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Effect of Some Antioxidants on Some Biochemical Parameters and Growth Performance of Nile Tilapia (*Oreochromis Niloticus*)

Abdel-Aziz, F. ABDEL-AZIZ; Neven, S.; Amany A. El-Shebly

Abstract: *The present study was conducted to evaluate the effect of antioxidants (vitamin C, vitamin E and Selenium) added to the basal diet on some blood parameters and growth performance of Nile tilapia (Oreochromis niloticus). Basal diet (25% protein) was formulated as control. The experiment design included 8 treatments in duplicates Antioxidants were added to the basal diet as follow (1- control (basal diet), 2-basal diet+ vit C, 3-basal diet+ Vit E, 4-basal diet + Se, 5-basal diet + vitamins C& E, 6-basal diet +vitamins C &Se, 7-basal diet +vitamins E& Se and 8-basal diet + vitamins C, E &Se). A number of 480 adult male tilapia with an average initial weight of 164.78±13gm were randomly raised in 16 fiberglass tanks (5m^3 each) in 8 treatments as duplicate (30 fish /tank) under laboratory conditions and fed the experimental diets daily at 3% body weight. After 4 months of the experiment, the fish fed vitamins C, E and C&E supplemented diets had significantly ($p<0.05$) higher final weight, daily gain in weight, specific growth rate and condition factor than other feeds, whereas the diet 4 (supplemented with Se) showed the poorest performance WG and SGR. Results of the present study suggest that diet supplemented with antioxidants produce the best growth response in Oreochromis niloticus .Hematological indices (red blood cells count, Hb, Hematocrit, , white blood cell count) showed a significant increase ($p< 0.05$) with dietary vitamins C and E. The results showed that vitamin E is limiting factor in improving fish growth and blood parameters.*

Keywords: *Nile tilapia, antioxidants, growth performance, blood parameters*

I. Introduction

Fish is an important aquatic organism, fish products are an important source of protein for human consumption (Duran, *et al*., 2009). Nile tilapia is classified as economically important fish species that has great commercial demands. Tilapia culture is widely practiced in many tropical and subtropical regions in the world and constitutes the third largest group of farmed finfish, right after carp and salmonids (Bittencourt *et al*., 2003). Tilapia fish are well adapted to enclosed water. They are fast growing, resistant to disease and handling, easy to produce in captivity, and are able to tolerate a wide range of environmental conditions (Elsayed, 1999). Nile tilapia (*Oreochromis niloticus*) is the most widely cultured species of tilapia as good fish for warm water aquaculture. They are easily spawned, used a wide variety of natural food (plankton, detritus, planktonic and benthic invertebrates and decomposed organic matter) as well as artificial feeds. They tolerate poor water quality, and grow rapidly in warm temperature (Kayode and Shamusideen, 2010). These attributes, along with relatively low input cost, have made it the most widely cultured freshwater fish.

Several types of antioxidative compounds are found in all fish species to protect their lipids against damage caused by reactive oxygen species. Changes in environment temperature and oxygen concentration may result in oxidative stress affecting the fishes (Sudha and Deepali, 2013). Antioxidants vitamins C and E are among the most important nutrients that influence the immune system of animals, including fish. These vitamins provide cellular defense against reactive oxygen species which damage biological membranes (Chhorn *et al*., 2010).

Antioxidants vitamins (mainly C + E vitamins) can scavenge ROS and upregulate the activities of antioxidant enzymes (Kelestemur and Ozdemir, 2013). These vitamins are among the most important nutrients influencing the organism immune system and their supply can reduce fish mortality and improve performance. Vitamin C is known as a key element in modern aquaculture that promotes survival and growth performance (Verlhac and Gabaudan, 2006). Vitamin C has immune modulators role (Anbarasu and Chandran, 2001; Chen *et al*., 2003 and Lin Chiiu, 2004), antioxidant (Yamaguchi *et al*., 1995) and a role in producing quality gametes (Dabrowski and Cierezko, 2001). Lack of Vitamin C was reported by (Fracalossi *et al*., 1994) in case of fishes

with structural deformities (Scoliosis, abnormalities in the cartilage, eyes gills or fins), abnormal pigmentation, increased fragility of the capillaries, reduce the immune response and reproductive performance.

