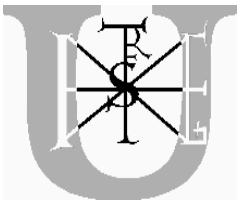

**SZENT ISTVÁN UNIVERSITY**
**FACULTY OF AGRICULTURAL AND ENVIRONMENTAL SCIENCES**
**DEPARTMENT OF FISH CULTURE & FISHERIES**

# EFFECTS OF SOME PROOXIDANTS AND ANTIOXIDANTS ON TWO FINFISH LARVAE AND FINGERLINGS

**Ph.D. Dissertation**

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# 1. INTRODUCTION

Interest in fish and shellfish nutrition has increased markedly over the past two decades, largely due to the global increase in aquaculture production. Although several cold water and warm water fish are cultured commercially, diet formulation for many of these species are based on limited information on the nutrient requirements. The need for proper diets to improve health of farmed aquatic animals is widely recognized. Nutritional and physical characteristics of diets can modulate susceptibility of fish to infectious diseases. In the most severe cases, diets that are inadequate with respect to essential nutrients (protein, amino acid, essential fatty acids, vitamins and minerals) lead to gross malnutrition and high disease susceptibility. In general, gross malnutrition is no longer a problem, however, fish nutritionists faced with the more challenging task of determining the more subtle effects those micronutrients and their interactions with other dietary components have on the fish health and growth.

Selenium is a trace element that is normally and widely distributed in aquatic system. It is now well established that selenium in both beneficial and harmful to various species, including fishes. Such effects may result from either insufficient or excessive amount of selenium in the organism. The average selenium content of natural water is usually not more than 0.1 $\mu g\ L^{-1}$, but high amount (5-130 $\mu g\ L^{-1}$) of selenium of water can cause disturbances in the whole ecosystem, including toxic symptoms in fishes (SAIKI and LOWE, 1987). Ecotoxicological effects of selenium toxicity occur at all levels of biological organisation, from molecular to ecosystem level. The bioavailability is the key factor responsible for ecotoxicological effect (FENT, 2002). Field and laboratory studies have indicated a wide variety of toxic effects of selenium in fish (BALOGH *et al*., 2002; LEMLY, 2002), which include spiral swimming, low growth rate and high mortality (TUCKER, 1998). On the other hand, selenium is also required for the normal growth and performance of fish. Selenium deficiency causes reduced growth, hemorrhagic exudates, decrease in glutathione-peroxidase activity which causes marked decrease of the activity of the biological antioxidant defense system (STADTMAN, 1991), also increases the incidence of "Hitra" disease in farmed Atlantic salmon (BELL *et al*., 1985; LOVELL, 1996; POSTON *et al*., 1976).

Ascorbic acid (AA, vitamin C) is essential for most vertebrates including fish as an important water soluble antioxidant and acts as a co-factor in various hydroxylation reactions in living tissue (SANDNES, 1992; WAAGBØ, 1994; ANDERSEN *et al*., 1998; LIM *et al*., 2000). Fish are unable to synthesize AA due to the lack of enzyme L-gulonolactone oxidase, which is necessary to convert L-gulonic acid to AA (TOLBERT, 1979; DABROWSKI *et al*., 1988), therefore, as have been indicated in numerous of studies, AA is required in diet to maintain the physiological processes of most fish (WAAGBØ, 1994). The dietary requirement for ascorbic acid depends on various factors such as fish species, fish size, growth rate, water temperature, storage conditions of diets and the occurrence of stress in the environment of fish (BLANCO and MEADE, 1980; MAHAJAN and AGRAWAL, 1980; SATO *et al*., 1983).

Iron is essential element involved in oxygen transport and cellular respiration. Iron is also involved in lipid oxidation reactions such as formation of hydroperoxides and free radical peroxides in the presence of oxygen (LEE *et al*., 1981, ANDERSEN *et al*., 1998, LIM *et al*., 2000). Increasing supplementation of ferrous sulphate has been shown to

increase lipid oxidation in diets (DESJARDINS *et al*., 1987) and concentration of lipid peroxidation product malondialdehyde (MDA) in African catfish (BAKER *et al*., 1997).

Several vitamin-mineral interactions in fish have been reported (DE SILVA and ANDERSON, 1995; LAYGREN *et al*., 1999 and 2000). The interaction between vitamin C and minerals was reviewed by HILTON (1984). The major interaction occurs in fish between ascorbic acid and iron (DE SILVA and ANDERSON, 1995). Ascorbic acid is involved in metabolism of iron metabolism as strong reducing agent which keep the ferrous iron in that highly absorbed and utilized form in fish (HARPER, 1975; MONSEN, 1982; HILTON, 1989; DE SILVA and ANDERSON, 1995; WATANABE *et al*., 1997). The apparent interaction between iron and ascorbic acid metabolism may be due to the effects of iron supplementation on diet rancidity and dietary ascorbic acid stability (DESJARDINS *et al*., 1987; DABROWSKI *et al*., 1996; WATANABE *et al*., 1997).

Vitamin E (α-tocopherol) functions as a lipid-soluble antioxidant in animal tissue, including fish (COWEY, 1986; WAAGBØ, 1994). It protects the organism 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).

Lipids are usually used to provide the necessary energy instead of proteins, in order to cover the metabolic needs of the fish and to provide essential fatty acids, which are particularly important for normal cell membrane function (SARGENT, 1995). The effects of dietary lipids in fish have been reported for both warm and coldwater fish species (DE SILVA *et al*., 1991; DE SILVA *et al*., 1991; HEMRE and SANDNES, 1999; GAYLORD and GATLIN, 2000). Data from these studies, together with recent technological advances in the fish-feed manufacturing industry, have produced commercial diets containing more than 30% lipids (ALSTED *et al*., 1995; WEATHERUP *et al*., 1997; HARDY, 1999), with satisfactory results.

Fats may contain high level of long chain polyunsaturated fatty acids (PUFAs), which are prone to peroxidation due to the presence of many double bonds. This suggests that the PUFAs found in fats will interact strongly with the antioxidant defense systems of the body leading to consumption of tissue antioxidants. However, fish oil containing n-3 PUFAs could act to induce tissue antioxidant protective mechanisms. Indeed, there exists a contradiction in the literature about whether the intake of fish oil acts to enhance (CHEN *et al*., 1994) or to deplete (IBRAHIM *et al*., 1997) tissue antioxidant defenses and the glutathione redox system in different organisms.

### 1.1. Objectives of the investigations

1. There are numerous factors in the environment which are having undesirable effects in fish production. Among those factors selenium contamination of water has also importance as industrial waste material. According to that the first objective was to investigate the effect of excess selenium on growth and mortality of fishes.
2. The effect of selenium was investigated comparatively in different age groups and also different fish species for determination of the different sensitivity.
3. The toxic effect of different selenium compounds is different as it was found in some vertebrate species. One of the objectives of my investigation was to

determine the toxic effect of different inorganic and organic selenium compounds also the effect of different route of selenium absorption on toxicity in two different fish species.

4. Investigation of the possible prooxidant and antioxidant processes occurring in African catfish during hatching and larval development.
5. Iron and ascorbic acid are essential micro-nutrients of fishes but in some extent iron also can be toxic in present of a particular amount of ascorbic acid. The mode of action of iron/ascorbic acid toxicosis is free radical generation and loading of the antioxidant defence mechanism – including glutathione redox system. According to that fact the purpose of that experiment was to investigate the possible pro-oxidant effect of different ratio between iron and ascorbic acid in African catfish fingerlings.
6. One of the objectives of my investigations was to study the effect of different ratios of vitamin E and selenium in the feed on the rate of lipid peroxidation and on the amount/activity of the glutathione redox system in African catfish fingerlings.
7. The main energy sources in fish nutrition are fats and oils. Otherwise, the different fatty acid composition of fat sources result different susceptibility of oxidation for which reason one of my objectives of my investigatoins was to study the effects of different fat sources using different ratios of dietary soybean oil/cod liver oil on the antioxidant system of African catfish fingerlings.

## 2. MATERIALS AND METHODS

### 2.1. Experiment 1. Subchronic selenium toxicity in African Catfish larvae

The experiments were done at the facilities of the Department of Fish Culture, Szent István University, Gödöllő, Hungary. Larvae of African catfish were obtained through artificial reproduction and kept in a plastic tank (200 L). Upon yolk resorption (approximately 2 days after hatching), the larvae were stocked in aquaria (volume=10 L) containing aerated dechlorinated tap water, at density of about 110 larvae per aquarium. At this time, the larvae average weight and length in the experiments were: 2.05 mg, 7.86 mm respectively. Water was changed daily, and the temperature was maintained at 27.5 ± 0.5° C.

The larvae were fed for 10 days. Feeding was done four times a day at 4-h intervals between 08:00 and 20:00 h. Before each feeding all feed remnants, faeces and debris were removed from the aquaria. Thirty aquaria (10 L) were used and each aquarium (treatment) had three replicates.

### 2.1.1. Treatments

Treatments and feeding regime were done as described in Table 1.

