



## EFFECTS OF DIETARY EXCESS OF VITAMIN E AND SELENIUM ON SOME PRODUCTION TRAITS, LIPID PEROXIDE STATE AND AMOUNT/ACTIVITY OF THE GLUTATHIONE REDOX SYSTEM IN AFRICAN CATFISH *CLARIAS GARIEPINUS* (SILURIFORMES, CLARIIDAE) FINGERLINGS

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**Abstract:** Purpose of present study was to investigate the effect of excess dietary amount of vitamin E and selenium also short-term starvation on some production traits and parameters of the lipid peroxide state and glutathione redox system in African catfish fingerlings. Three purified diets were formulated: diet 1 (control) contained 114 mg.kg<sup>-1</sup> tocopherol and 0.43 mg.kg<sup>-1</sup> selenium, diet 2 contained high level of tocopherol (245mg kg<sup>-1</sup>) and control level of selenium (0.5 mg kg<sup>-1</sup>), while diet 3 contained high level of selenium (2.5 mg kg<sup>-1</sup>) and control level of tocopherol (170 mg kg<sup>-1</sup>). Fingerlings of *C. gariepinus* (3.16-3.93 g) were investigated in four groups, three consumed the above mentioned diets and the fourth served as unfed control. The experiment lasted 30 days except in the case of the unfed group which was terminated on the 15<sup>th</sup> day because of high mortality. The vitamin E / selenium treated groups showed significantly ( $P \leq 0.001$ ) lower final body weight and specific growth rate as compared to the control. Vitamin E and selenium content of the fish body in diet 2 group increased significantly ( $P \leq 0.001$ ) as compared to the control. Vitamin E content did not change in the unfed group, but selenium content decreased significantly ( $P \leq 0.001$ ) during the 15-days starvation period. Reduced glutathione (GSH) content increased significantly ( $P \leq 0.05$ ) only in the high selenium group. Glutathione-disulphide (GSSG) content, and glutathione peroxidase activity did not change neither during the period of investigation and nor as effect of treatments, but the value of GSH/GSSG ratio was significantly higher ( $P \leq 0.05$ ) in the high selenium as compared to the high vitamin E group. Lipid peroxide state of the body, as was measured by the malondialdehyde content, changed as effect of age and/or maturation but not as effect of the treatments.

**Keywords:** African catfish, Vitamin E, Selenium, Glutathione, Malondialdehyde

### INTRODUCTION

The main function of vitamin E ( $\alpha$ -tocopherol) is a lipid-soluble antioxidant in animal tissues, including fish (Cowey 1986, Waagbø 1994). It protects the organism, mainly membrane phospholipids against uncontrolled oxidation by free radicals, which are generated through normal metabolism, and by oxidative challenge such as infection, tissue damage, and pollution (Waagbø 1994, Hamre *et al.*, 1997, Lygren *et al.*, 2001).

Selenium is an essential trace element, mainly as an integral part of glutathione peroxidase (GPx) (Flohé *et al.*, 1973, Chow & Tappel 1974), but in excess amount it has high toxicity in fish (Balogh *et al.*, 2002). Bioavailable forms of selenium include both inorganic (e.g. selenate and selenite) and organic (e.g. selenomethionine and selenocysteine) forms from different dietary sources (Arteel & Sies, 2001). The function of selenium is interrelated with that of vitamin E (Chow, 2001). However, selenium complements vitamin E in the cellular antioxidant defense system

(Chow & Hong 2002). Watanabe (1997) reported that selenium in conjunction with vitamin E is essential for preventing nutritional muscular dystrophy. The National Research Council (1993) has recommended a minimum requirement of 50 mg vitamin E kg<sup>-1</sup>, while the selenium recommendation is 0.1–0.25 mg kg<sup>-1</sup> diet, on dry matter basis.

The purpose of this experiment was to study the effect of excess dietary amounts of vitamin E, selenium and short-term starvation on some production traits and on some parameters of the lipid peroxide state and glutathione redox system in African catfish *Clarias gariepinus*, Burchell, 1822 fingerlings.