Vitamin C has been shown to stimulate immune response in fish (Waagboo *et al*., 1993; Verlhac *et al*., 1996; Orluno *et al*., 1999). Vitamin C (ascorbic acid) has been used in fish foods for improving fish immunity and growth (Wang *et al*., 2003; Zhou *et al*., 2003). Fish cannot synthesize vitamin C, because they don't have the L-glutonolactone oxidase enzyme, which is required to convert L-gutonic acid to ascorbic acid (Sato *et al*., 1976; Shiou and Lin, 2006). For this reason, it is needed to add vitamin C in fish feed.

Vitamin C deficiency has been shown to produce various abnormal signs in various fish including slow growth rate (Gouittou – Coustans *et al*., 1998), impaired wound healing (Wahlie *et al*., 2003), increased susceptibility to bacterial diseases (Ai *et al*., 2006), lower survival rates (Wang *et al*., 2003; Ai *et al*., 2006). Vitamin E is among the most important a nutrient influencing the fish immune system and its supply can reduce mortality and improve fish performance (Ortuno *et al*., 2001; Shiou and Itsu, 2003; Puangkaew etal., 2004).

Vitamin E is potent antioxidant that offer protection against oxidative damage to various fish tissues (Adham *et al*., 2000), enhance resistance of red blood all membranes (Kiron *et al*., 2004), and protect leucocyte functions (Sahoo and Mukherjee, 2002). Vitamin E functions as a lipid-soluble antioxidant, protecting biological membranes, lipoproteins and lipid stores against oxidation (Shiau and Lin, 2006).

Vitamin E has proven beneficial in protecting cellular membrane against oxidation, increase the resistance to stress (Choi *et al*., 2004; Fang *et al*., 2002; Henrique *et al*., 1998).

Selenium (Se) is an essential micronutrient for vertebrates including fish (Wiseman *et al*., 2011). This element may play a significant role in antioxidant defense in fish with physiological non-enzymatic antioxidant properties (Mruk *et al*., 2002; Nogueira *et al*., 2003; Cheung *et al*.; 2004).

Selenium (Se) is an essential trace element required in the diet for normal growth and physiological function of fish (Hilton *et al*., 1980; Bell *et al*., 1985; Vlang and Lovell, 1997 and Mohsen Wafeek, 2008).

In recent years, there has been a great deal of studies carried out on antioxidant system (Kelestemur and Ozdemir, 2013).

Hossein and Aberoumand (2011) studied the natural antioxidants in fish. Antioxidative compounds are found in all fish species to protect their lipids and other compounds than contain double bonds, against damage caused by reactive oxygen species (Hossein and Aberoumand, 2011).

Aquatic organisms can provide systems for investigation of how reactive oxygen species (ROS) damage cellular compounds, how cells respond, and how oxidative stress can lead to disease (Shapour *et al*., 2013).

Toleost fish have proved to be good models to evaluate the antioxidants action since their biochemical responses are similar to those of mammals and of other vertebrates (Sancho, *et al*., 2000).

Fish constitute an excellent model to understand the oxidative stress in aquatic ecosystems (Sudha and Deepali, 2013).

The study of blood parameters in fishes has been widely used for the detection of physiological attentions in different conditions of stress (Nussey *et al*., 1995). Hematological parameters such as hematocrit, hemoglobin, number of erythrocytes and white blood cells are indicators of the effect of toxicants (Sancho *et al*., 2000; Barcellos *et al*., 2003).

The exposure of fish to several types of chemical agents may induce changes in several hematobiological variables (Health, 1995), which are frequency used to evaluate fish health (Martinez and Sauza, 2002).

Hematological indices are important parameters for the evaluation of physiological status of aquatic environment (Golovina, 1996; Luskova, 1997; Vosyliene, 1999; Hrubec *et al*., 2001; Vazquez, 2007).

Aim of the work: the present study was conducted to evaluate the effect of antioxidants on growth performance and some biochemical parameters of Nile tilapia (*Orechromis niloticus*).

II. Materials and Methods

Experimental design:

The experimental design included 8 treatments in duplicates. The experimental design is shown in table (1).

