Synergistic Effects of Vitamins C and E and Selenium on the Reproductive Performance of Nile Tilapia, *Oreochromis niloticus*

Abdel Hakim E. El-Gamal, Zeinab A. El-Greisy and El-Sayed H. El-Ebiary

National Institute of Oceanography and Fisheries, Alexandria, Egypt.

**Abstract:** Effect of addition of different combinations between vitamin C (L-ascorbic acid, 120 mg/kg diet), vitamin E (dl-alpha-tocopherol acetate, 34 mg/kg diet) and selenium (0.2 mg/kg diet) to the diet on the reproductive performance of *Oreochromis niloticus* has been investigated in the present study after 120 days of treatment. Gonadosomatic index (GSI), Hepatosomatic index (HSI), egg diameter, fecundity, fertilization percentage, hatching rate and survival percentage of the fry have been investigated at the different treatments compared to the control group. Histological examination of the gonads has been demonstrated in both sexes at different treatments. The present study showed that all the reproductive parameters as well as the histological examination were affected positively by the supplementation of the additives. However, the combination between vitamin E, vitamin C and selenium showed the best results among all treatments compared to the control group, which was devoid from any supplementary additives. In females, egg diameters increased in fish fed with vitamin C or in combination with vitamin E and selenium. The diameter of most ripe eggs were ranged between 1500 and 2000 µ, while some others exceeded 2000 µ. The highest percentage of large eggs which have diameters exceeded 2000 µm is recorded in the group supplemented with vitamin E + vit. C and selenium. The fecundity increased significantly in case of combination of vitamins E and C and selenium. A strong correlation coefficient was obtained either in its relation to the total length (r=0.945) or its relation to the gutted weight (r=0.982). Also, an increase was recorded in the spawning rate, hatching rate and the survival of the fries. Histologically, the ovaries of the fish fed with vitamins E and C and selenium contained post vitellogenic and ripe oocytes with majority of the egg diameter exceeded 2000 µ. However, little number of young oocytes was recorded. In males, GSI values significantly increased in case of the group treated with vitamin E with selenium, as well as in case of combination of selenium with vitamin C (p<0.05). Histologically, the testes of the treated fish contained semineferous tubules filled with spermatozoa compared to the control group which contained young spermatogenic stages.

**Key words:** fish, Nile tilapia, *Oreochromis niloticus*, reproduction, vitamins, vitamin C, Vitamin E, Selenium.

**INTRODUCTION**

The success of intensive tilapia culture depends to a large extent on supplemental feeding which lead to a great amount of fry production[7]. Among factors considered to be important in fry production are brood fish age and size, brood fish stocking density, broad fish sex ratio, frequency of removing fry from breeding unit, type of container, water quality, rate of water exchange and brood fish nutrition.

Diets are often supplemented with vitamins as essential dietary requirements for fish. Vitamins C (ascorbic acid) and E (α-tocopherol) are among the important vitamins required for fish reproduction. Ascorbic acid is a co-factor in the biosynthesis of steroid hormone and neurohormones[34]. In fishes, it plays an important role in fish reproduction[5,6,9,19]. Ascorbic acid is an essential dietary component for most fish species[11]. However, its direct mechanism has not been well understood. Ascorbyl monophosphate is an excellent source of stable ascorbic acid and is efficiently transferred to eggs and offspring[10]. The direct correlation between hatching rate and egg concentration of ascorbic acid was studied[5]. On the other hand, a decrease in ascorbic acid concentration might be responsible for a decrease in sperm viability[3].

Previous studies reviewed the biological functions of vitamin C in living organisms as a general water soluble redox reagent, cofactor in collagen synthesis, growth activator, regulator of hormone synthesis, modular of hexose monophosphate shunt and indicator of hepatic microsomal hydroxylase and as immunostimulator in fish[21].