### MATERIALS AND METHODS

#### Diets, fish and experimental procedures:

The basal diet was prepared as described in Table 1, at the Department of Nutrition at the Research Institute for Fisheries, Aquaculture and Irrigation,

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Szarvas, Hungary (HAKI). Three purified diets were formulated to contain approximately 46.7% crude protein based on the feedstuff values reported by Lovell et al., (1984) and Lovell (1989) as follows: diet 1 (control) contained no additional supplemental dietary  $\alpha$ -tocopherol or selenium; diet 2 (H-TOC/SE) contained high levels of tocopherol (245 mg kg<sup>-1</sup>) and control level of selenium (0.5 mg kg<sup>-1</sup>); diet 3 (H-SE/TOC) contained high level of selenium (2.5 mg kg<sup>-1</sup>) and control level of  $\alpha$ -tocopherol (170 mg kg<sup>-1</sup>). Proximate composition of the diets and concentrations of  $\alpha$ -tocopherol and selenium (supplied as sodium selenite, Na<sub>2</sub>SeO<sub>3</sub>) are shown in Table 1. All feed ingredients were thoroughly mixed, and, after the addition of 40% water, the mixture was extruded through a pasta machine. The strands thus obtained were air-dried, ground and sieved to obtain suitable particle size fractions in the range of 1-2 mm, which were stored at -20°C until used. Diets were prepared according to the vitamin and mineral premix contents of vitamin E and selenium.

**Table 1:** Diet composition and proximate nutrient analysis of the basal diet used in the experiment

Ingredient*		g kg <sup>-1</sup> dry diet			
Gelatin		80.0			
Dextrin		280.0			
Casein		420.0			
Cellulose		68.0			
Soybean oil		45.0			
Cod liver oil		35.0			
Carboxymethyl cellulose		20.0			
Vitamin premix <sup>1</sup>		10.0			
Mineral premix <sup>2</sup>		40.0			
Red colorant		2			
Total		1000			
Diet	Vitamin E (mg kg <sup>-1</sup> )	Selenium (mg kg <sup>-1</sup> )	Fat g kg <sup>-1</sup>	Protein g kg <sup>-1</sup>	Ashg kg <sup>-1</sup>
Control	114	0.43	80	463	87
H-TOC/SE	248	0.48	83	460	84
H-SE/TOC	173	2.31	85	465	88

\*All purified feed ingredients were purchased from Sigma-Aldrich Chemie (Schnelldorf, Germany) except for the vitamin premix (HAKI, Szarvas, Hungary), gelatin and minerals (Merck, Darmstadt, Germany), soybean oil (Oleificio Zucchi Spa., Rome, Italy), cod liver oil (Molar plc, Debrecen, Hungary)

<sup>1</sup>Vitamin premix expressed per kg of the mixture: thiamine, 4g; riboflavin, 5.5g; pyridoxine, 1g; calcium d-pantothenate, 10g; niacin, 10g; biotin, 0.1g; cholin chloride, 200g; folic acid, 1g; vitamin B12, 5mg; vitamin C, 10g; vitamin A, 200000 IU; menadione sodium bisulphite, 4g; vitamin E, 12g; vitamin D, 200000 IU; Fe, 5g; I, 0.1g; Co, 0.3g; Cu, 0.4g; Mn, 4.5g; Se, 0.05g; Zn, 5g.

<sup>2</sup>Mineral premix expressed per kg of the mixture: CaHPO<sub>4</sub>·2H<sub>2</sub>O, 368.75g; CaCO<sub>3</sub>, 263.68g; KH<sub>2</sub>PO<sub>4</sub>, 178.31g; KCl, 17.68g; NaCl, 106.58g; MnSO<sub>4</sub>·H<sub>2</sub>O, 6.57g; FeSO<sub>4</sub>·7H<sub>2</sub>O, 8.91g; 4MgCO<sub>3</sub>·Mg(OH)<sub>2</sub>·5H<sub>2</sub>O, 43.24g; KI, 0.18g; CuSO<sub>4</sub>·5H<sub>2</sub>O, 0.54g; ZnSO<sub>4</sub>·7H<sub>2</sub>O, 5.35g; CoCl<sub>2</sub>·6H<sub>2</sub>O, 0.05g; Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O, 0.15g.