Table (1): The experimental design and diets combinations

Diet no	Antioxidants additives
1	Basal diet without antioxidants (Control)
2	Basal diet + Vitamin C
3	Basal diet + Vitamin E
4	Basal diet + Se
5	Basal diet + Vitamins C + E
6	Basal diet + Vitamin C + Se
7	Basal diet + Vitamin E + Se
8	Basal diet + Vitamins C + E + Se

Fish and Cultural Conditions:

This study was carried out at the wet fish laboratory, Mataria Fish Research Station, National Institute of Oceanography and Fisheries to investigate the effects of antioxidants on some blood parameters and growth performance of Nile tilapia *(Oreochromis niloticus*). Adult male Nile tilapia (*O. nitoticus*) of initial weight 164.78 g were collected from Lake Manzala, fish was acclimated to the experimental conditions for a period of two weeks. During this period they were fed with commercial basal diet. At the beginning of the experiment, 480 Nile tilapia fish were randomly divided into eight different groups with two replicates containing 30 fish/each. Fish were kept in 16 fiberglass tanks (5 m^3/ tank). Fish were then fed with 3% of body weight per day. Each diet was fed twice a day at 9:00 (a.m) and 16:00 (p.m) for four months to duplicate groups of fish. An air stone continuously aerated all tanks. Tanks were cleaned up every day in the morning by siphoning off accumulated waste materials. Each group of fish was weighted every two weeks and the amount of diet fed was adjusted accordingly. A photo period of 12 hours light, 12 hours dark.

Experimental Diets

Basal diet was formulated to contain about 25% crude protein (Tables 2, 3)

Table (2): Ingredients composition of the basal diet

Ingredients	Kg/ton	ingredients	Kg/ton
Fish meal	60	Yellow corn	180
Soybean meal	250	Corn gluten	40
Wheat bran	200	Salts & molasses	20
Rice bran	250	**Total**	**1000**

Table (3): Chemical analysis of experimental (basal) diet

Nutrient composition	% on dry matter basis
Dry matter (DM)	90.50
Crude protein (CP)	25.41
Ether extract (EE)	9.0
Crude fiber	7.29
Ash	9.13
Total carbohydrates	49.17

Water quality

Water quality parameters in the experimental tanks were determined according to the methods of APHA (1992). Water temperature was recorded daily in each tank. Also, dissolved oxygen was measured daily by oxygen meter (HANNA H199/46-04- Romania) and pH by pH meter (Consort C 860- Belgium). Unionized ammonia was measured weekly.

Growth performance measurements

Total weight gain, average daily gain and specific rate were calculated according to **Recker, (1995) and Castell and Tiews, (1980).**

Total gain (g/fish) = (W_2-W_1)

Where: W_2: final weight of fish in grams and W_1: initial weight of fish in grams.

Average daily gain (ADG) (g/fish/day) = total gain / duration period in day, Specific growth rate (SGR, % / day) = 100 × (ln W_2 – ln W_1) / duration period/day. Where (ln) is the natural log.

- The chemical analysis of fish samples muscles were analyzed according to A.O.A.C, (2000) method. For dry matter, crude protein and ether extract of 3 fishes from each tank were used for this process.

Blood parameters determination

At the end of the experiment, blood samples from the fish of the different groups were collected from the caudal peduncle. Adequate amounts of whole blood were collected in small plastic vials containing heparin used for the determination of RBCs and WBCs cells count, hemoglobin concentration (Hb); (g/dl), Hematocrit (Ht) and platelets. Other blood samples were collected and then centrifuged at 3500 rpm for 15 min to obtain blood serum for determination of total protein (Gornell rt al., 1949), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and growth hormone (GH) (Varley, 1976) using a spectrophotometer (model 5010, germany) and commercial kits.

Statistical Analysis

Statistical analysis of growth performance parameters, blood parameters and nutritive value data were done using analysis of variance of data according to **Steel and Torrie (1980**). Statistical analysis was applied using the SAS (1997) with factorial design including comparisons between significant means.