Table 1. Treatments and feeding of experiment 1.

| Aquarium | Treatments | Feeding |
|---|---|---|
| 1 | 2.19mg $L^{-1}$ sodium-selenite ($Na_2SeO_3$)[b], dissolved in the water | decapsulated *Artemia* cysts[a] |
| 2 | 23.9mg $L^{-1}$ sodium selenate ($Na_2SeO_4$)[b], dissolved in the water | decapsulated *Artemia* cysts[a] |
| 3 | 21.9mg $L^{-1}$ sodium-selenite ($Na_2SeO_3$)[b], dissolved in the water | decapsulated *Artemia* cysts[a] |
| 4 | 2.39mg $L^{-1}$ sodium selenate ($Na_2SeO_4$)[b], dissolved in the water | decapsulated *Artemia* cysts[a] |
| 5 | - | 300mg $day^{-1}$ selenium enriched yeast (seleno-yeast)[c] |
| 6 | - | 30mg $day^{-1}$ selenium enriched yeast (seleno-yeast)[c] |
| 7 | - | 15mg $day^{-1}$ selenium enriched yeast (seleno-yeast)[c] |
| 8 | - | sodium selenite (0.1mg $mL^{-1}$) enriched *Artemia* cysts |
| 9 | - | sodium selenite (0.05mg $mL^{-1}$) enriched *Artemia* cysts |
| 10 | No additional selenium supply and served as a control | decapsulated *Artemia* cysts[a] |

[a] Aquafauna Bio-Marine, Inc., CA, decapsulation of the cysts was done according to the method described by LAVENS and SORGELOOS (1996)

[b] Sigma, St. Louis

[c] Sel-Plex®, Alltech, Lexington

An experiment was performed regarding the enrichment of the cysts as follows: four flasks A, B, C and D were filled with 100 mL of distilled water then sodium selenite was added in different amounts: 0.1, 0.5, 1.0 and 0 mg $mL^{-1}$, respectively; after that 4 g of dried decapsulated cysts were put in each flask and after 2 hours the cysts were filtered and kept in the freezer for selenium determination. Details of the enrichment of the cysts

procedure was described previously by BARDÓCZ *et al*., (1999) when they enriched decapsulated *Artemia* cysts by vitamin C.

### 2.1.2. Measurements

The growth rate was controlled on experimental days 0, 5, 10 according to individual wet weight and length of 10 larvae randomly sampled per aquarium. The mortality rate was checked daily by counting and removing the dead larvae from each aquarium.

## 2.2. Experiment 2. Subchronic selenium toxicity in common carp larvae

The experiments were done at the facilities of the Department of Fish Culture, Szent István University, Gödöllő, Hungary. Common carp spawned eggs were obtained from fish induced to spawn by hypophysation and incubated in a separate recirculated-water in 1-L Zuger-jars at 22ºC (HORVÁTH *et al.*, 1992). After hatching, larvae were kept in a plastic tank (200 L). Upon yolk resorption (3 days after hatching), the larvae were randomly stocked in aquaria (volume= 40 L) containing aerated dechlorinated tap water, at density of about 350 larvae per aquarium. The larvae had an initial individual mean weight of 2.7±0.1 mg and length of 6.7±0.5 mm (mean ± SD).Water was changed daily and the temperature was maintained at 23±1.0ºC.

The larvae were fed for 10 days. Feeding was done four times a day at 4-h intervals between 08:00 and 20:00 h. Before each feeding all feed remnants, faeces and debris were removed from the aquaria.

### 2.2.1. Treatments

Twelve aquaria were used and each aquarium (treatment) had three replicates treated as follows:

Treatment A and B: animals fed with *Artemia* cysts (Aquafauna Bio-Marine Inc., CA) after they were being decapsulated according to the method described by LAVENS and SORGELOOS (1996). These groups were treated daily with 0.62, 0.68 mg $L^{-1}$ sodium selenite, sodium selenate (Sigma, St. Louis) respectively, dissolved in the water of the aquaria.

Treatment C: animals fed with sodium selenite (0.1 mg $mL^{-1}$) enriched *Artemia* cysts.

Treatment D: animals fed with decapsulated *Artemia* cysts and *Artemia* nauplii without additional selenium supply and served as a control.

### 2.2.2. Measurements

The growth was controlled on experimental days 0, 5 and 10 according to individual wet weight and length of 10 larvae randomly sampled per replicate. The mortality rate was checked daily by counting and removing the dead larvae from each aquarium. Specific growth rate (SGR) was computed based on the formula of RICKER (1979). By the end of the experiment fish larvae were collected and kept at –20$^{o}$C for whole-body selenium determination.

## 2.3. Experiment 3. Ontogenesis of antioxidant system in African catfish larvae

The larvae were stocked in triplicate containers (15 l) connected to re-circulation system. The water temperature was 27±1.0ºC, and oxygen was maintained near saturation level

throughout the experiment. The flow rate of water of each container was approximately 0.8 l $min^{-1}$. The light regime was maintained at a 12:12 h light: dark schedule.

### 2.3.1. Treatments

Samples of fertilised eggs (embryos) just prior to hatch (group 1.), newly hatched larvae before the onset of exogenous feeding (group 2.), feeding larvae fed on decapsulated *Artemia* cysts for one week (group 3.), larvae fed with reduced glutathione enriched *Artemia* nauplii (group 4.) for two weeks and larvae fed on starter commercial diets for three weeks (group 5.) were taken.

The enrichment of the *Artemia* with reduced glutathione was done according to the method described in STEWART *et al*. (2001).

### 2.3.2. Measurements

The samples were analysed to determine the reduced glutathione, glutathione disulphide and malondialdehyde amount also glutathione peroxidase activity.

## 2.4. Experiment 4. Effects of dietary ascorbic acid/iron on the antioxidant system in African catfish fingerlings

### 2.4.1. Experimental diets

Three purified diets were formulated to contain approximately 46.7% crude protein based on feeding stuff values reported by LOVELL *et al*. (1984) and LOVELL (1989) as follows:

Diet 1 (control) contained no added supplemental dietary ascorbic acid or iron;

Diet 2 (H-AA/FE) contained high levels of ascorbic acid (600mg $kg^{-1}$) and low levels of iron (218mg $kg^{-1}$) supplied as ferric citrate ($FeC_6H_5O_7$);

Diet 3 (H-FE/AA) contained high levels of iron (364mg $kg^{-1}$) and low levels of ascorbic acid (300mg $kg^{-1}$).

All feed ingredients were thoroughly mixed, and, after the addition of 40% water, the mixture was extruded through a pasta machine. The strands were air-dried, ground and sieved to obtain suitable particle size fractions in the range of 1-2 mm (Figure 9). The particles were stored at -20ºC until used. Diets were prepared according to the vitamin and mineral premix AA and iron contents.

### 2.4.2. Experimental animals

Fingerlings of African catfish (*Clarias gariepinus)* with an initial body weight of 3.62±0.32 g were divided randomly among 12 plastic containers (15 liters each) at a stocking density of 30 fish per container. All containers were connected to a re-circulating system. Water temperature was 27±1.0ºC, and oxygen was maintained near saturation throughout the experiment. The flow rate into each container was approximately 0.8 l $min^{-1}$ and photoperiod was maintained at a 12L:12D.

After acclimatisation to the experimental diets and conditions for one week, the fish were assigned in triplicate containers 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 for 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$^{-1}$) 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 dry feed fed (g)/wet weight gain (g). Mortality was recorded throughout the experiment.

### 2.4.3. Measurements

Feed samples were taken from each batch of diets at the start and stored at -20ºC until the analysis for ascorbic acid and iron content.
The fish samples were stored at -80ºC for determination of whole body reduced glutathione, glutathione disulphide and malondialdehyde content also for glutathione peroxidase activity (3x5 fish) as well as for ascorbic acid (3x3 fish) and iron content (3x2 fish).

## 2.5. Experiment 5: Effects of dietary vitamin E/selenium on antioxidant system of African catfish fingerlings

### 2.5.1. Experimental diets

The basal diet was prepared at the Department of Nutrition at the Research Institute for Fisheries, Aquaculture and Irrigation, 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 added supplemental dietary α-tocopherol or selenium;
Diet 2 (H-TOC/SE) contained high levels of DL-α-tocopheryl-acetate (245 mg kg$^{-1}$) and low levels of selenium (0.5mg kg$^{-1}$) supplied as sodium-selenite;
Diet 3 (H-SE/TOC) contained high levels of selenium (2.5mg kg$^{-1}$) supplied as sodium-selenite and low levels of DL-α-tocopheryl-acetate (170mg kg$^{-1}$).
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 -20ºC until used. Diets were prepared according to the vitamin and mineral premix contents of vitamin E and selenium.

### 2.5.2. Experimental animals

Fingerlings of African catfish (*Clarias gariepinus)*, with an initial body weight 3.16±0.32 g, 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±1.0ºC, and oxygen was maintained near saturation throughout the experiment. The flow rate of each container was approximately 0.8 l min$^{-1}$. The photoperiod was maintained at a 12:12 h light: dark schedule.
After their acclimatisation 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.

### 2.5.3. Sample collection and measurements

Feed samples were taken from each batch of diets at the start and stored at -20ºC 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.
Two groups of five fish were sampled from each container (30, 3 replicates each of 10 fish). The fish were sacrificed and then stored at -80ºC in order to determine whole body content of reduced glutathione, glutathione disulphide and malondialdehyde also glutathione peroxidase activity (3x5 fish), as well as for α-tocopherol (3x3 fish) and selenium (3x2 fish) content.
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 $day^{-1}$) 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= Dry feed fed (g)/wet weight gain (g). Mortality was recorded throughout the experiment.

## 2.6. Experiment 6. Effects of dietary soybean oil/cod liver oil on the antioxidant system of African catfish fingerlings

### 2.6.1. Experimental diets

The basal diet was prepared at the Department of Nutrition, HAKI. Three purified diets were formulated to contain approximately 46.7% crude protein.
Control diet contained 35 g cod liver oil (CLO) and 45 g soybean oil (SOY) per kg;
H-CLO diet contained high levels of CLO (60g $kg^{-1}$) and low level of SOY (20g $kg^{-1}$);
H-SOY diet contained high levels of SOY (60g $kg^{-1}$) and low levels of CLO (20g $kg^{-1}$).
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 until obtaining suitable particle size fractions in the range of 1-2 mm. The particles were stored at -20ºC until they were used.