Fingerlings of African catfish *C. gariepinus*, ranging in weight from 3.16-3.93g, were divided randomly among 12 modified plastic containers (15 liters each) at a stocking density of 30 fish per container at the fish hatchery of HAKI. All containers were connected to the re-circulating system in the farm, where the water is purified by a series of filtration treatments including UV irradiation and a bio-filter system. Therefore, the water quality was similar to that in other tanks on the farm. Water temperature was 27±1.0°C, and oxygen was maintained near saturation throughout the experiment. The flow rate of each container was approximately 0.8 L min<sup>-1</sup>. The photoperiod was maintained at a 12:12 h light: dark schedule.

After their acclimatization to the experimental diet and conditions for one week, the fish in triplicate containers were assigned to each of the three experimental diets and fed at 8% of their body weight daily using automatic feeders for a period of 30 days. An additional triplicate group was kept unfed as a negative control that was investigated for only 15 days.

All fish were weighed by group (container) at 5-day intervals until the end of the experiment. Growth was calculated as daily specific growth rate (SGR; % body weight day<sup>-1</sup>) using the equation:  $SGR = 100 (\ln W_f - \ln W_i) t^{-1}$ , where  $W_i$  and  $W_f$  were the initial and final mean body weights (g), respectively, after  $t$  days (Ricker 1979). The feed conversion ratio (FCR) was calculated as follows:  $FCR = \text{feed intake (g)} / \text{wet weight gain (g)}$ . Mortality was recorded throughout the experiment.

#### Sample collection, biochemical and statistical analyses:

Feed samples were taken from each batch of diets at the start and stored at -20°C until the analysis of vitamin E and selenium content. The fish were starved for 12 h before samples were collected at the start of the experiment and after 30 days of feeding, while the unfed group samples were taken after 15 days. Two groups of five fish were sampled from each container (n=30, 3 replicates each of 10 fish). The fish were sacrificed and then stored at -80°C in order to determine whole body glutathione redox system parameters and malondialdehyde (MDA) content, as well as vitamin E and selenium content.

GPx (E.C.1.11.1.9) activity was measured in the 10,000 g supernatant fraction of tissue homogenate (1:9 in 0.65% NaCl) using reduced glutathione and cumene hydroperoxide as co-substrates in an end point direct assay following the system of Lawrence & Burk (1976). The loss of glutathione was measured using Ellmann's reagent (Sedlak & Lindsay 1968). The enzyme activity was expressed as unit which means 1 nmol glutathione oxidation per minute at 25°C. The enzyme activity was calculated to protein content of the supernatant

fraction of tissue homogenate, which was measured using the Folin-phenol reagent (Lowry *et al.*, 1951). Bovine serum albumin served as the standard.

Reduced glutathione (GSH) content of the tissue homogenate (1:9 in 0.65 % NaCl) was determined based on the color complex formation of glutathione with Ellmann's reagent (Sedlak & Lindsay 1968). The glutathione disulfide (GSSG) content of the tissue homogenate was determined using the Tietze (1969) recycling assay, with glutathione reductase and NADPH as hydrogen donor. The total amount of glutathione was measured using the Sedlak & Lindsay assay (1968), and the GSSG content was calculated.

The MDA content of the tissue homogenate (1:9 in 0.65 % NaCl) was measured based on the color complex formation of malondialdehyde with 2-thiobarbituric acid in an acidic environment at high temperatures (Placer *et al.*, 1966). The standard was 1, 1, 3, 3-tetraethoxypropane (Fluka, Buchs).