Corresponding Author: Abdel Hakim E. El-Gamal, National Institute of Oceanography and Fisheries, Alexandria, Egypt.
In the cichlid family, ascorbic acid requirement have been studied in Tilapia zillii[1], Oreochromis mossambicus[20], Oreochromis aureus[21], Oreochromis niloticus[20] and a hybrid of Oreochromis aureus and Oreochromis niloticus[27,28].

Two experimental diets differing in supplementation of ascorbic acid and a third commercial diet were fed to rainbow trout broodstock. A supplementation level of 115 mg ascorbic acid/kg significantly increased the number of hatching eggs compared to eggs from fish without dietary ascorbic acid supplementation[23]. It concluded that broodstock fish should be fed on adequate amounts of the vitamin to provide eggs with more than 20 μg ascorbic acid/g.

Vitamin E is known to have a great role for fish reproduction[32]. It is the most important one among the essential nutrients related to the development of reproductive organs. It also plays a role in relation to spawning and egg qualities, as also was observed in higher animals. Reared the parent ayu (Plecoglossus altivelis) with diets containing different levels of vitamin E for three months before spawning have estimated that this fish requires 3.4 mg of vitamin E in 100 g diets in terms of hatching rate and the survival of hatched larvae[32].

As for carp, 17-months feeding trials have shown that vitamin E deficiency in diets resulted in the retardation of ovarian development[34]. The gonad weight and gonadosomatic index of carp had low values in case of vitamin E-deficient group compared to the control group. Selenium is a trace element found widely in the environment. Although Selenium is an essential element, at high concentrations, it could be toxic to fish, cause mortality, growth retardation and reproductive impairment[2]. It is incorporated into a number of enzymes; including glutathione peroxidase. This enzyme helps protect cell membranes from damage by free radicals. Selenium also is involved in the immune system, thyroid metabolism and in reproduction[35].

Little is known about the effect of providing nutritionally adequate food on reproduction and growth of sexually mature tilapia in captivity, therefore the aim of the present study is to evaluate the effects of supplementation of vitamin E, vitamin C and selenium in diets on the gonadosomatic index, fecundity, egg size, spawning efficiency and hatchability as well as histological examination of Nile tilapia Oreochromis niloticus.

MATERIAL AND METHODS

Brood fish of Nile tilapia, Oreochromis niloticus were obtained from Lake Manzalla during the prespawning season. The total number of collected fish was 750 male and female. The fish were acclimatized for one week in the tank, at Al-Mataria Research Station. Before the beginning of the experiment, twelve glass aquaria (80 liters each) were prepared to be used in the experiment. Twenty fish were placed in each aquarium and the aquaria were divided into six groups. The broodstock males were put together with broodstock females in each aquarium, the sex ratio were (1 M: 3 F). In each aquarium, water was partially changed once every day and was siphoned and renewed every 48 hours. Aeration was provided using a blower and the water temperature was thermostatically controlled between 25-28°C. The average initial lengths of brood fish Nile tilapia was 11.28±0.52 cm and average initial weight was 22.89±4.20 g.

Six diets were formulated to contain 35% crude protein and 9.0% crude lipid. A control diet (diet no.1) which contained 30% fish meal, 30% solvent-extracted Soybean meal, 18% wheat bran, 13% yellow corn, 6% corn oil, 2% vitamin and mineral premix and 1% carboxy methyl cellulose. The other tested diets from no. 2 to no. 6 contained the following:

diet (1): Control diet.
diet (2): Control diet + vitamin E (34 mg/kg diet).
diet (3): Control diet + vitamin C (120 mg/kg diet).
diet (4): Control diet + vitamin E (34 mg/kg diet) + vitamin C (120 mg/kg diet).
diet (5): Control diet + vitamin E (34 mg/kg diet) + selenium (0.2 mg/kg diet).
diet (6): Control diet + vitamin E (34 mg/kg diet) + vitamin C (120 mg/kg diet) + selenium (0.2 mg/kg diet).