### 2.6.2. Experimental animals

African catfish (*C. gariepinus)* fingerlings with an initial body weight 3.59±0.30 g, were divided randomly among 12 modified plastic containers (15 liters each) at a stocking density of 30 fish per container. All the containers were connected to a re-circulation 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. The water temperature was 27±1.0ºC, and oxygen was maintained near saturation throughout the experiment. The flow rate of each container was approximately 0.8 l $min^{-1}$. The light regime was maintained at a 12:12 h light: dark schedule.
After their acclimatisation to the experimental diet and conditions for one week, the fish were assigned in triplicate containers 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 only for 15 days.

### 2.6.3. Sample collection and measurements

Feed samples were taken from each batch of diets at the start and stored at -20ºC until an analysis of fatty acids composition was carried out. The fish were starved for 12 hours before the samples were collected at the start of the experiment and after 30 days of feeding, 15 days in the case of the unfed group. Two groups of five fish were sampled from each container (n=30, three replicates of 10 fish each). The fish were sacrificed and then stored at -80ºC to determine their whole body reduced glutathione, glutathione disulphide and malondialdehyde content also glutathione peroxidase activity (3x5 fish).
All the 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 $day^{-1}$) 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= Dry feed fed (g)/wet weight gain (g). Mortality was also recorded throughout the experiment.

## 2.7. Methods of Analyses

### 2.7.1. Determination of selenium

Selenium was extracted from the samples using microwave acid digestion apparatus (Milestone MLS-1200 MEGA) and measured using flameless atomic absorption spectrophotometry with Zeeman correction (UNICAM 939 QZ AA spectrometer) using the method of OSTER *et al.* (1988). The internal standard was egg yolk powder (reference material donated by Dr. J. Dixon, Scottish Agricultural College, Auchincruive, Scotland).

### 2.7.2. Determination of iron

To determine iron content, the samples were homogenized by chopper, and the moisture content of the sample was determined from this homogenized mixture. The completely dried material (dried until reaching its constant weight at 105ºC) was milled, and 1,000 g was measured out for digestion under pressure by a vapour phase microwave process, applying 1 g sample material and a mixture containing 5 ml of 63% nitric acid and 2ml of 30% hydrogen-peroxide. The digested sample solution was then filled with distilled water up to 10 ml volume. The measurements were carried out by a JY24 ICP spectrometer.

### 2.7.3. Determination of α-tocopherol

The tocopherol content of feed and whole fish body samples was determined according to the HUNGARIAN NATIONAL STANDARD (2002) and MCMURRAY *et al*. (1980). The samples were saponified at 70 $^{o}$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 $^{o}$C). The petroleum layer was taken out and evaporated under nitrogen at 40 $^{o}$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. The fluorescent method (excitation: 292 nm ; emission: 330 nm) was used for detection. The standard was DL-α-tocopherol (Sigma, St. Louis) in methanol.

**2.7.4. Ascorbic acid content**

The ascorbic acid content of feed, water and tissue samples was determined using a 2,4 dinitrophenyl-hydrasine reagent in an acidic solution, following the method described by OMAYE *et al*. (1979).

**2.7.5. Reduced glutathione content**

Reduced glutathione (GSH) content of the tissue homogenate (1:9 in 0.65 % NaCl) was determined after deproteinisation with trichloro-acetic acid based on the colour complex formation of glutathione with Ellmann's reagent (5,5'-dithiobis-2 nitrobensoic acid) using the method of SEDLAK and LINDSAY (1968).

**2.7.6. Glutathione disulphide content**

Glutathione-disulphide (GSSG) content of tissue homogenate was determined using the recycling assay of TIETZE (1969), with glutathione-reductase and NADPH as the hydrogen donor. The total amount of glutathione was measured using the method as described above and GSSG content was calculated.

**2.7.7. Glutathione peroxidase activity**

Glutathione peroxidase (GSHPx) (E.C.1.11.1.9) activity was measured in the 10,000 g supernatant fraction of tissue homogenate (1:9 in physiological saline), using reduced glutathione and cumene hydroperoxide as co-substrates in an end point direct assay following the LAWRENCE and BURK (1976) system. The loss of glutathione was measured using Ellmann's reagent (SEDLAK and LINDSAY, 1968). The enzyme activity was expressed as nmol glutathione oxidation per minute at 25 $^{o}$C. The enzyme activity was calculated to protein content of the supernatant fraction of tissue homogenate.

Protein content of the 10.000 g supernatant fraction of tissue homogenate (1:9 in physiological saline) was measured using the Folin-Ciocolteau phenol reagent (LOWRY *et al*., 1951). Bovine serum albumin served as the standard.

**2.7.8. Malondialdehyde content**

Malondialdehyde content of the tissue homogenate (1:9 in physiological saline) was measured based on the colour 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).

### 2.8. Statistical analysis

Descriptive Statistics was used to determine means using SPSS Graduate Pack 8.0 for Windows (SPSS Inc.USA, 1998).

All data were subjected to one-way variance analysis (ANOVA), and differences between means was calculated with the Tukey test at a 95% interval of confidence ($p<0.05$). The statistical analyses were performed using the GraphPad InStat software package for Windows version 3.01. Data are reported as mean ± SD.

## 3. RESULTS

### 3.1. Experiment 1 Subchronic selenium toxicity in African Catfish larvae

#### 3.1.1. Selenium content of the selenium-enriched decapsulated Artemia cysts

The results (Table 2) showed that selenium was diffused into the cysts and also attached on the wall of the decapsulated *Artemia* cysts. Whereas, in samples A,B,C, and D, where the dried decapsulated *Artemia* cysts were put in 0.1, 0.5, 1.0 and 0 mg $mL^{-1}$ of selenium showed 58, 70, 207 and 0.5 µg $g^{-1}$ of selenium found after the analysis of the cysts whereas D is control (no additional Se).

Table 2. Selenium content of selenium enriched decapsulated *Artemia* cysts

| | Selenium Content | |
|---|---|---|
| | µg $g^{-1}$ | Dry |
| Selenium-enriched decapsulated cysts | matter | |
| A (0.1 mg $mL^{-1}$) | 58 | 20% |
| B (0.5 mg $mL^{-1}$) | 70 | 19.9% |
| C (1.0 mg $mL^{-1}$) | 207 | 20.5 % |
| D (control, no additional Se) | 0.5 | 19.2% |

#### 3.1.2. Mortality, body weight and length

Selenium in the forms of selenite and selenate have dose-dependent effect on mortality, therefore groups 1 and 2 showed high mortality (100%) at the higher dose (21.9, 23.9 mg $L^{-1}$ respectively) while at the lower dose (2.19, 2.39 mg $L^{-1}$ respectively) in groups 3 and 4, selenite showed significant difference (Tables 3,4 ), in mortality (20%). Mortality in selenium-enriched yeast treated groups 5, 6 and 7 was significantly higher (49%, 42%, and 48% respectively) as compared to other treatments (Table 4). Selenium enriched *Artemia* cysts (0.1 mg $mL^{-1}$) in group 8 showed lower mortality (12%) than the other treatments but not significantly different from the control group, whereas larvae treated with 0.05 mg $mL^{-1}$ selenium enriched *Artemia* cysts in group 9 showed higher mortality

rate (18%) as compared to group 8. However, there were no significant differences ($P$>0.05) in mortality between treatment groups 3 and 9 (Table 4).
The body weight of the larvae in the groups (3 and 4) treated with the lower dose of selenite and selenate (2.19, 2.39 mg $L^{-1}$ respectively) and groups 8 and 9 fed with selenium-enriched *Artemia* cysts (0.05 mg $mL^{-1}$) showed no significant difference ($P$>0.05) as compared to control on day 5, but it was significantly different and lower than the control on day 10 ($P$<0.05).

Table 3. Length and wet weight (mean ± SD, n=20) of African catfish larvae treated different selenium sources at day 5

| Group number | Length (mm) | Weight (mg) |
|---|---|---|
| 3 | $9.35\pm0.62^{b}$ | $19.77\pm1.21^{ac}$ |
| 4 | $8.60\pm2.63^{b}$ | $24.90\pm1.38^{ac}$ |
| 5 | $9.00\pm1.50^{b}$ | $14.82\pm0.93^{b}$ |
| 6 | $8.10\pm0.65^{b}$ | $14.79\pm0.98^{b}$ |
| 7 | $8.79\pm0.75^{b}$ | $14.91\pm1.05^{b}$ |
| 8 | $11.40\pm2.49^{a}$ | $21.69\pm3.93^{a}$ |
| 9 | $10.80\pm2.23^{a}$ | $19.96\pm0.93^{ac}$ |
| 10 | $12.80\pm0.67^{a}$ | $24.76\pm1.13^{a}$ |

Values with same superscripts in the same column are not significantly different ($P$<0.05; $n$=20). 3: 2.19 mg $L^{-1}$ sodium selenite; 4: 2.39 mg $L^{-1}$ sodium selenate; 5: 300 mg $day^{-1}$ selenium-enriched yeast; 6: 30 mg $day^{-1}$ selenium-enriched yeast; 7: 15 mg $day^{-1}$ selenium-enriched yeast; 8: 0.1 mg $mL^{-1}$ sodium selenite enriched *Artemia* cysts; 9: 0.05 mg $mL^{-1}$ sodium selenite enriched *Artemia* cysts; 10: control.

Selenate (group 4) has significant marked effect on length, it showed slower growth up to the end of the investigation (Tables 3 and 4). The two selenium-enriched yeast fed groups (300 and 30 mg $day^{-1}$) showed that it has significant marked effect on decrease of body weight and length ($P$<0.05; Tables 3 and 4). Selenium-enriched yeast treated groups (5-7) showed the lowest body weight and length up to the end of the investigation even at lower dose (15 mg $day^{-1}$). Selenium enriched *Artemia* cysts (0.1 mg $mL^{-1}$) showed significantly the highest weight and length of the fish larvae compared to other treatments.