In order to determine the selenium content, the samples were homogenized by chopper, and the moisture content of the sample was determined. The completely dried material (dried until reaching its constant weight at 105°C) was grinded, and 1.000 g was measured out for digestion under pressure by a vapor phase microwave process applying 1 g sample material and a mixture containing 5 ml of 63% nitric acid and 2 ml of 30% hydrogen-peroxide. The digested sample solution was then filled with distilled water up to a 10 ml volume. The measurements were carried out by a UNICAM QZ ETA spectrometer.

The vitamin E content of feed and whole fish body samples was determined according to the Hungarian National Standard 2002 and McMurray *et al.*, 1980. The samples saponified at 70°C for 30 minutes with 15 ml 60 % (w/v) KOH in the presence of 20 ml 5% (w/v) pyrogallol in absolute ethanol. The extraction was carried out with 35 ml distilled water and 25 ml light petroleum (bp. 40-70°C). The petroleum layer was taken out and evaporated under nitrogen at 40°C. Quantitative measurement was carried out using a HPLC apparatus equipped with a BST SI-100 10 µm C8 reverse phase column (BST, Budaörs, Hungary). The mobile phase was 95:5 (v:v) methanol: water at 1 ml/min flow rate. A fluorescent method (excitation: 292 nm; emission: 330nm) was used for detection. The

standard was DL- $\alpha$ -tocopherol (Sigma, St. Louis) in methanol.

All data were subjected to a one-way variance analysis (ANOVA), and differences between means were compared by the Tukey test at a 95% confidence interval ( $p < 0.05$ ). The statistical analyses were performed using the GraphPad InStat software package for Windows, release 3.01.

**Table 2:** Growth performance of the African catfish fingerlings fed on dietary excess of vitamin E and selenium (mean $\pm$ SD, n=3 replicates)

Parameters	Dietary group (30-day)			Unfed*
	Control	H-TOC/SE	H-SE/TOC	
Initial weight (g)	3.65 $\pm$ 0.08	3.71 $\pm$ 0.03	3.93 $\pm$ 0.12	3.16 $\pm$ 0.63
Final weight (g)	11.63 $\pm$ 0.46 <sup>a</sup>	10.70 $\pm$ 0.20 <sup>b</sup>	10.70 $\pm$ 0.32 <sup>b</sup>	2.60 $\pm$ 0.45 <sup>c</sup>
SGR % (d <sup>-1</sup> )	3.86 $\pm$ 0.20 <sup>a</sup>	3.54 $\pm$ 0.03 <sup>b</sup>	3.30 $\pm$ 0.00 <sup>c</sup>	-1.26 $\pm$ 0.16 <sup>d</sup>
FCR	1.80 $\pm$ 0.11 <sup>a</sup>	2.02 $\pm$ 0.09 <sup>a</sup>	2.09 $\pm$ 0.17 <sup>a</sup>	-

\* Final weight was taken after 15 days.

Values with different superscripts in the same row means significant difference at  $P < 0.05$  level

## RESULTS AND DISCUSSION

Mortality during the course of the experiment was negligible in the groups were fed with the experimental diets, while mortality of unfed fingerlings appeared by day 5 and reached 47% by day 15; furthermore, signs of cannibalism were observed. The treated groups (H-TOC/SE and H-SE/TOC) revealed significantly lower ( $P \leq 0.001$ ) final body weight after a 30-day period as compared to the control (Table 2). For that reason, the SGR was significantly lower ( $P \leq 0.001$ ) in both treated groups, and the FCR was numerically, but not significantly, higher (Table 2.). These results on the effect of dietary H-TOC/SE and H-SE/TOC on growth rate in African catfish agree with the results of previous studies on channel catfish *Ictalurus punctatu* (Gatlin & Wilson 1984). On the other hand, Roem *et al.*, 1990 reported better growth of tilapia *Oreochromis aureus* that were fed diets containing low levels of vitamin E and polyunsaturated fatty acids. The values of vitamin E and selenium in this experiment were much higher than the dietary vitamin E and Se requirements of African catfish (NRC 1993). Thus, there was an effect on the growth rate of the fish during the study.