Fish in each group (1-6) at aquaria were hand fed their pelleted feeds twice daily, six days a week at a rate of 3 % of body weight on a dry weight basis for 120 days. Feeding rate were adjusted weekly after the biomass in each aquarium was determined. At the end of the experiment, fish in each aquarium were netted, counted and weighted. The total length of the fish was recorded to the nearest cm. The fertilization and hatching rates were recorded.

The gonadosomatic index (GSI) was calculated as a percentage of the gonad weight to the gutted weight of the fish. The hepatosomatic index was recorded as a percentage of the liver weight to the gutted weight of the fish. The oocyte stage were described[27] and the oocyte diameters were determined by using eye piece micrometer under stereomicroscope. The fecundity was calculated and assigned as two terms[22].

For histological examination, small pieces from both of ovaries and testes were fixed for about 48 hours, dehydrated in ascending concentrations of ethanol, cleared in xylene and embedded in parablast
paraffin (m.p. 56-58°C). Transverse sections were cut at 6-8 microns and stained with hematoxylin[2], aqueous solution of eosin was used as counter stain.

RESULTS AND DISCUSSION

Biological parameters and histological examinations were studied in case of fish fed with control diet and fish fed with diets with supplementary vitamins and selenium.

1. Biological parameters

1.1. Gonado-and hepato-somatic indices

**1.1.1. Females:** Effect of addition of different combination of vitamin C and E and selenium on the values of GSI and HSI are shown in table (1). Significant difference in the values of GSI of the females were recorded in the group fed with diet 3 (vit. C) and the group fed with diet 6 (C + E + Se) compared to the control group (diet 1), p < 0.05.

According to HSI values, a significant difference was recorded in the group fed with diet 2 (Vit. E) and the group fed with diet 6 (C + E + Se) compared to the control group (diet 1).

The group fed with diet 6 (C + E + Se) showed the highest values of both gonado- and hepato-somatic indices.

**1.1.2. Males:** Effect of addition of vitamin C and E and selenium on the values of gonado- and hepato-somatic indices of male *Oreochromis niloticus* are shown in table (2).

As for GSI values, it was observed that these values increased in all treatments, but the highest value was recorded in group received diet 6 (C + E + Se). A significant difference (p < 0.05) was recorded in case of the group fed on diet 5 (E + Se) and diet 6 (C + E + Se).

According to HSI values, the case was different since not all of the values increased more than the control value. However, difference was insignificant among treatments involving the maximum values. The average of group 6 (C + E + Se) showed significant difference compared to the control group (p < 0.05).

**1.2. Egg diameter:** In case of the control group, the majority of eggs have diameters less than 1500 µm.

### Table 1: Effect of addition of vitamin C, vitamin E and selenium in diet on gonadosomatic index (GSI) and hepatosomatic index of *Oreochromis niloticus* females after 120 days of treatments.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>No. of fish</th>
<th>GSI</th>
<th>HSI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Min.</td>
<td>Max.</td>
</tr>
<tr>
<td>Diet 1: control (CTR)</td>
<td>8</td>
<td>0.451</td>
<td>1.848</td>
</tr>
<tr>
<td>Diet 2: (CTR + vit.E)</td>
<td>7</td>
<td>0.681</td>
<td>1.801</td>
</tr>
<tr>
<td>Diet 3: (CTR + vit.C)</td>
<td>7</td>
<td>1.437</td>
<td>3.083</td>
</tr>
<tr>
<td>Diet 4: (CTR + vit.C + E)</td>
<td>6</td>
<td>1.600</td>
<td>2.468</td>
</tr>
<tr>
<td>Diet 5: (CTR + vit.E + Se)</td>
<td>7</td>
<td>0.405</td>
<td>1.792</td>
</tr>
<tr>
<td>Diet 6: (CTR + vit.C + vit.E + Se)</td>
<td>6</td>
<td>2.189</td>
<td>3.333</td>
</tr>
</tbody>
</table>

LSD * 1.07 1.57

* Least significant difference.
In the same column insignificant differences between treatment with same letter (p<0.05).