Table 4. Length, wet weight and mortality (mean ± SD, n=20) of African catfish larvae treated different selenium sources at day 10

| Group number | Length (mm) | Weight (mg) | Mortality (%) |
|---|---|---|---|
| 3 | 12.10±2.37$^{b}$ | 34.35±2.79$^{c}$ | 20±3.77$^{ac}$ |
| 4 | 11.50±1.08$^{b}$ | 33.14±3.96$^{c}$ | 27±2.50$^{c}$ |
| 5 | 11.40±1.17$^{bc}$ | 19.65±3.87$^{b}$ | 49±2.19$^{b}$ |
| 6 | 10.37±0.95$^{c}$ | 20.24±0.84$^{b}$ | 42±2.45$^{b}$ |
| 7 | 10.22±0.87$^{c}$ | 20.41±1.00$^{b}$ | 48±3.76$^{b}$ |
| 8 | 15.75±1.18$^{a}$ | 41.76±7.89$^{a}$ | 12±2.43$^{b}$ |
| 9 | 15.10±1.79$^{a}$ | 38.82±1.38$^{a}$ | 18±4.52$^{ac}$ |
| 10 | 16.11±1.18$^{a}$ | 53.96±3.32$^{a}$ | 11±5.05$^{a}$ |

Values with same superscripts in the same column are not significantly different ($P$<0.05; $n$=20). 3: 2.19 mg $L^{-1}$ sodium selenite; 4: 2.39 mg $L^{-1}$ sodium selenate; 5: 300 mg $day^{-1}$ selenium-enriched yeast; 6: 30 mg $day^{-1}$ selenium-enriched yeast; 7: 15 mg $day^{-1}$ selenium-enriched yeast; 8: 0.1 mg $mL^{-1}$ sodium selenite enriched *Artemia* cysts; 9: 0.05 mg $mL^{-1}$ sodium selenite enriched *Artemia* cysts; 10: control.

### 3.2. Experiment 2. Subchronic selenium toxicity in common carp larvae

Selenium as dissolved in the form of selenite or selenate in aquarium water has a relatively low effect on mortality; therefore it was significantly ($P$<0.05) higher when selenium was given in the form of selenite as compared to selenate (Tables 5). Beside, the growth rate was also the lowest in selenite treated group (Treatment A) and those larvae accumulate the highest amount of selenium (Table 5).

Selenium enriched *Artemia* cysts (Treatment C) caused low mortality and showed significantly ($P$<0.05) the highest growth rate even as compared to the control (Treatment D) group (Tables 5 and 6).

Table 5. Length, wet weight and specific growth rate (SGR) of common carp larvae treated with different selenium compounds at day 5 (mean ± SD)

| | Length (mm) | Weight (mg) | SGR (%) |
|---|---|---|---|
| Treatment A | 9.0±0.8[a] | 5.8±0.3[a] | 15.29 |
| Treatment B | 8.6±0.9[a] | 6.0±0.5[a] | 15.97 |
| Treatment C | 10.5±0.6[b] | 10.0±0.5[b] | 26.18 |
| Treatment D | 8.8±1.0[a] | 8.1±0.6[b] | 21.97 |

Values with same superscripts in the same column are not significantly different ($P$<0.05)
Treatment A: 0.62 mg $L^{-1}$ sodium-selenite dissolved in water
Treatment B: 0.68 mg $L^{-1}$ sodium-selenate dissolved in water
Treatment C: sodium-selenite (0.1 mg $mL^{-1}$) enriched *Artemia* cysts.
Treatment D: control

Table 6. Length, wet weight specific growth rate (SGR) and mortality of common carp larvae treated with different selenium compounds at day 10 (mean ± SD)

| | Length (mm) | Weight (mg) | SGR (%) | mortality (%) |
|---|---|---|---|---|
| Treatment A | 9.8±0.6[a] | 8.0±0.6[a] | 10.87 | 10±0.6[a] |
| Treatment B | 10.2±0.4[a] | 8.6±0.4[a] | 11.68 | 5.0±0.9[ab] |
| Treatment C | 14.4±0.7[b] | 17.3±0.8[b] | 18.57 | 3.0±0.6[b] |
| Treatment D | 13.2±0.8[b] | 12.3±0.5[c] | 15.23 | 0.00[b] |

Values with same superscripts in the same column are not significantly different ($P$<0.05)
Treatment A: 0.62 mg $L^{-1}$ sodium-selenite dissolved in water
Treatment B: 0.68 mg $L^{-1}$ sodium-selenate dissolved in water
Treatment C: sodium-selenite (0.1 mg $mL^{-1}$) enriched *Artemia* cysts
Treatment D: control

### 3.3. Experiment 3. Ontogenesis of antioxidant system in African catfish larvae

Reduced glutathione content and GSH/GSSG ratio was determined to be higher in embryos than at further stages of development, however both glutathione disulphide content and GSHPx activity increased in both reduced glutathione enriched *Artemia* or artificial feed fed groups (Table 7) during the development from embryo to the free swimming larvae and to the exogenous feeding larvae. Lipid peroxidation as measured by MDA in the fertilized eggs was high and decreased after hatching then increased after feeding with artificial diets and glutathione enrichment of *Artemia* (Table 7). Reduced glutathione content did not increase as effect of glutathione enrichment of *Artemia*.

Table 7. Changes of reduced glutathione, glutathione disulphide and malondialdehyde content and glutathione peroxidase activity of the African catfish eggs and larvae during development (mean ± SD)

| Group | GSH ($\mu$mol $g^{-1}$) | GSSG ($\mu$mol $g^{-1}$) | GSH/GSSG | GSHPx (U $g^{-1}$) | MDA (mmol $g^{-1}$) |
|---|---|---|---|---|---|
| 1 | 755.0±118.8$^{a}$ | 18.5±3.1$^{b}$ | 40.8± 0.7$^{a}$ | 0.37±0.1$^{a}$ | 6.42±1.5$^{a}$ |
| 2 | 260.0±2.2$^{b}$ | 32.5±1.5$^{b}$ | 8.00± 0.4$^{b}$ | 0.20±0.0$^{a}$ | 4.61±0.5$^{b}$ |
| 3 | 459.2±91.2$^{c}$ | 67.1±34.2$^{a}$ | 10.0±5.6$^{b}$ | 1.14±0.4$^{b}$ | 4.57±1.2$^{b}$ |
| 4 | 369.7±1.2$^{c}$ | 75.6±1.7$^{a}$ | 4.90±0.1$^{c}$ | 1.00±0.0$^{b}$ | 6.00±0.3$^{a}$ |
| 5 | 409.0±3.3$^{c}$ | 207.4±4.0$^{c}$ | 2.00±0.1$^{c}$ | 1.30±0.3$^{b}$ | 4.78±0.2$^{b}$ |

Groups: 1: Fertilised eggs; 2: newly hatched larvae; 3: decapsulated *Artemia* cysts feeding larvae; 4: reduced glutathione enriched *Artemia* cysts feeding larvae; 5: artificial diets feeding larvae.
Abbreviations: GSH= reduced glutathione; GSSG= glutathione disulphide; GSHPx= glutathione peroxidase; MDA= malondialdehyde.
Values with same superscripts in the same column are not significantly different ($P$<0.05)

### 3.4. Experiment 4. Effects of dietary ascorbic acid/iron on the antioxidant system in African catfish fingerlings

All diets were palatable and were readily consumed by fish throughout the experiment. All the fish, except the unfed group, were active and appeared healthy at the end of the experiment. Mortality during the course of the experiment was negligible in all fed groups, while mortality in the unfed fingerlings appeared by day 5 and reached 47% by day 15; furthermore, signs of cannibalism were observed.

There were no significant differences (P>0.05) in the average final weight among the groups (Table 8), except the unfed group, which was determined on day 15. The SGR was significantly (P≤ 0.05) lower in the H-AA/FE group compared to the other dietary groups. The FCR values did not differ significantly (Table 8). The SGR in the unfed group was the lowest and showed highly significant differences (P<0.001, Table 8) as compared to the dietary groups because of the lack of feed intake. The results of this study showed no effects of high iron (364 mg $kg^{-1}$) in the diets on the growth of *C. gariepinus* (Table 8).

After 30 days of feeding, there were significant differences in fish body ascorbic acid content between the treated groups as compared to the initial value. The H-AA/Fe dietary group had significantly higher (P≤0.001) body ascorbic acid content than the other groups (Table 9). While the whole body ascorbic acid content of fishes in the unfed group was significantly higher than that of the fish at the beginning of the trial (Table 9). The form of iron used in the present feeds was ferric iron. The results showed that the total iron content of the fish body increased significantly in comparison to the initial value, except in the unfed group (Table 9). The difference as compared to the control was significant (P≤0.001) only in the case of the H-Fe/AA group, but not with the H-AA/Fe group. The difference between the H-AA/Fe and H-Fe/AA groups was also significant (P≤0.001, Table 9).