**Table 3:** Vitamin E, selenium, malondialdehyde, glutathione content and glutathione peroxidase activity of the African catfish fingerlings fed on dietary excess of vitamin E and selenium (mean±SD, n=6-9)

Parameters	Initial	30-day			
		Control	H-TOC/SE	H-SE/TOC	Unfed (15 D)
Vitamin E <sup>1</sup>	5.32±0.46 <sup>a</sup>	5.40±0.35 <sup>a</sup>	13.18±4.86 <sup>b</sup>	5.69±0.20 <sup>a</sup>	5.34±0.67 <sup>a</sup>
Selenium <sup>2</sup>	74.18±2.90 <sup>b</sup>	88.42±2.61 <sup>a</sup>	87.80±3.24 <sup>a</sup>	171.86±5.17 <sup>c</sup>	60.62±0.92 <sup>d</sup>
GSH <sup>3</sup>	362.78±3.71 <sup>ac</sup>	300.64±44.34 <sup>a</sup>	362±7.65 <sup>ab</sup>	517.65±229.01 <sup>bc</sup>	430.14±82.00 <sup>ba</sup>
GSSG <sup>3</sup>	7.87±0.38 <sup>a</sup>	6.40±1.42 <sup>a</sup>	7.52±0.22 <sup>a</sup>	6.10±4.18 <sup>a</sup>	7.00±1.88 <sup>a</sup>
GSH/GSSG	46.13±2.30 <sup>a</sup>	50.71±21.67 <sup>a</sup>	48.23±1.43 <sup>a</sup>	145.08±109.63 <sup>b</sup>	65.47±21.30 <sup>a</sup>
GPx <sup>4</sup>	1.15±0.15 <sup>a</sup>	0.82±0.50 <sup>ab</sup>	0.57±0.04 <sup>b</sup>	1.16±0.26 <sup>a</sup>	0.82±0.43 <sup>ab</sup>
MDA <sup>5</sup>	10.14±0.33 <sup>a</sup>	4.85±1.53 <sup>b</sup>	4.00±1.45 <sup>b</sup>	4.32±0.26 <sup>b</sup>	5.21±1.52 <sup>b</sup>

Values with different superscripts in the same row means significant difference at  $P < 0.05$  value

Abbreviations: GSH= reduced glutathione; GSSG= glutathione disulphide; GPx= glutathione peroxidase; MDA= malondialdehyde. <sup>1</sup>(mg g<sup>-1</sup>), <sup>2</sup>(µg kg<sup>-1</sup>), <sup>3</sup>(µmol g<sup>-1</sup> protein), <sup>4</sup>(U g<sup>-1</sup> protein), <sup>5</sup>(mmol g<sup>-1</sup>)

The vitamin E content of the fish body in the H-TOC/SE treated group was significantly ( $P \leq 0.001$ ) higher as compared to the control and H-SE/TOC groups after the 30-day experimental period (Table 3). This effect was reflected by the dietary input, which is in agreement with studies reporting that African catfish (Baker & Davies 1997) and channel catfish (Gatalin et al., 1992) accumulate  $\alpha$ -tocopherol in the liver and muscle tissues corresponding to dietary vitamin E levels. It was interesting to find that the vitamin E content of the unfed group did not decrease during the 15-day starvation period as compared to the initial value (Table 3). This finding means that vitamin E reservoir of the tissues did not deplete during that period of time. Yoshida et al., 1992 observed that the capacity of the liver in rats to store  $\alpha$ -tocopherol continues to increase for several months after the first feeding and may be linked to induction of a hepatic tocopherol binding protein. The selenium content of the fish body also increased significantly during the 30-day feeding period in all of the groups as compared to the initial level, but it decreased significantly in the unfed group ( $P \leq 0.001$ ) during the 15-day starvation period (Table 3). This result indicates that selenium store in the fish body is depleted much more quickly than vitamin E without further addition. The H-SE/TOC group showed the highest selenium content, and the difference was significant ( $P \leq 0.001$ ) when compared to both the control and H-TOC/SE groups after the 30-day experimental period (Table 3).