III. Results

Water Quality Parameters:

During the feeding trial, water quality parameters averaged water temperature 26.5± 2.7 °C, pH 7.6± 0.2, Dissolved oxygen (DO) 6.8 ± 0.2 mg/Land unionized ammonia 0.002± 0.001mg/L. The water quality parameters are within the optimum levels for fish culture.

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Growth Performance

As presented in tables 4 and 5 average of final body weight (g/fish), total weight gain (g/fish), average daily gain (g/fish/day) and specific growth rate (%/day) as affected with antioxidants indicate that feeding group No. 5 (basal diet + vitamins C and E) showed the highest growth performance parameters ($P< 0.05$) followed in significant ($P< 0.05$) decreasing order by group 3 feed (basal diet + Vit. E) And group 2 feed (basal diet + Vit. C) respectively.

The lowest growth performance parameters were recorded in feeding group 4 (basal diet + Selenium) the other feeding treatments (control, C + Se, E + Se and C + E + Se) showed no significant difference between them.

Table (4): Growth rate of Nile tilapia (*Oreochromis niloticus*) fed different antioxidants

Groups	At zero time		First month		Second month		Third month		Fourth month		K
	W	L	W	L	W	L	W	L	W	L	
1- Control	164.78±3.7	20.48	201.32^{ef} ±3.94	22	242.86^{d} ±6.88	23.5	287.28^{ef} ±5.48	24.7	327.08^{c} ±3.63	26.14	1.84^{e} ±0.02
2- Vit C	164.78±3.7	20.48	210.68^{bc} ±6.11	22.8	250.68^{c} ±4.91	23.8	297.64^{bc} ±4.97	25	352.48^{b} ±3.25	26.31	1.93^{cd} ±0.03
3- Vit E	164.78±3.7	20.48	216.44^{ab} ±4.68	22	265.34^{b} ±7.22	24	302.58^{ab} ±1.8	24.5	356.30^{ab} ±3.42	26.00	2.03^{a} ±0.04
4- Se	164.78±3.7	20.48	196.12^{f} ±4.25	21	231.48^{e} ±4.62	23	289.72^{de} ±3.84	25	315.78^{d} ±5.67	25.30	1.92^{d} ±0.04
5- C+E	164.78±3.7	20.48	217.82^{a} ±2.98	22.3	272.20^{a} ±5.60	24.5	307.62^{a} ±4.47	25.3	360.10^{a} ±7.42	26.20	1.99^{abc} ±0.01
6- C+Se	164.78±3.7	20.48	210.24^{cd} ±3.36	21.5	242.76^{d} ±3.83	23	281.58^{f} ±6.07	24	322.04^{c} ±3.54	25.63	1.94^{cd} 0.03±
7- E+Se	164.78±3.7	20.48	212.42^{abc} ±2.64	21.5	250.18^{c} ±3.37	23.3	295.42^{cd} ±4.82	24.5	327.00^{e} ±2.92	25.30	2.00^{ab} ±0.02
8- C+E+Se	164.78±3.7	20.48	204.84^{de} ±5.08	22	$236.76d^{e}$ ±3.20	23	286.16^{ef} ±2.98	24.7	325.02^{c} ±3.95	25.60	$1.96b^{cd}$ ±0.10

Table (5): Growth performance of Nile tilapia (*Oreochromis niloticus*) fed different antioxidants

Treatments	Control	Vit C	Vit E	Se	C +E	C + Se	E +Se	C+ E +Se
Average initial weight (g)	164.78 ±3.7	164.78 ±3.7	164.78 ±3.7	164.78 ±3.7	164.78 ±3.7	164.78 ±3.7	164.78 ±3.7	164.78 ±3.7
Average final weight (g)	327.08^{c} ±3.63	352.48^{b} ±3.25	356.30^{ab} ±3.42	315.78^{d} ±5.67	360.10^{a} ±7.42	322.04^{c} ±3.54	327.00^{c} ±2.92	325.02^{c} ±3.95
Gain in weight (G)	162.30^{c}	187.70^{b}	191.52^{ab}	151.00^{d}	195.22^{a}	157.26^{c}	162.22^{c}	160.24^{c}
% gain in weight	98^{c}	114^{b}	116^{ab}	91^{d}	119^{a}	96^{c}	99^{c}	97^{c}
Daily gain in weight/g	1.35^{c}	1.56^{b}	1.59^{ab}	1.25^{d}	1.63^{a}	1.31^{c}	1.35^{c}	1.34^{c}
S G R	0.57^{c}	0.63^{b}	0.64^{ab}	0.54^{d}	0.65^{a}	0.56^{c}	0.57^{c}	0.57^{c}
Condition factor (K)	1.84^{e} ±0.02	1.93^{cd} ±0.03	2.03^{a} ±0.04	1.92^{d} ±0.04	1.99^{abc} ±0.01	1.94^{cd} 0.03±	2.00^{ab} ±0.02	1.96^{bcd} ±0.10