Table 8. Live weight, specific growth rate (SGR) and feed conversion rate (FCR) of fish, initial body weight 3.62±0.32 g (mean±SD)

| Parameters | Dietary group | | | |
|---|---|---|---|---|
| | Control | H-AA/FE | H-FE/AA | Unfed* |
| Final weight (g) | 11.63±0.71[a] | 11.67±0.55[a] | 11.67±0.62[a] | 2.60±0.45[b] |
| SGR % ($d^{-1}$) | 3.86±0.82[a] | 3.62±1.22[b] | 3.84±0.69[a] | -1.26±0.16[c] |
| FCR | 1.80±0.5[a] | 1.65±0.4[a] | 1.74±0.72[a] | - |

* Final weight was taken after 15 days.
Values with same superscripts in the same row are not significantly different (*P*<0.05)

Table 9. Ascorbic acid, iron, reduced glutathione, glutathione disulphide and malondialdehyde content and glutathione peroxidase activity of whole fish fed diets with different iron and ascorbic acid content (mean ± SD)

| Parameters | Initial | 30-day | | | Unfed* |
|---|---|---|---|---|---|
| | | Control | H-AA/FE | H-FE/AA | |
| AA (mg $g^{-1}$) | 17.65±0.83$^{a}$ | 38.90±2.10$^{b}$ | 124.05±1.51$^{c}$ | 59.22±2.33$^{d}$ | 24.78±0.77$^{e}$ |
| Iron (µg $kg^{-1}$) | 71.54±1.18$^{a}$ | 93.32±1.86$^{b}$ | 92.86±1.52$^{b}$ | 223.35±2.32$^{c}$ | 63.86±1.90$^{a}$ |
| GSH (µmol $g^{-1}$) | 362.78±3.71$^{a}$ | 300.64±44.34$^{a}$ | 285.01±10.0$^{a}$ | 345.52±4.01$^{a}$ | 430.14±82.00$^{a}$ |
| GSSG (µmol $g^{-1}$) | 7.87±1.42$^{a}$ | 6.40±1.42$^{a}$ | 4.92±0.62$^{a}$ | 5.80±1.00$^{a}$ | 7.00±1.88$^{a}$ |
| GSH/GSSG | 46.13±1.22$^{a}$ | 50.71±21.67$^{a}$ | 58.50±4.24$^{a}$ | 62.00±6.20$^{a}$ | 65.47±21.30$^{a}$ |
| GSHPx ($Ug^{-1}$) | 1.15±0.15$^{a}$ | 0.82±0.50$^{ab}$ | 0.45±0.56$^{bc}$ | 0.54±0.40$^{c}$ | 0.82±0.43$^{ac}$ |
| MDA (mmol $g^{-1}$) | 10.14±0.33$^{a}$ | 4.85±1.53$^{ab}$ | 2.90±0.73$^{b}$ | 5.36±1.83$^{b}$ | 5.21±1.52$^{b}$ |

*15-day
Values with same superscripts in the same row are not significantly different ($P$<0.05)
Abbreviations: AA= ascorbic acid; GSH= reduced glutathione; GSSG= glutathione -disulphide; GSHPx = glutathione peroxidase; MDA = malondialdehyde

GSH and GSSG content, as well as the ratio between reduced glutathione and glutathione disulphide (GSH/GSSG) in the fish body, did not differ significantly (P>0.05) as compared to the initial value or to the control (Table 9.). This resulted non-significant (P>0.05) differences in the GSHPx activity of the fish body. Otherwise, the results show that the average GSH content was highest in the unfed groups. The average rate of oxidation of GSH in the H-AA/Fe group was lower than in the H-Fe/AA group. The lipid peroxidation processes decreased significantly as an effect of the AA and iron supplementation and as an effect of age and/or maturation of the fish, which is supported by the significant (P≤0.001) difference between the initial and 30-day control values of the amount of MDA. However, the lowest MDA content was in the H-AA/Fe group. The amount of MDA was also significantly (P≤0.001) lower in the unfed group as compared to the initial value.

### 3.5. Experiment 5. Effects of dietary vitamin E/selenium on antioxidant system of African catfish fingelings

The mortality during the course of the experiment was negligible for the experimental diet fed groups, 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) produced significantly lower ($P \leq 0.001$) final body weight after a 30-day period as compared to the control (Table 10). For that reason, the SGR was significantly lower ($P \leq 0.001$) in both treated groups, and the FCR was higher, but not significantly (Table 10).

The values of vitamin E and selenium in this experiment were much higher than the dietary vitamin E and Se requirements in African catfish (NRC 1993). Thus, there was an effect on the growth rate of the fish during the study.

Table 10. Growth performance of the African catfish fingerlings fed on dietary excess of vitamin E and selenium (mean±SD), initial body weight 3.59±0.30g

| Parameters | Dietary group (30-day) | | | |
|---|---|---|---|---|
| | Control | H-TOC/SE | H-SE/TOC | Unfed* |
| Final weight (g) | $11.63 \pm 0.46^{a}$ | $10.70 \pm 0.20^{b}$ | $10.70 \pm 0.32^{b}$ | $2.60 \pm 0.45^{c}$ |
| SGR % ($d^{-1}$) | $3.86 \pm 0.20^{a}$ | $3.54 \pm 0.03^{b}$ | $3.30 \pm 0.00^{c}$ | $-1.26 \pm 0.16^{d}$ |
| FCR | $1.80 \pm 0.11^{a}$ | $2.02 \pm 0.09^{a}$ | $2.09 \pm 0.17^{a}$ | - |

* Final weight was taken after 15 days.
Values with same superscripts in the same row are not significantly different ($P<0.05$)

The vitamin E content of the fish body in the H-TOC/SE treated groups increased significantly ($P \leq 0.001$) as compared to the control and H-SE/TOC groups after the 30-day experimental period (Table 11). α-tocopherol content of the unfed group did not decrease during the 15-day period as compared to the initial value (Table 11.). The selenium content of the fish body also increased significantly during the 30-day 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 period of investigation (Table 11.). 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 11).

After the 30 days 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 11).

Table 11. 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)

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

*15-day

Values with same superscripts in the same row are not significantly different ($P<0.05$)
Abbreviations: GSH= reduced glutathione; GSSG= glutathione disulphide; GSHPx= glutathione peroxidase; MDA= malondialdehyde. [1](mg $g^{-1}$), [2](µg $kg^{-1}$), [3](µmol $g^{-1}$ protein), [4](u $g^{-1}$ protein), [5](mmol $g^{-1}$).

GSSG content did not change during the period of investigation or as an effect of the treatments, including starvation (Table 11). The value of the GSH/GSSG ratio remains unchanged in the groups (Table 11). The value of the ratio was significantly higher ($P\leq0.05$) in the H-SE/TOC group, as compared to the H-TOC/SE group, at the end of the investigation, which was caused by the higher GSH content in the H-SE/TOC group. Although the GSHPx activity was a little higher in the H-SE/TOC treated group, that difference was not significant (Table 11) during the investigation period or as a result of the treatments. The lipid peroxidation status of the whole body of fishes, as measured by the MDA content, was lower in all of the groups (including unfed one) as compared to the initial value.

### 3.6. Experiment 6. Effects of dietary soybean oil/cod liver oil on the antioxidant system of African catfish fingerlings

The fatty acid compositions of the different diets are shown in Table 12. The only notable differences between the H-CLO and H-SOY diets were the preponderance in n-3 or n-6 PUFAs and the degree of unsaturation, which was higher in the diets containing H-CLO.

Table 12. Fatty acid composition of diet samples (g / 100 g )

| Fatty acids | control | H-CLO | H-SOY |
|---|---|---|---|
| *Saturated* | | | |
| C14:0 Miristic acid | 2,00 | 3,54 | 1,26 |
| C15:0 Pentadecaenoic acid | 0,18 | 0,39 | 0,12 |
| C16:0 Palmitic acid | 11,50 | 12,59 | 11,01 |
| C17:0 Heptadecanoic acid | 0,37 | 0,58 | 0,26 |
| C18:0 Stearic acid | 3,26 | 2,89 | 3,48 |
| C20:0 Arachidic acid | 0,28 | 0,23 | 0,31 |
| C22:0 Behenic acid | 0,26 | 0,16 | 0,30 |
| *Total* | 17,84 | 20,38 | 16,74 |
| *Unsaturated* | | | |
| C16:1n-7 Palmitoleic acid | 2,80 | 4,92 | 1,74 |
| C18:1n-9 Oleic acid | 20,00 | 18,04 | 20,83 |
| C20:1n-9 Eicosenoic acid | 2,57 | 4,47 | 1,72 |
| C22:1n-9 Erucic acid | 0,28 | 0,51 | 0,18 |
| C24:1n-9 Nervonic acid | 0,36 | 0,64 | 0,23 |
| Total | 26,01 | 28,58 | 24,70 |
| *Unsaturated (n-3)* | | | |
| C18:3n-3 a-Linolenic acid | 5,18 | 3,55 | 5,98 |
| C20:5n-3 Eicosapentaenoic acid | 4,32 | 7,70 | 2,62 |
| C22:6n-3 Docosahexaenoic acid | 6,30 | 11,62 | 3,87 |
| *Total* | 15,80 | 22,87 | 12,47 |
| *Unsaturated (n-6)* | | | |
| C18:2n-6t Linolelaidic acid | 0,25 | 0,44 | 0,17 |
| C18:2n-6c Linoleic acid | 35,64 | 19,79 | 43,18 |
| C20:2n-6 Eicosadienoic acid | 0,24 | 0,39 | 0,16 |
| C20:4n-6 Arachidonic acid | 4,22 | 7,55 | 2,58 |
| *Total* | 40,35 | 28,17 | 46,09 |

The different oil supplementation of the feed did not cause changes in the final weight after a 30-day period as compared to the control, except in the H-SOY group, which showed a significantly ($P \leq 0.05$) heavier final weight than the other groups.
SGR was significantly lower ($P \leq 0.001$) in the H-SOY group, as compared to both the control and the H-CLO groups. The FCR were moderately, but not significantly, lower in the treated groups as compared to the control (Table 13). The mortality during the course of the experiment was negligible for the experimental diet fed groups, while the mortality of the unfed fingerlings appeared by day 5 and reached 47% by day 15; furthermore, signs of cannibalism were observed.

Table 13. Growth performance of the African catfish fingerlings fed on different dietary fat sources (mean±SD), initial body weight 3.59±0.30.

| Parameters | Dietary group | | | |
|---|---|---|---|---|
| | Control | H-CLO | H-SOY | Unfed* |
| Final weight. (g) | 11.63±0.46[a] | 11.22±0.10[a] | 11.80±0.22[a] | 2.60±0.45[b] |
| SGR % ($d^{-1}$) | 3.86±0.20[a] | 3.56±0.07[b] | 3.86±0.09[a] | -1.26±0.16[c] |
| FCR | 1.80±0.11[a] | 1.69±0.25[a] | 1.60±0.15[a] | - |

*Final weight was taken after 15 days

Values with same superscripts in the same row are not significantly different ($P$<0.05)

The GSH content of the fish body did not change significantly during the 30-day period in the treated groups or during the 15-day period in the unfed group (Table 14). However, the unfed control did not show a significantly higher GSH content compared to the other groups. The GSH content was lower (P≤0.05) in the cod liver oil group than in all other groups.