The biochemistry of selenium and the glutathione system in relation to antioxidant defense has been reviewed by Arteel & Sies 2001. After the 30 day experimental period, the H-SE/TOC treated group showed significantly higher ( $P \leq 0.05$ ) GSH content than the control group, while the GSH content of the fish body did not change significantly in the other groups, including the unfed group, which was investigated for 15 days (Table 3). These results agree with previously described studies (Lygren et al., 2001, Hamre et al., 1997) with Atlantic salmon (*Salmo salar* L.). The results in the present study show that the loss of GSH is a slow process because the organism tries to maintain the free sulfhydryl group, in this case glutathione, content

of the tissues even during starvation in order to maintain the antioxidant state of the cells and tissues. Moreover, the excess amount of vitamin E/selenium did not cause higher rates of oxidation, but the excess selenium caused a moderate increase in GSH content. This increase could possibly be the result of the adaptive mechanism, a higher rate of GSH biosynthesis, against the effect of glutathione-selenium complex formation. Yet, Combs & Combs 1984 reported that an excess amount of selenium reduces the glutathione content of the animal tissues because of the formation of selenium-diglutathione to bound glutathione.

GSSG content did not change during the period of investigation or as an effect of the treatments, including starvation (Table 3). These findings support the hypothesis that the oxidative processes during starvation become lower due to the lower basal metabolic rate in that situation. The results also suggest that an excess amount of vitamin E and selenium did not cause a higher rate of GSH oxidation; namely, either it did not cause oxidative stress in the tissues or this stress was not manifested in the glutathione redox system. The value of the GSH/GSSG ratio remains unchanged in the groups (Table 3). This result suggests the well-known hypothesis that the cells of aerobic organisms try to keep the GSH/ GSSG ratio at the same level, even during a starvation period, in order to avoid the dangerous loss of the free SH group level. The value of the ratio was significantly higher ( $P \leq 0.05$ ) in the H-SE/TOC group, as compared to the H-TOC/SE group, at the end of the investigation, which caused the higher GSH content in the H-SE/TOC group.

Although the GPx activity was moderately higher in the H-SE/TOC treated group, but did not change significantly ( $P \leq 0.05$ , Table 3) during the investigation period or as a result of the treatments. These results agree with results from previous studies on channel catfish (Gatlin & Wilson 1984, Gatlin et al., 1986, Wise et al., 1993). The results from the present study suggest our previous hypothesis that the excess vitamin E or selenium did not alter the amount and/or activity of the glutathione redox system. GPx activity in blood plasma

has been attributed to a protein immunologically distinct from, but homologous to, classical GPx (Takahashi et al., 1990).

The lipid peroxidation state of the body, as measured by the MDA content, changed as an effect of age or/and maturation, but not as an effect of the treatments. This means that neither excess vitamin E nor excess selenium, in the doses that were used, nor the starvation, caused lipid peroxidation processes. This was also shown earlier by Hamre et al., 1997, who found that MDA concentration in the liver of Atlantic salmon was higher in fish fed 0 mg kg<sup>-1</sup> α-tocopherol. It was noted that rainbow trout *Oncorhynchus mykiss* liver glutathione transferase inhibited MDA formation in the NADPH-stimulated microsomal lipid peroxidation system (Bell et al., 1985).

In conclusion, it can be stated that higher vitamin E and lower selenium or lower vitamin E and higher selenium supply in the diet caused a decrease in some production traits. However, there were no changes in the amount/activity of the antioxidant defence system or in the lipid peroxide state of the bodies of African catfish fingerlings. On the other hand, short term starvation depleted the selenium but not the vitamin E stores, and it had no measurable effect on the glutathione redox system.

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