### Table 2: Effect of addition of vitamin C, vitamin E and selenium in diet on gonadosomatic index (GSI) and hepatosomatic index (HIS) of *Oreochromis niloticus* males after 120 days of treatment.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>No. of fish</th>
<th>GSI</th>
<th>HSI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Min.</td>
<td>Max.</td>
</tr>
<tr>
<td>Diet 1: control (CTR)</td>
<td>7</td>
<td>0.107</td>
<td>0.325</td>
</tr>
<tr>
<td>Diet 2: (CTR + vit.E)</td>
<td>6</td>
<td>0.104</td>
<td>0.581</td>
</tr>
<tr>
<td>Diet 3: (CTR + vit.C)</td>
<td>8</td>
<td>0.058</td>
<td>0.569</td>
</tr>
<tr>
<td>Diet 4: (CTR + vit.C + E)</td>
<td>7</td>
<td>0.268</td>
<td>0.419</td>
</tr>
<tr>
<td>Diet 5: (CTR + vit.E + Se)</td>
<td>6</td>
<td>0.487</td>
<td>1.04</td>
</tr>
<tr>
<td>Diet 6: (CTR + vit.C + vit.E + Se)</td>
<td>6</td>
<td>0.363</td>
<td>1.095</td>
</tr>
</tbody>
</table>

LSD * 0.50 1.53

* Least significant difference.
In the same column, insignificant differences between treatments with same letter (p<0.05).
Table 3: Effect of additional vitamins (vitamin C, vitamin E and selenium) on the percentage of egg sizes distribution of Oreochromis niloticus.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>No. of fish</th>
<th>Total length (cm)</th>
<th>Gut wt (gm)</th>
<th>Gonad wt. (gm)</th>
<th>GSI</th>
<th>&lt;1500</th>
<th>1500-2000</th>
<th>&gt;2000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet 1: control (CTR)</td>
<td>3</td>
<td>11.6</td>
<td>21.5</td>
<td>0.15</td>
<td>0.697</td>
<td>67</td>
<td>33</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15.5</td>
<td>61.5</td>
<td>0.45</td>
<td>0.731</td>
<td>57</td>
<td>43</td>
<td>-</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>14.53</td>
<td>48.4</td>
<td>0.43</td>
<td>0.84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diet 2: (CTR + vit E)</td>
<td>3</td>
<td>15</td>
<td>62</td>
<td>0.53</td>
<td>0.854</td>
<td>57</td>
<td>33</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16</td>
<td>66.5</td>
<td>0.68</td>
<td>1.01</td>
<td>51</td>
<td>35</td>
<td>14</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>14.50</td>
<td>53.3</td>
<td>0.53</td>
<td>0.91</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diet 3: (CTR + vit C)</td>
<td>3</td>
<td>12.3</td>
<td>36.5</td>
<td>0.38</td>
<td>1.04</td>
<td>35</td>
<td>50</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14.5</td>
<td>50.6</td>
<td>0.68</td>
<td>1.34</td>
<td>27</td>
<td>52</td>
<td>21</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>13.43</td>
<td>42.53</td>
<td>0.50</td>
<td>1.17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diet 4: (CTR + vit C + vitE)</td>
<td>2</td>
<td>13.5</td>
<td>38</td>
<td>0.51</td>
<td>1.34</td>
<td>25</td>
<td>35</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14.7</td>
<td>45.5</td>
<td>0.48</td>
<td>1.05</td>
<td>47</td>
<td>30</td>
<td>23</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>14.10</td>
<td>41.75</td>
<td>0.50</td>
<td>1.20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diet 5: (CTR + vit E + Se)</td>
<td>3</td>
<td>13.8</td>
<td>37.8</td>
<td>0.31</td>
<td>0.82</td>
<td>63</td>
<td>37</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12.2</td>
<td>28.5</td>
<td>0.22</td>
<td>0.76</td>
<td>65</td>
<td>35</td>
<td>-</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>13.67</td>
<td>42.77</td>
<td>0.40</td>
<td>0.89</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diet 6: (CTR + vit C + vit. E + se)</td>
<td>3</td>
<td>15</td>
<td>63</td>
<td>0.93</td>
<td>1.476</td>
<td>21</td>
<td>35</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12.5</td>
<td>43</td>
<td>0.67</td>
<td>1.54</td>
<td>18</td>
<td>30</td>
<td>52</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>13.30</td>
<td>49.17</td>
<td>0.72</td>
<td>1.46</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSD</td>
<td></td>
<td>0.76</td>
<td>7.26</td>
<td>0.17</td>
<td>0.37</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Third of the fish of the control group developed eggs with diameters exceeded 2000 µm. The same case was still observed in the group fed with diet 2 (vit. E) and in case of the group fed with diet 5 (E+Se). On the other hand, the groups fed with diet 3 (C), diet 4 (E + C) and diet 6 (C + E + Se) developed eggs exceeded 2000 µm in all fish of those groups table (3).