The GSSG content did not change as an effect of aging or starvation (Table 14). The different fat sources with different fatty acid profiles caused significant changes. GSSG was higher (P≤0.001) in the H-CLO supplemented group than in either the control or the H-SOY groups.

The value of the GSH/GSSG ratio did not change as an effect of aging or starvation, but it was markedly affected by the different fatty acid sources (Table 14). The H-CLO supplemented group showed the lowest GSH/GSSG ratio. Otherwise, the unfed group showed the highest average ratio value.

The GSHPx activity did not significantly decrease as an effect of age and/or maturation, and it increased significantly (P≤0.05) as an effect of H-SOY supplementation as compared to the control and H-CLO (Table 14).

The lipid peroxidation state of the body, as measured by the MDA content, changed as an effect of age and/or maturation also as effect of the treatments. The differences compared to the control were not statistically significant, but in the H-CLO group, the MDA content was significantly (P≤0.01) lower than in the H-SOY group (Table 14).

Table 14. Reduced glutathione, glutathione disulphide and malondialdehyde content and glutathione peroxidase activity of the African catfish fingerlings fed on different dietary fat sources (mean±SD)

| | GSH[1] | GSSG[1] | GSH/GSSG | GSHPx[2] | MDA[3] |
|---|---|---|---|---|---|
| Initial | 362.78±3.71$^{a}$ | 7.87±0.38$^{a}$ | 46.13±2.30$^{a}$ | 1.15±0.15$^{ac}$ | 10.14±0.33$^{a}$ |
| Unfed-15 d | 430.14±82.00$^{a}$ | 7.00±1.88$^{a}$ | 65.47±21.30$^{a}$ | 0.82±0.43$^{a}$ | 5.21±1.52$^{b}$ |
| Control-30 d | 300.64±44.34$^{a}$ | 6.40±1.42$^{a}$ | 50.71±21.67$^{a}$ | 0.82±0.50$^{a}$ | 4.85±1.53$^{b}$ |
| H-CLO-30 d | 264.50±2.81$^{b}$ | 17.04±0.53$^{b}$ | 15.53±0.60$^{b}$ | 0.70±0.06$^{a}$ | 3.01±0.14$^{b}$ |
| H-SOY-30 d | 344.60±8.04$^{a}$ | 6.36±0.16$^{a}$ | 54.20±1.00$^{a}$ | 1.44±0.05$^{bc}$ | 5.35±0.10$^{b}$ |

Values with same superscripts in the same column are not significantly different ($P$<0.05)
Abbreviations: GSH= reduced glutathione; GSSG= glutathione disulphide; GSHPx= glutathione peroxidase; MDA= malondialdehyde. [1](μmol $g^{-1}$ protein), [2](u $g^{-1}$ protein), [3](mmol $g^{-1}$).

## 4. DISCUSSION

### 4.1. Experiment 1. Subchronic selenium toxicity in African catfish larvae

The results showed that dissolved selenium content decreased gradually which may be caused by the formation of volatile selenium compounds (FAN *et al*., 1997) and also by the intake and/or diffusion into the larvae. The results showed that sodium-selenate is more stable as compared to sodium-selenite and also suggest that the animals absorb more selenite than selenate from the water. Using the selenium enriched decapsulated *Artemia* cyst the selenium content of enriched cysts was the highest at the time of feeding which means that selenium was present either attached on the wall of the cysts or inside them and partly dissolved into water.

The results of this experiment also showed that decapsulated *Artemia* cysts would be a good carrier of selenium for African catfish larvae and the incorporation of selenium into *Artemia* cyst showed a dose-dependent manner. These results support the previous proposal of BARDÓCZ *et al*. (1999) about the decapsulated *Artemia* cysts as possible carrier of biologically active materials (ascorbic acid) for young fishes.

Selenium uptake from water is very rapid especially when present in sublethal concentrations. However, equilibrium concentrations in tissues are not likely reached for at least 60 days (LEMLY, 1982).

The treatment of larvae with different chemical forms of selenium showed that first it has a dose dependent manner and also showed that selenium diffuse into the larvae both through the intestinal tract and also through the skin and/or gill lamellae. The moderate, but not significant difference in the mortality rate of two inorganic selenium

sources can be explained by the different stability of selenite and selenate in water solution, because more stable form absorbed effectively for a longer period of time. In respect of weight gain and body length the two inorganic selenium species both decrease growth rate of African catfish larvae. The effect of organic selenium source – selenium-enriched yeast – which contains mainly seleno-methionine, showed that through the gastrointestinal tract that form of selenium can also be absorbed and may cause toxicity and marked decrease in the growth rate of larvae.
It has been hypothesized that selenium is being incorporated into selenoproteins (LUNDE, 1972). The time periods for selenium up take and deposition to various tissues differs, thus acute toxicity tests may be the results of variable peripheral involvement of factors (i.e., superficial gill tissue, differential levels of immediate metabolic stress resulting from rapid physiological or chemical changes) and not reflect true interspecific sensitivity differences.
The inorganic selenium in selenium enriched *Artemia* cysts showed significantly lower mortality than other treatments, which suggest that *Artemia* cysts can be enriched with a dose of 0.1 mg $mL^{-1}$ selenium without undesirable effects, namely toxicity. The above mentioned dose of selenium for enrichment of *Artemia* cysts can also improve the growth rate of the African catfish larvae.

### 4.2. Experiment 2. Subchronic selenium toxicity in common carp larvae

The results of this experiment showed that the effect of different selenium sources was almost the same in common carp as it was found in the case of African catfish larvae. Selenite and selenate dissolved in aquarium water have relatively low effect on mortality, but it was significantly ($P<0.05$) higher in the case of selenite as compared to selenate. That result supports some previous findings which showed that selenite is one of the most toxic selenium compounds in other animal species as compared to other selenium compounds including organic ones, such as selenium enriched yeast (MAHAN and PARRETT, 1996; LÁSZTYITY *et al*., 1998). Beside the lower survival rate, the growth rate was also the lowest in the selenite treated group. The possible cause of the highest mortality and highest depressive rate of growth is that larvae in that group accumulate the highest amount of selenium.
In freshwater organisms, approximately 36% of total selenium is present as selenate, the rest as selenite and selenide (EISLER, 1985). Selenium can occur as its elemental state, although the most probable form is as an inorganic selenite because of its low redox potential. Selenite is generally more toxic to early life stages (NIIMI and LAHAM, 1976) and at higher temperatures (ADAMS, 1976). The biochemical mechanism of selenium toxicity is not clear yet, but some previous investigations suggested that selenium – particularly selenite – react with some thiol compounds (e.g glutathione) and inhibits the protein synthesis (VERNIE *et al*., 1975). It was found first in the literature that gastrointestinal absorption of selenium enriched *Artemia* cysts caused low mortality and showed significantly ($P<0.05$) the highest growth rate even as compared to the controls. That result suggests that selenium from decapsulated *Artemia* cysts release and absorb slowly from the gastro-intestinal tract and accumulate significantly ($P<0.05$) smaller amount in the body of carp larvae. The positive effect of selenium enriched *Artemia* cysts suggested, that selenium demand more accurately the actual requirement of the animal.

The accumulation of selenium into the whole body of larvae was the lowest in the case of selenium-enriched *Artemia* cyst treatment. It means that the absorption of selenium is more effective through the skin and/or gill directly from water –as supported by some previous results (HODSON and HILTON, 1983) - than from the gastrointestinal tract after intake and ingestion of *Artemia* cysts.

### 4.3. Experiment 3. Ontogenesis of antioxidant system in African catfish larvae

There are few previous studies in other fish species that have been undertaken to examine the glutathione redox system in fish larvae. In the present study, first in the literature about African catfish, it was found that the amount and/or activity of the components of the glutathione redox system were higher in embryos as compared to the larval stages and may be indicative of decreasing need to detoxify $O^{\cdot}{}_2^-$ during development from embryo to feeding larvae. The possible explanation of that finding is the different oxygen loading. It was found previously that following the fertilisation, the oxygen uptake by turbot embryo increases (PETERS and LIVINGSTONE 1996) and peaks after the embryo hatches. Increasing GSH activity during the development of the embryo to larvae would indicate a progressive need to remove oxygen free radicals ($H_2O_2$ and lipid peroxides) from the tissues also as an effect of the diet given to the larvae. During turbot embryo development, rates of oxygen consumption increased 3-fold from fertilization to hatching and then reached a maximum between 3-4 days post hatch (RØNNESTAD *et al*., 1992). The increased rate of oxygen uptake may increase prooxidant processes such as $O_2^-$ production, which in African catfish are counteracted by GSHPx activity present in the embryos and larvae. Oxygen availability has been demonstrated to be one of the factors effecting embryo hatching (YAMAGAMI, 1988). That hypothesis supports the finding of the present study that the marker of lipid peroxidation, malondialdehyde content, increased as effect of aging and/or as effect of the diets given to the larvae.
The results of that experiment also showed that glutathione enrichment of decapsulated *Artemia* is not a useful way to improve the amount of the glutathione redox system of larvae.