Blood Hematological and Biochemical Parameters:

Data of blood hematological and biochemical parameters of the Nile tilapia (*Oreochromis niloticus*) which fed diets containing antioxidants are shown in tables 6, 7, 8 and 9.

Average of red blood cell count (RBCs) showed the maximum value was recorded in the feeding group number 5 (basal diet + vitamins C + E) followed in significant ($P<0.05$) decreasing order by group 3 (basal diet + Vit. E) and group 8 (basal diet + C + E + Se). The value of hemoglobin (HGB) (g/dL) recorded the maximum value in group 5 (basal diet + Vit. C + E) followed in significant decreasing order by groups 3 + 8. The lowest value of HGB was recorded in the control (basal diet without antioxidants).

The count of WBCs (10^3/ uL) recorded the maximum count in group 5 followed in significant orders by groups 6 and 8. The lowest value was recorded in the feeding group 3 (basal diet + Vit. E) followed in significant deceasing ($p<0.05$) order by groups 5 + 4 respectively. Concerning the hematocrit (%) the maximum percentage with record in groups 3, 5 and 6 with no significant difference, followed in significant ($p<0.05$) decreasing order by groups 1, 2 , 4, 7, 8.

Concerning the effects of antioxidants on kidney function of Nile tilapia recorded as creatinine it is showed that antioxidants improve kidney function where the maximum value of creatinine was recorded in control group(without antioxidants) followed significantly ($p<0.05$) in decreasing order by group 8. The lowest value of serum creatinine was recorded in group 3 (basal + E).

Table 8 shows the serum total protein which recorded its maximum value in feeding group 5 followed in significant ($p<0.05$) order by groups 1 + 7 with no significant difference. The lowest value of serum total protein was recorded in group 3. The values of liver functions were recorded in table 9. Alanine aminotransferase ALT recorded the maximum value in groups 1 + 4, the best value of ALT was recorded in groups 4 + 7. Aspartate aminotransferase AST rerecorded in the maximum value in groups 2, 4 and 8, the lowest value was recorded in group 7 (basal diet + Vit. E + Se).

Table (6): Average Complete Blood Count of Nile tilapia (*Oreochromis niloticus*) fed different antioxidant

Treatment	RBCs 10^6/uL	HGB g/dL	WBCs 10^3/uL	PLt 10^3/uL	HCT%
1 - Control	2.19^{cd} ±0.17	9.75^{e} ±0.22	58.37^{e} ±5.31	7.12^{c} ±0.92	38.50^{b} ±2.29
2 - VIT C	2.20^{bcd} ±0.10	10.12^{d} ±0.05	65.48^{cd} ±1.02	3.04^{e} ±0.11	35.40^{b} ±2.76
3 - VIT E	2.37^{ab} ±0.06	10.91^{b} ±0.07	67.75^{c} ±2.19	9.02^{a} ±0.33	46.80^{a} ±3.65
4 - Se	2.27^{abcd} ±0.04	10.56^{c} ±0.13	60.32^{de} ±0.99	8.07^{b} ±0.37	36.17^{b} ±3.43
5 - C + E	2.42^{a} ±0.05	11.20^{a} ±0.12	88.35^{a} ±1.06	8.09^{b} ±0.11	46.60^{a} ±2.26
6 - C + Se	2.16^{d} ±0.08	10.37^{cd} ±0.24	80.35^{b} ±6.00	7.01^{c} ±0.47	48.17^{a} ±2.68
7 - E + Se	1.75^{e} ±0.08	8.46^{f} ±0.25	57.63^{e} ±0.73	7.02^{c} ±0.12	34.80^{b} ±3.94
8 - C + E + Se	2.34^{abc} ±0.11	10.88^{b} ±0.15	80.85^{b} ±0.65	5.10^{d} ±0.30	37.40^{b} ±1.83