1.3. Fecundity: The present study considered the females of the group that fed with diet 6 (E + C + Se) for fecundity calculations, since this group recorded the highest values of GSI and egg diameter as described before.

1.3.1. Relation between the fecundity and the total length: The absolute fecundity increased with the increase of lengths according to the following equation:

\[ F_{abs} = a L^b \]

Logarithmic transformation of the above equation was applied:

\[ \log F_{abs} = \log a + b \log L \]

The above equation yields a straight line and gave a strong correlation coefficient \((r = 0.991)\) and the formula representing this relationship was as follows:

\[ \log F_{abs} = 15.377 + 1.612 \log L \]

The average of the absolute fecundity varied from 665 to 1502 for fish length ranged from 10.5-17.5 cm as shown in table (4).

1.3.2. Relation between the fecundity and the gutted weight: The relation between the absolute fecundity and the gutted weight is represented by the following equation:
The relationship between absolute fecundity and total length of *Oreochromis niloticus* fed with control diet in addition to vitamin C and/or E and selenium.

<table>
<thead>
<tr>
<th>Length interval (cm)</th>
<th>No. of fish</th>
<th>Mid interval length</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Average±SD</th>
<th>Calculated absolute fecundity</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.1-10.9</td>
<td>4</td>
<td>10.5</td>
<td>550</td>
<td>750</td>
<td>665±80.46</td>
<td>681</td>
</tr>
<tr>
<td>11-11.9</td>
<td>4</td>
<td>11.5</td>
<td>720</td>
<td>850</td>
<td>800±49.47</td>
<td>789</td>
</tr>
<tr>
<td>12-12.9</td>
<td>5</td>
<td>12.5</td>
<td>790</td>
<td>930</td>
<td>878±55.57</td>
<td>902</td>
</tr>
<tr>
<td>13-13.9</td>
<td>5</td>
<td>13.5</td>
<td>960</td>
<td>1150</td>
<td>1022±79.81</td>
<td>1022</td>
</tr>
<tr>
<td>14-14.9</td>
<td>6</td>
<td>14.5</td>
<td>1200</td>
<td>1270</td>
<td>1243±32.09</td>
<td>1147</td>
</tr>
<tr>
<td>15-15.9</td>
<td>6</td>
<td>15.5</td>
<td>1260</td>
<td>1350</td>
<td>1304±45.6</td>
<td>1277</td>
</tr>
<tr>
<td>16-16.9</td>
<td>5</td>
<td>16.5</td>
<td>1330</td>
<td>1470</td>
<td>1370±75.82</td>
<td>1412</td>
</tr>
<tr>
<td>17-17.9</td>
<td>3</td>
<td>17.5</td>
<td>1430</td>
<td>1550</td>
<td>1502±49.47</td>
<td>1553</td>
</tr>
</tbody>
</table>

\[ F_{abs} = a \cdot w^{b} \]

Logarithmic transformation of the above equation is represented as follows:

\[ \log F_{abs} = \log a + b \log w \]

The relation between absolute fecundity and gutted weight gave a strong correlation coefficient \((r = 0.982)\) as shown in table (5).