### 4.4. Experiment 4. Effects of dietary ascorbic acid/iron on the antioxidant system in African catfish fingerlings

The results of this experiment showed that the different ascorbic acid and/or iron content of the diets did not cause toxicity because of the negligible rate of mortality; also there were no significant differences in the final weight during the course of the experiment. The specific growth rate showed significantly lower value in the high ascorbic acid / low iron treated group which supports previous hypothesis about the importance of iron during the growing period of catfish (NRC, 1993). On the other hand the ascorbic acid content of experimental diet in that group was higher than the dietary vitamin C requirement of Channel catfish (*Ictalurus punctatus)* (LIM and LOVELL, 1978; NRC, 1993; ROBINON and LI 2002). That low specific growth rate is in contrast with earlier studies showed that no growth effects were found by adding dietary ascorbic acid beyond the requirement in Atlantic salmon *Salmo salar* L. (SANDNES *et al*., 1992). The results of this study also showed no effects of high iron in the diets on the growth of *C.*

*gariepinus*. This finding is in agreement with previous studies on iron nutrition in *Ictalurus punctatus* (LIM *et al*., 2000) and *Salmo salar* (ANDERSEN *et al*., 1996, ANDERSEN *et al.* 1998).

After 30 days of feeding, there were significant differences in fish body ascorbic acid content between the treated groups as compared to the initial value. The high ascorbic acid supply caused significantly ($P \leq 0.001$) higher body ascorbic acid content than the other groups. Similarly, SANDNES *et al*. (1992) reported that the concentration of ascorbic acid in the liver of *Salmo salar* clearly demonstrated a relationship between dietary levels and liver ascorbic acid content. In the study of SANDNES *et al*. (1992), contrary to our results, no tissue saturation of ascorbic acid was observed which can be explained by the species' differences.

The results showed that the total iron content of the fish body increased significantly in comparison to the initial value. The difference as compared to the control was significant ($P \leq 0.001$) only in the case of the high iron supplemented group. The increase of the fish body iron content found in this experiment might be explained either by more intestinal residue iron in the dietary groups or by the absorption of ferric iron (supplied in this experiment) through the intestine, thus affecting the body content of iron. This finding contrasts with the results of an earlier study by BAKER *et al.* 1997, which showed that liver as well as muscle and serum iron concentrations of *Clarias gariepinus* were unaffected by feeding diets containing 663.5 or 6354.4 mg iron $kg^{-1}$, using ferrous iron.

The form of iron naturally present in fish and fish meal is mainly hem bound iron (STEFFENS, 1989). Hem and non-hem iron have different absorption mechanisms (CONDRAD and UMBREIT, 1993). Intestinal absorption of hem iron is not affected by ascorbic acid, while absorption of non-hem iron has been found to be enhanced by ascorbic acid (BOTHWELL, 1995). DABROWSKI and KOCK (1989) showed that ascorbic acid increased the absorption of $Fe^{2+}$ in rainbow trout, while ANDERSEN *et al*. (1998) found no effect of increasing dietary ascorbic acid on the absorption of dietary iron in fish, when ferrous iron was used. The late hypothesis was supported by the results of the present study.

Otherwise, the rate of mortality in the unfed fingerlings was really high and signs of cannibalism were also observed and the final weight was significantly lower. The specific growth rate of the fishes in the unfed group was the lowest as compared to the dietary groups because of the lack of feed intake. The whole body ascorbic acid content of unfed fishes at the $15^{th}$ day of experiment was significantly higher than the initial value. Similarly, a relatively high concentration of ascorbic acid in the bodies of common carp *Cyprinus carpio* L. larvae kept unfed was found after 7 days of feeding trials (GOUILLOU-COUSTANS *et al*., 1998). Plausible explanations for such prevalence would be cannibalism during the starvation period. Otherwise the iron content of the whole body did not show differences in the unfed group.

The high iron content of the diets may initiate lipid peroxidation processes and can be cause impairment of the amount and/or activity of the glutathione redox system (BAKER *et al*., 1997). The interactive effect of ascorbic acid on iron metabolism is also known (DE SILVA and ANDERSON, 1995). The results of present study showed that GSH and GSSG content, as well as the ratio between reduced glutathione and glutathione disulphide in the fish body, did not differ significantly as compared to the initial value or to the control. This result suggests that the age and/or maturation process in fish and the

different ratio between ascorbic acid and iron in the feed did not cause significant differences in the synthesis and oxidation of glutathione. This hypothesis also is also supported by the non-significant differences in the GSHPx activity, which means that the supplementation of feed with different amounts of ascorbic acid and iron did not alter the amount and/or activity of the glutathione redox system. The sensitivity of GSH metabolism to vitamin C supplementation was low, according to a study on *Salmo salar* (HAMRE *et al*., 1997). Otherwise, the results show that the average GSH content was highest in the unfed groups, which can be explained as an effect of different age and/or maturation, as the unfed group samples were taken after 15 days of the experimental period, or partly as a result of cannibalism or starvation stress. Many studies have reported changes in antioxidant enzyme activities in relation to season, age and/or maturation (RITOLA *et al*., 2002), e.g., in rainbow trout *Oncorhynchus mykiss* (Walbaum) (OTTO and MOON, 1996). The average rate of oxidation of GSH in the high ascorbic acid / low iron group was lower than in the high iron / low ascorbic acid fed, which supports the hypothesis regarding the well-known antioxidant effect of ascorbic acid (SANDNES *et al*., 1992). This phenomenon is also supported by the lower average activity of GSHPx in the high ascorbic acid / low iron group.
The rate of the lipid peroxidation processes was significantly lower as an effect of the ascorbic acid and iron supplementation and as an effect of age and/or maturation of the fish. That result is supported by the significant ($P \leq 0.001$) difference between the initial and 30-day control values of the amount of MDA. However, the lowest MDA content was found in the high ascorbic acid / low iron group, which supports the hypothesis regarding the antioxidant capacity of ascorbic acid, as indicated earlier (ANDERSEN *et al*., 1998). At high concentrations, ascorbic acid reacts directly with peroxy radicals at the lipid/water interface, resulting in a decreased production of total MDA (REED, 1992). CHO and CHOI (1994) showed that organisms that were fed fish oil consumed more glutathione than those that were fed soy oil, which strongly emphasises the importance of considering water soluble antioxidants when discussing fatty acid peroxidation *in vivo*. The amount of MDA was also significantly ($P \leq 0.001$) lower in the unfed group as compared to the initial value, which was possibly caused by the lack of fatty acid and carbohydrate intake and a decrease in fat stores during the starvation period. A lack of fatty acids means a lack of substrate for oxidation, therefore effect on MDA level as it was found in mammals (DWORSCHÁK *et al*., 1988). The metabolic rate also decreased as an effect of starvation, which caused a lower rate of oxygen free radical formation in the cells and, therefore, a lower rate of fatty acid oxidation.

### 4.5. Experiment 5. Effects of dietary vitamin E/selenium on antioxidant system of African catfish fingelings

The results of this experiment, which is the first in the literature with African catfish, showed that the different vitamin E and/or selenium content of the diets did not cause toxicity because of the negligible rate of mortality. Otherwise, both treated groups (H-TOC/SE and H-SE/TOC) showed significantly lower ($P \leq 0.001$) final weights after a 30-day period as compared to the control which means that the applied dose of vitamin E and/or selenium have some undesirable effect. The specific growth rate was also

significantly lower (P≤0.001) in both treated groups.The feed conversion rate was moderately, but not significantly, higher as compared to the control value.

These results on the negative effect of dietary tocoherol and/or selenium on growth rate in African catfish agree with the results of previous studies on Channel catfish (GATLIN and WILSON, 1984). ROEM *et al*. (1990) also reported better growth of tilapia (*Oreochromis aureus* Steindachner) that was fed diets containing low levels of vitamin E and polyunsaturated fatty acids. Explaining the results of present experiment it can be stated that the amount of vitamin E and selenium in the feed used in this experiment was much higher than the dietary vitamin E and Se requirements of catfish (NRC, 1993).

The rate of mortality of the unfed fingerlings appeared by day 5 and reached 47% by day 15; furthermore, signs of cannibalism were observed. For that reason the body weight, specific growth rate were also the lowest in that group because the lack of feed intake.

The vitamin E supplementation was effective which showed by the α-tocopherol content of the whole fish body. The highest value was found in the H-TOC/SE treated group which differed significantly (P≤0.001) as compared to the control also to the H-SE/TOC groups. The effective accumulation of α-tocopherol is in agreement with previous studies reporting that African catfish (BAKER and DAVIES, 1997) and channel catfish (GATALIN *et al*., 1992) accumulate α-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 period as compared to the initial value. This finding shows that the 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 α-tocopherol continues to increase for several months after the first feeding.

The selenium accumulation was also effective and its content in the whole fish body increased significantly during the 30-day period in both treated and control groups as compared to the initial level. Selenium accumulation showed a dose-response manner because the H-SE/TOC group showed the highest selenium content as compared to the H-TOC/SE also to the control groups after the 30-day experimental period. Contrary to vitamin E, selenium content decreased significantly in the unfed group during the 15-day period of investigation. This result indicates that selenium store in the fish body is depleted much more quickly than vitamin E without further addition.

Selenium has a direct effect on the glutathione redox system, while vitamin E acts only indirectly as the main lipid-soluble antioxidant compound in the cells (ARTEEL and SIES, 2001). That effect of selenium is supported by the results of present study when it was found that high level of selenium supplementation resulted significantly higher (P≤0.05) GSH content than the control group, while did not change significantly in the other, including the unfed groups. These results agree with the results of some previous studies on Atlantic salmon (LYGREN *et al*., 2001; HAMRE *et al*., 1997). 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 and 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. The results of the unfed group showed 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 which support the finding that GSSG content did not change in the treated groups during the period of investigation. Starvation also did not alter the GSSG content which support the hypothesis that the oxidative processes during starvation become lower due to the lower basal metabolic rate. 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. This result suggests the well-known hypothesis that the aerobic cells maintain the GSH/ GSSG ratio at the same level, even during starvation order to avoid the 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 GSHPx activity as seleno-enzyme was a little higher as effect of the high selenium supplementation its activity did not change significantly during the investigation or as a result of the treatments. These results agree with results from previous studies on channel catfish (GATLIN and WILSON, 1984; GATLIN *et al*., 1986; WISE *et al*., 1993). The results from the present study support my previous hypothesis that the excess vitamin E or selenium as applied in present study did not alter the amount and/or activity of the glutathione redox system.

