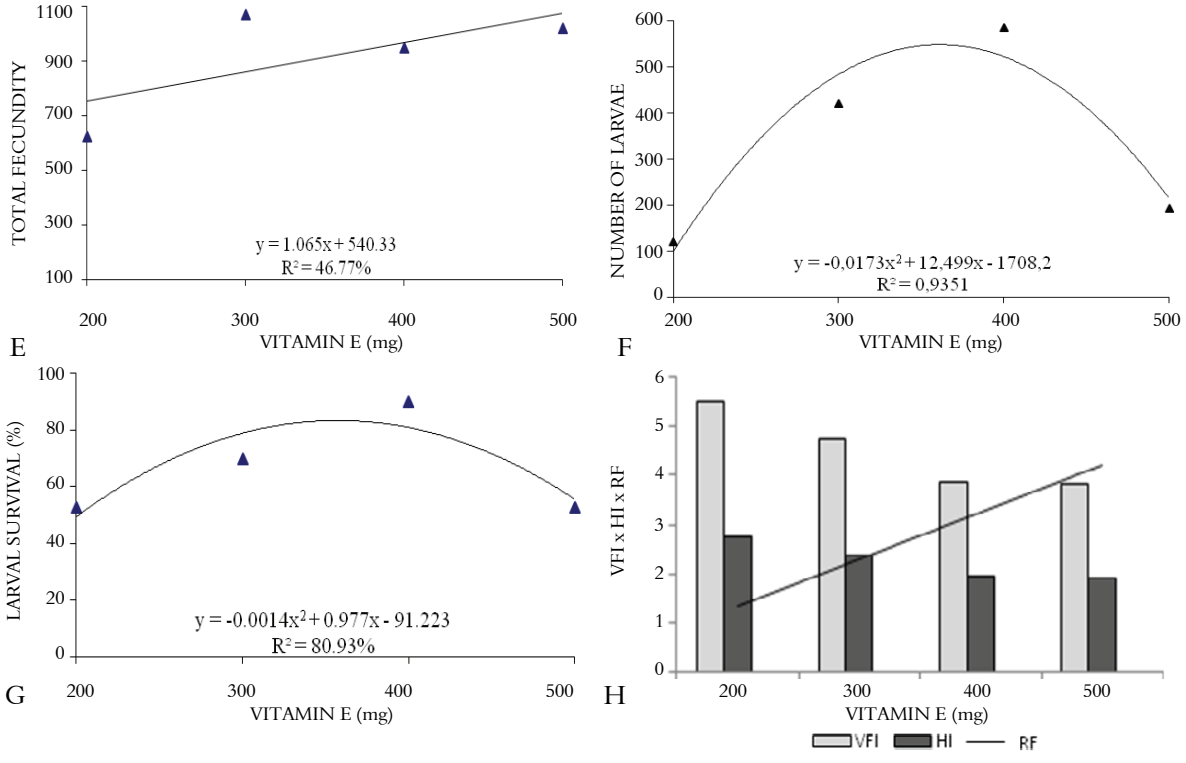

Figure 2. E - Total fecundity, F - number of larvae per female, G - larval survival and H - relationship between hepatosomatic index (HI), visceral fat index(VFI) and relative fecundity (RF) of Nile tilapia fed diets containing different levels of vitamin E.

Conclusion

It is known that the nutritional status of fish embryo, necessary for the proper development of the animals, depends on the transfer of nutrients from breeders to gametes. Thus, the diet of breeding individuals must not only meet the nutritional requirements or gonadal development, but also the eggs and the embryonic development. With the results of this study, we concluded that 400 mg kg^{-1} diet of vitamin E during the reproductive period of female Nile tilapia are sufficient to ensure the best reproductive performance, providing efficient production of a larger number of larvae in individuals of this species.

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