1.4. Ability of spawning and hatchability:
Spawning success occurred in the fish groups fed on diets 3 (C), diet 4 (C + E) and diet 6 (C + E + Se). The number of eggs increased in the treatments, especially when the additives were combined together. The fertilization percentage, hatching percentage and the survival percentage of the fries increased in the same trend. All the spawned egg diameters exceeded 2000 µm table (6).
Fig. 1: Histological appearance in the ovaries of Oreochromis niloticus at different supplementary diets, stained with Harris haematoxylin-Eosin. Arranged as follows:

a) Cross section in the ovary of fish fed with control diet, showing early stages of young oocytes (Arrows), few oocytes appeared in ripe stage (Arrowheads) X 100.

b) Section in the ovary of fish fed with diet supplemented with vitamin E (Vit. E), showing early and late peri-nucleolus stage (Arrows), yolk vesicle stage (Yv) and few of ripe oocytes stage (Arrowhead) X 100.

c) Section in the ovary of group 3 (vitamin C), showing the early young oocyte stage (Arrows), yolk vesicle stage (Yv) and ripe stage (Arrowheads) X 50.

d) Section in the ovary of group 4 (E+C), showing ripe oocytes (Arrows) increased in number, yolk vesicle stage (Yv) and young oocytes were also observed (Arrowheads) X 50.

2. Histological examination of the gonads

2.1. Ovaries: Histological examination of the ovaries in fish fed with diets supplemented with vitamin E and/or C with selenium were protected to be atretic (Fig.1 b, c and d). For fish fed on vitamin E (Fig.1 b), it was observed that most oocytes were maintained at early young stages, but few of them appeared at ripe stage. In case of fish fed either on vitamin (C) or (E + C) (Fig.1 c and d), the ovaries contained more advanced oocytes at final maturation stage (i.e. yolk vesicle, primary and secondary yolk granules stage). In addition, young oocyte stages were dominant after the fish had fed on vitamin C (Fig.1 c) and were more than in ovaries of fish fed on vitamin E and C in combined together. Moreover, the number of young oocytes in case of both treatments is less than the number of young oocytes in the control group (Fig.1 a), or those fed with vitamin E (Fig.1 b). After the fish fed with vitamin E and selenium (Fig.2 a), the histology of the ovary was similar to that described when fish were fed with control diet or those fed with vitamin E, in which early young oocyte stages were dominant (Fig.2 a).

In case of (E + C +Se), most of the young oocyte become more developed and reached to the final

Fig. 2: Histological appearance in gonads of O. niloticus fed with different diets as follows:

a) Cross section in the ovary of fish group 5 (E + Se), showing oocytes at early young stage (Arrow), few of large oocytes appeared in yolk vesicle stage (Yv) and ripe oocyte stage (Arrowheads) X 50.

b) Cross selection in the ovary of fish group 6(E + C + Se), showing that most oocytes appeared in the tertiary and ripe oocyte stages (Arrows), few oocytes appeared at early stage. X 50.

c) Magnified portion of the preceding plate (b), showing tertiary and ripe oocytes stages. X 100.

d) Cross section in the testis of males fed with control diet (no additives), showing the seminiferous lobules (Sm) devoid from sperms. X 100.
maturation (i.e. tertiary and ripe oocytes). It was observed that about 90% of oocytes reached final maturation (Fig. 2b and c).

2.2. Testes: In case of the control group, the seminiferous lobules are devoid from sperms. However, few sperms were observed only in spermatic duct. The wall of seminiferous lobules are lined with spermatogenic cells at various stages of development (Fig.2 d).

In case of fish group 2 (vitamin E) or group 3 (vitamin C), most of spermatogenic cells appeared at primary spermatocyte, spermatids and some sperms appeared towards the seminiferous lobules (Fig.3 a and b). For fish of group 4 (E+C), the sperms were greatly accumulated in the most of seminiferous lobules (Fig.3 cand d).