Table (7): Average kidney function of Nile tilapia (*Oreochromis niloticus*) fed with different antioxidant

Treatment	1 - Control	2 – VIT C	3 – VIT E	4 – Se	5 – C + E	6 – C + Se	7 – E + Se	8 – C + E + Se
Creatinine	0.54^{a} ±0.02	024^{ef} ±0.02	0.23^{f} ±0.00	0.25^{ef} ±0.01	0.36^{c} ±0.01	0.27^{d} ±0.00	0.25^{e} ±0.00	0.49^{b} ±0.01

Table (8): Average Serum total protein of Nile tilapia (*Oreochromis niloticus*) fed different antioxidant

Treatment	1 - Control	2 – VIT C	3 – VIT E	4 – Se	5 – C + E	6 – C + Se	7 – E + Se	8 – C + E + Se
Serum total protein	3.93^{b} ±0.15	3.80^{bc} ±0.10	3.30^{d} ±0.10	3.60^{c} ±0.10	4.30^{a} ±0.10	3.70^{c} ±0.10	3.93^{b} ±0.15	3.73^{bc} ±0.06

Table (9): Average Liver Function Test of Nile tilapia (*Oreochromis niloticus*) fed different antioxidant

Treatment	1 – Control	2 – VIT C	3 – VIT E	4 – Se	5 – C + E	6 – C + Se	7 – E + Se	8 – C + E + Se
ALT	14.05^{a} ±0.95	13.46^{ab} ±0.43	12.51^{bc} ±0.24	14.07^{a} ±0.05	12.07^{c} ±0.12	13.49^{ab} ±0.67	12.05^{c} ±0.53	13.09^{ab} ±0.73
AST	23.07^{d} ±0.35	44.79^{a} ±4.80	34.04b ±0.61	42.54^{a} ±1.26	28.62^{c} ±2.08	21.53^{de} ±1.12	18.05^{e} ±0.65	42.03^{a} ±2.55
Albumin	1.07^{d} ±0.06	1.99^{a} ±0.11	1.20^{cd} ±0.13	1.44^{d} ±0.10	1.26^{c} ±0.07	1.23^{cd} ±0.10	1.24^{cd} ±0.06	1.12^{cd} ±0.11

IV. Discussion

Water quality parameters were not significantly different between treatments and were within the recommended ranges for the culture of Nile tilapia. The present results recorded improved growth performance with the addition of antioxidants which agree with the findings of Galaz *et al*., (2010) who recorded that growth performance of fish fed 25 mg/kg vitamin E were significantly higher than control.

- Malayoglu *et al*., (2009) recorded that body weight and body weight gain WG were significantly improved with vitamin E.
- Faramarz 2012 recorded an increase in weight gain for the fish fed diets with vitamin C. He also recorded high specific growth rate (SGR)
- Desimira *et al*., 2013 recorded that the lowest values for SGR were recorded at control (without antioxidants).
- Kelestenur and Ozdemir, 2013 recorded that lower weight gain (WG) was observed in fish fed vitamin E free diet.
- Hamilton, 2004 recorded that elevated levels of selenium concentration can result in toxic effects.
- Liin *et al*., 2010 recorded that supplementation of 100 mg vitamin C/kg to the basal diet was sufficient to increase growth, but adding 50 mg vitamin E/kg was necessary to increase survival.
- The present result agree with finding of (Ispir *et al*., 2011) who recorded an increase ($P<0.05$) on the red blood cells count and on the hemoglobin concentration was obtained on treatments with 80 mg vitamin E/kg.
- Ombe *et al*., 2009 recorded that fish fed vitamin C supplemented diets had significantly ($P<0.05$) higher indices showed a significant increase ($P<0.05$) with dietary vitamin C.
- Abdel-Tawwab and Wafiek, 2008 recorded that antioxidants supplementation enhanced fish growth and reduced creatinine, AST and ALT.

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