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 supports my previous hypothesis that neither excess vitamin E nor excess selenium in the doses that were used and even the starvation did not cause measurable increase in the lipid peroxidation processes of fishes. This was also shown earlier by HAMRE *et al*. (1997), who found that MDA concentration in the livers of Atlantic salmon, was higher in fish fed α-tocopherol free diet.

### 4.6. Experiment 6. Effects of dietary soybean oil/cod liver oil on the antioxidant system of African catfish fingerlings

The fatty acid compositions of the different diets showed that the high soybean oil diet contains more n-3 or n-6 PUFAs but the higher total percentage of unsaturated fatty acids was in the diet containing higher amount of cod liver oil. The different oil supplementation of the feed showed that high soybean diet with higher essential fatty acid content caused not significantly higher final weight than the other groups. The apparent digestibility of fats from different sources (fish oil, soybean oil) is the same (CRUZ, 1975) but approximately 1-2 % linolenic acid is required for optimal growth of catfish (SATOH *et al*., 1989) while higher can cause the opposite effect. This is supported by results of present study that significantly lower ($P \leq 0.001$) specific growth rate resulted in the highest unsaturated fatty acid, cod liver, containing, diet. These results are similar to those obtained in *Clarias gariepinus* fed dietary palm oil (LIM *et al*., 2001), seabass (*Dicentrarchus labrax* L.) fed on different dietary oils (PARPOURA and ALEXIS, 2001), and Eurasian perch (*Perca fluviatilis* L.) fed high dietary fat levels (XU *et al*., 2001).

The mortality during the course of the experiment was negligible for the experimental diet fed groups, while the mortality of the unfed fingerlings appeared by day 5 and reached 47% by day 15; furthermore, signs of cannibalism were observed.

Different fat sources in the feed change the fatty acid composition of the fish body and its susceptibility to oxidation (SANT'-ANA and MANCINI-FILHO, 2000). The results of present investigation support some previous hypothesis that different fatty acid profiles produce different levels of susceptibility to fatty acid oxidation and oxygen free radical formation, which can activate the glutathione redox system.

The GSH content was lower ($P \leq 0.05$) in the cod liver oil supplemented feed consumed group as compared to the soybean group. This means that a different fatty acid profile, and, therefore, different oil oxidation susceptibility, caused different oxidation and /or formation of glutathione. The GSH content of the fish body did not change significantly during the 15-day period in the unfed group. That result means that the organism tried to maintain the amount of the free sulfhydryl (SH) groups, in this case glutathione, in the tissues, even during starvation in order to maintain the antioxidant state of the cells and tissues.

GSSG content did not change as an effect of aging or starvation. The different fat sources with different fatty acid profiles otherwise caused significant changes. GSSG content was higher ($P \leq 0.001$) in the high cod liver oil supplemented group than in either the control or the high soybean oil supplemented groups. These findings support the above-mentioned hypothesis regarding the oxidative processes of different fat sources and it means that high cod liver oil not only increase the oxidation but also the recycling and/or synthesis of glutathione.

The value of the GSH/GSSG ratio did not change as an effect of aging or starvation, but it was markedly affected by the different fatty acid sources. The high cod liver oil supplemented group showed the lowest GSH/GSSG ratio, which means that cod liver oil causes the higher rate of glutathione oxidation. Otherwise it was also found that the unfed group showed the highest average ratio value, which suggests the above mentioned 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 a dangerous loss of the free SH group level. The above finding does not agree with some previous data from mammalian species, where starvation was described as an inducer of free radical formation (KERCKAERT *et al*., 1982) and had a depleting effect on glutathione (IGARASHI *et al*., 1983) and GSHPx activity (GODIN and WOHAIEB, 1988).

The GSHPx activity did not decrease significantly as an effect of age and/or maturation, but it increased significantly ($P \leq 0.05$) as an effect of high soybean oil supplementation as compared to the control and high cod liver oil. This is in contrast to previous studies which showed that GSHPx activity in rat liver fed on dietary fish oil decreased significantly (LUOSTARINEN *et al*., 1997; VENKATRAMAN and PINNAVAIA, 1998; GONZALEZ *et al*., 1992). The results suggest that in the case of high cod liver oil supplementation, the oxygen free radical (e.g. alkyl-peroxyl and alkyl-hydroperoxyl radicals) level was higher because of the high unsaturated fatty acid content, and the glutathione recycling system was not adequate, while in the case of high soybean oil supplementation the activation of GSHPx, together with the recycling enzymes (e.g. glutathione reductase), keeps the glutathione content stable.

The lipid peroxidation state of the body, as measured by the MDA content, changed as an effect of age and/or maturation and as an effect of the treatments. The differences as compared to the control were not statistically significant, but in the high cod liver oil group, the MDA content was significantly ($P \leq 0.01$) lower than in the high soybean oil group. This finding supports my above-mentioned hypothesis regarding the changes produced in the antioxidant, mainly glutathione redox system using different fat sources with different fatty acid profiles and different susceptibility to oxidation. The lower MDA content means that the antioxidant defence was more effective in the high cod liver oil group, on the other hand, the process caused a higher rate of glutathione oxidation. MOURENTE *et al*. (2000) showed that altering the PUFA/vitamin E ratios in gilthead sea bream livers significantly affected their peroxidation status. Fish fed a diet rich in PUFA and low in vitamin E showed higher values of lipid peroxidation without, however, producing significant effects on liver antioxidant defence enzyme activities. In the present study the vitamin E supply was the same in all groups for that reason some alteration in the PUFA/vitamin E ratio would be possible and can be a further possible cause of different response to different oil supplementation.

## 5. New scientific results

1. The rate order of selenium accumulation in African catfish and common carp larvae is selenite> selenate> seleno methionine from selenium enriched yeast > selenium enriched decapsulated *Artemia* cysts.
2. It can be stated that the amount of ascorbic acid and iron in the diet of African catfish fingerlings can be increased to the value of 591 mg $kg^{-1}$ ascorbic acid and 360 mg $kg^{-1}$ iron (supplied as ferric citrate) without any negative effects.
3. High vitamin E (248 mg $kg^{-1}$) and high selenium (2.31 mg $kg^{-1}$) supplies in the diet caused a decrease in growth rate of African catfish fingerlings without effect on the amount / activity of glutathione redox system.
4. Short term starvation – 15 days - depleted the selenium but not the vitamin E stores of African catfish fingerlings.
5. High cod liver oil (60 g $kg^{-1}$) supplementation of the diet of African catfish fingerlings caused changes in the glutathione redox system which showed some oxidative loading of the cellular antioxidant defence.

## 6. SUMMARY

The results of experiments with African catfish and common carp larvae showed and supported some previous findings with other animal species that selenium in the form of selenite is the most toxic form.

It was found that the rate order of selenium accumulation in larvae is selenite> selenate> seleno methionine from selenium enriched yeast > selenium enriched decapsulated *Artemia* cyst.

The results suggested and supported some previous studies in different species that the absorption of selenium is more effective through the skin and/or gill directly from water

than from the gastrointestinal tract after intake and ingestion of selenium enriched yeast or *Artemia* cysts.

The results also showed, first in the literature, that decapsulated *Artemia* cysts would be a good carrier of selenium for African catfish larvae and the incorporation of selenium into *Artemia* cyst showed a dose-dependent manner. *Artemia* cysts can be enriched with a dose of 0.1 mg $mL^{-1}$ selenium without undesirable effects, namely toxicity. The above mentioned dose of selenium for enrichment of *Artemia* cyst also improves the growth rate of the larvae.

It was found that contrary to selenium glutathione enrichment of *Artemia* is not a useful way to improve the amount of the glutathione redox system of African catfish or common carp larvae. Also, it was observed that reduced glutathione content and GSH/GSSG ratio was higher in embryos than further stages of development of African catfish, however both glutathione disulphide content and GSHPx activity increased in both reduced glutathione enriched *Artemia* or artificial feed fed groups during the development from embryo to the free swimming larvae to the exogenous feeding larvae. Lipid peroxidation as measured by MDA in the fertilized eggs was high and decreased after hatching

Results of the experiment with African catfish fingerlings shows that the tissue stores of ascorbic acid and / or iron increased as a result of feed supplementation, and the ratio between them did not alter the actual lipid peroxidation processes and the amount / activity of the glutathione redox system. The higher amount of ascorbic acid suggests some well known antioxidant effect.

It can be stated based on the results of experiments that the amount of ascorbic acid and iron in the diet of African catfish fingerlings can be increased to the value of 591 mg $kg^{-1}$ ascorbic acid and 360 mg $kg^{-1}$ iron (supplied as ferric citrate) without any negative effects.

It can be stated that high vitamin E (248 mg $kg^{-1}$ and low selenium (0.48 mg $kg^{-1}$) and the low vitamin E (173 mg $kg^{-1}$) and high selenium (2.31 mg $kg^{-1}$) supplies in the diet caused a decrease in some production traits of African catfish but did not cause changes in the amount/activity of the glutathione redox system or in the lipid peroxide state of the whole body of African catfish fingerlings.

It was also found that short term starvation – 15 days - depleted the selenium but not the vitamin E stores of African catfish fingerlings but it has no effect on the glutathione redox system.

The different oil supplementations (cod liver oil, soybean oil) of the diet of *Clarias gariepinus* fingerlings did not show significant changes in the production traits and also did not significantly alter the lipid peroxidation state of the whole body. However, the changes in the glutathione redox system showed some oxidative loading of the cellular antioxidant defence mainly as an effect of cod liver oil supplementation.

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