For the fish fed on diet 5 (E+selenium), the testes developed sperms which were accumulated in seminiferous lobules and spermatic duct (Fig.4 a and b). In group 6 (E+C+selenium), the testes appeared in ripe stage. The accumulated sperms appeared as a bulk in the lumen of the seminiferous lobules and spermatic duct (Fig.4 c and d).
Discussion: Vitamins are the most important micronutrients in the diets, the deficiencies or excessiveness of which has profound impact on physiological reactions of the fish. Deficiency in these vitamins can result in poor feed conservation, poor growth, decreased resistance to stress, high mortality, impaired wound healing and low reproductive performance[12,24,31].

Very little attention has been paid to vitamin requirements of tilapia broodstock, despite their positive effects on the reproductive performance of farmed fish[10,29].

Ascorbic acid may act as a cofactor or as a regulator in the biosynthesis of oestrogens in the follicle cells[20]. Vitamin C functions in the maintenance of membrane integrity in all cells. Dietary vitamin C exhibits protective effects on pesticide intoxication of both organochlorine and organophosphorus compounds. It can antagonize when administered at high dose[21]. Deficiency in vitamin C in tilapia feeding can cause scoliosis, lordosis, reduced growth, reduced wound repair, internal and external hemorrhage, caudal fin erosion, exophthalmia, anaemia and reduced egg hatchability[29,30]. On the other hand, supplementation of ascorbic acid in the broodstock feed significantly increased the egg hatchability.

The hatching rate of eggs from broodstock rainbow trout given no dietary ascorbic acid for 4 months before ovulation was significantly reduced compared to a control group[16,23].

Lower survival in vitamin C and E deficient diets were attributed to impaired metabolism in yellow perch[8]

A combination between vitamin C, vitamin E and selenium was known to increase the reproductive performance, such as ovarian growth in common carp, Cyprinus carpio[20], eyed-stage survival and hatchability in ayu, Plecoglossus altivelis[32] and higher percentage of normal eggs and fecundity in gilthead seabream, Sparus aurata[15].

In the present study, gonado- and hepatosomatic indices were affected differently between males and females by various combinations between vitamin E and C and selenium.

Egg diameter showed obvious changes related to the different combinations between the additives. The diameters were recorded in each treatment within three different categories of egg sizes. The first egg size group was measured less than 1500 µ, the second egg size group ranged from 1500 to 2000 µ and the third egg size group exceeded 2000 µ. The egg diameter of the majority of fish of group 6 (vitamin E + vitamin C + selenium) exceeded 2000 µ with a percentage of 22-52%. It was followed by the group of fish supplemented with vitamin C + E with a percentage of 23-40%, then the fish group supplemented with vitamin C alone with a percentage of 15-22%. Only one fish in each of group 1 (control), group 2 (vitamin E) and group 5 (vitamin E + selenium) showed a small percentage of egg diameter exceeded 2000 µ.

The absolute fecundity of fish group 6 (vitamin E + C + selenium) showed a positive correlation in relation to fish length and weight. The present study indicates the fitness of the equations expressed the relations between the absolute fecundity and the length as well as the gutted weight of the fish.

Spawning was lacked in case of control group, group 2 (vitamin E), as well as group 5 (vitamin E + selenium). On the other hand, spawning process was observed in case of group 3 (vitamin C) with a percentage of 20%, group 4 (vitamin E + C) with a percentage of 50% and group 6 (vitamin E + C + selenium) with a percentage of 75%.

The present study showed that dietary vitamin C with a dose of 120 mg/kg diet increased the reproductive performance, of Nile tilapia, but vitamin E with a dose of 34 mg/kg diet did not. However, 34 mg of vitamin E /kg diet was not efficient to make improvement in the reproductive performance when supplemented alone, but it was efficient when mixed with vitamin C and selenium. However, an accurate quantification of the requirement is dependent upon the evaluation of several factors, such as type of diet, processing, moisture content of the diet, storage time of the diet and environmental toxicants and possibly the size, age and genetic makeup of the fish[13].

The present study comes in agreement with other studies. For example, higher fertilization and hatching rates by dietary vitamin C rather than vitamin E was recorded in yellow perch[19]. Generally a positive effect of ascorbic acid in fish eggs on hatching performance has been demonstrated in previous studies[23,33].

It was indicated that ascorbic acid was related to endocrine functions in maturing fish. It was suggested that ascorbic acid may have a role in steroid synthesis and secretion and/or act as stabilizer, protector, enhanced as an inhibitor in relationship to the high local concentrations of steroids in endocrine systems[20]. The requirement for vitamin C depends on fish age[33]. This study reported that adult rainbow trout which were fed with a diet devoid of ascorbic acid for 21 months including the stages of gonadal development, showed no macroscopic signs of avitaminosis C and no increased mortality compared to the control group.

The ovaries undergo an annual cycle which exerts a profound impact upon most metabolic pathways in

the fish. During gonadal growth in oviparous teleosts, the precursors of the egg yolk proteins, vitellogenin, is synthesized in the liver under the control of oestrogenic hormones[8].

The endocrine tissues normally contain high levels of ascorbic acid. After the supplementation of ascorbic acid, this high levels found in the ovaries have been considered to be a reflection of the endocrine functions of this organ[20].

The broodstock dietary ascorbic acid is transferred to the eggs where it is stored for use during growth and development of the larvae until the first feed intake[23,29].

The dose of vitamin E required to be added singly to lead Oreochromis niloticus to spawn might be higher than the present dose. Further investigations of the effect of vitamin E on the reproductive performance of Oreochromis niloticus are needed to situate vitamin E in the correct position according to the role and importance compared to other vitamins.

Higher levels of vitamin E had more protective effect on the RBC membrane against peroxidant induced lysis[21]. The phospholipids of mitochondria, the endoplasmic reticulum and plasma membranes possess special affinities for α-tocopherol. In salmonids, the higher vitamin E supplementation had an enhancement of lymphocyte proliferation[21].

It has been found that vitamin E deficiency in Oreochromis niloticus feed causes lack of sexual coloration (light skin color) and reduces reproductive activity[22].

Aquatic organisms accumulated selenium from inorganic and organic selenium species via aqueous and food-chain exposure routes[2]. Selenium has been reported to confer tolerance to toxicity of heavy metals[20]. The effects of dietary and waterborne selenium on the reproductive success of adult bluegills, Lepomis macrochirus, were evaluated in a chronic toxicity study. Before spawning, two-year-old bluegills were exposed for 60 days to six combinations of dietary and waterborne selenium[4]. Morphological measurements of adult fish, including length, weight, condition factor and gonadosomatic index, were measured at days 60 and 140 of exposure. Reproductive parameters, including spawning frequency, number of eggs per spawn, percentage of hatch and survival of resulting fry for 30 days after hatch were monitored during the 11-week spawning period. Selenium concentrations were determined in adult fish, eggs and 30 days-old fry. Only fry were significantly affected. Survival was severely reduced in fry of parents exposed to 10Fg/L waterborne selenium in combination with dietary exposure of 33.3 Fg/g seleno-L-methionine. These results support field observations that indicate food-chain accumulation of selenium can severely reduce reproductive success of bluegills.

Despite the essentiality of minerals for fish growth and general metabolism, few studies have been conducted on the effects of dietary mineral supplementation on the reproductive performance of tilapia. It is evident, therefore, that extensive work is urgently needed on quantitative vitamins and minerals requirements of tilapia.

In conclusion, an improvement in broodstock maturation and reproductive performance of Nile tilapia Oreochromis niloticus was observed as a result to the supplementation of vitamins E and C and selenium. Their mixing together in the diet is the most favourable choice for nutrition of Oreochromis niloticus to get the best reproductive performance. High fertilization and hatching rates as well as high survival percentage of the fries were observed in the diet containing the three components supplemented together. The synergistic effect of their supplementation together was clearly observed.

REFERENCES


