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INVESTIGATION ON THE EFFECTS OF SOME NUTRITIONAL FACTORS OF SELENIUM TOXICITY IN SOME VERTEBRATE FARM ANIMAL SPECIES

Ph.D. thesis

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1. SCIENTIFIC BACKGROUND

Toxicoses caused by selenium occur on several parts of the world. A part of these have a natural origin, which means that selenium taken up by plants of seleniferous fields leads to toxicosis in domestic animals. Other part of the toxicoses can be connected to environmental pollution and non-proper agricultural management caused by humans. Irrigation of seleniferous soils can result leaching out of selenium, which may later enrichen in swamps and lakes, leading to toxicity symptoms in animals /e.g. fish (May et al., 2001; Lemly, 2002) and birds (Ohlendorf et al., 1988; O'Toole and Raisbeck, 1997)/. Selenium can be enrichen in the environment due to burning of fossil fuels, as reported by Hodson (1988) and Terry et al. (2000). Importance of this problem is enhanced by the fact that only in California (USA) approximately 10.000 wild birds a year die due to selenium toxicosis (Bobker, 1993).

In Hungary – since our soils belong to the soils with low selenium concentration – in most cases, selenium toxicosis is caused by technological problems during the production of mixed feeds. One of the probable reasons is that, in order to avoid lack of selenium the inorganic or organic selenocompounds are mixed in the feed at non-proper rate. Other probable reason is the inhomogeneous mixture. Cases like this have occurred to some domestic animals (e.g. swine, broilers etc.) (Sályi et al., 1988; Sályi et al., 1993).

Reason of toxicity of selenium has been interesting for researchers for a long time. First Painter (1941), than Ganther (1968) reported, that the toxicity of selenium (selenite) is caused by its interaction with thiols, which results selenotrisulphides (RSeSR). According to the *in vitro* researches of Seko et al. (1989) while selenite is converted to selenide in the cells, it reacts several times with glutathione, than in presence of oxygen selenide is converted to selenium generating superoxid radical ($O_2^{\cdot -}$). This free radical reacts with unsaturated fatty acids of cell membranes, and breaks their integrity. Selenium toxicosis (acute or chronical) turns up when level of oxidative damage exceeds the capacity of antioxidant defense system, or exceeds the ability of the organism to build the potentially reactive selenocompounds in selenoproteins, or convert them to non-reactive selenoethers or selenium.

According to Spallholz and Hoffman (2002) excess amount of selenium in form of SeCys decreases of the methylation of selenium. As a result of this, hydrogen-selenide (as an intermediary metabolite) gathers up in the organism, which compound is hepatotoxic and also has other negative effect. Above mentioned authors reported that the background of occurring

teratogenic effects in birds may be that excess selenium (as sulphur-analog) can be built into structural proteins. Reaction of selenium with sulphydil (-SH) groups of proteins can lead to changes in the activity of several enzymes, especially in those enzymes which needs free SH-groups to their catabolic activity (e.g. methionine-adenosiltransferase, a succinate-dehydrogenase (SDH), a lactate-dehydrogenase (LDH) and NADP-isocitrate-dehydrogenase (Nebbia et al., 1990).

Though selenium can be found in the active site of several enzymes, excess amount of selenium decreases the activity of glutathione-peroxidase (which is important part of the antioxidant defense system, eliminating free radicals) and also decreases the amount of glutathione (GSH) in cells (especially in liver). Due to the decrease of GSHPx activity and GSH concentration the intensity of lipid peroxidation processes gets higher in cells. As an effect of oxidative stress, membranes (e.g. cell-organelle membranes) loose their integrity thus lysosomal enzymes can get out of them, causing serious necrotic type of damage in tissues (Mézes and Matkovics, 1986).

2. PURPOSE OF THE STUDY

The aim of my study was to investigate the biochemically traceable effects of selenium overdose in some economically important species (domestic fowl, African catfish, and common carp). Primer aim was to investigate the effect of selenium overdose on quantity and activity of parts of the biological antioxidant defense system.

Other aim was to study whether the same amount of different selenocompounds (selenium-dioxide, sodium-selenite, sodium-selenate, selenium-enriched yeast) cause any difference in the biochemical parameters investigated, and if they do, what kind of differences it means.

During my experiments with fish species, I tried to answer, whether the parameters of antioxidant defense system investigated (reduced glutathione concentration, glutathione-peroxidase activity) are suitable as biomarkers of prooxidant effect caused by selenium exposition, which means that their changes could be the base of evaluating the probable oxidative stress, or the seriousness of it.

3. MATERIALS AND METHODS

3.1. Studies carried out with domestic fowl

In four experiment done with domestic fowl (Chapter 3.1.1 - 3.1.4) the treated groups received inorganic selenocompounds /selenium-dioxide (Merck, Darmstadt) or sodium-selenite or sodium-selenite (Sigma, St. Louis)/ dissolved in drinking water, so that calculating on average daily water uptake for each animals, the average daily selenium intake should be 1 mg, which extremely exceeds the actual requirement.

3.1.1. Investigation of the effect of selenium-dioxide dissolved in drinking water

For this experiment 40 extensive type native Hungarian yellow genotype chickens were used at 38 days of age. The animals were kept in deep litter. The birds were divided in two groups (control and selenium-dioxide treated). Each group contained 20 animals.

3.1.2. Investigation of the effect of sodium-selenite dissolved in drinking water

The experimental protocol was the same as written in previous investigation, except the treated group received sodium-selenite dissolved in drinking water.

3.1.3. Investigation of the effect of sodium-selenite and sodium-selenate dissolved in drinking water

For this experiment intensive type Ross 308 broiler cockerels (n=65) were used at 21 days of age. The birds were divided in three groups (control, sodium-selenite and sodium-selenate treated). The control group contained 25, while the treated groups 20-20 animals.

3.1.4. Investigation of the effect of sodium-selenite and sodium-selenate dissolved in drinking water on the stationary free radical level of liver

For this experiment intensive type Ross 308 broiler cockerels (n=65) were used at 21 days of age. The birds were divided in three groups (control, sodium-selenite and sodium-selenate treated). Each group contained 20 animals.

3.1.5. Investigation of the effect of selenomethionine (selenium-enriched yeast) added in feed in higher concentrations

For this experiment intensive type TETRA H cockerels (n=65) were used at 21 days of age. The feed of the treated groups was supplemented with selenium-enriched yeast (Sel-Plex, Alltech), so that it should contain 24.5 mg (Group ’Sel-Plex-1’) and 49.0 mg selenium per kilogram (Group ’Sel-Plex-2’), which extremely exceed the actual requirement (0.3 mg Se/kg feed). The aim was that the average daily selenium-intake of the animals has to be the same which were in the previous experiments, and double of it, namely 1 and 2 mg.

3.1.6. Investigation of the effect of selenomethionine (selenium-enriched yeast) added in feed in lower concentration

For this experiment intensive type Ross 308 broiler cockerels (n=65) were used at 21 days of age. The feed of the treated groups was supplemented with selenium-enriched yeast (Sel-Plex, Alltech), so that it should contain 12.25 mg selenium per kilogram, which exceeds the actual requirement (0.3 mg Se/kg feed), but is exactly the half of the amount used in the previous experiment for the lowest selenium-treated group. To investigate the effect of the expected decrease in feed intake, a 'pair-fed' control group (n=20) was also used.

3.1.7. Investigation of the effect of sodium-selenite added in feed

For this experiment intensive type TETRA H cockerels (n=100) were used at 21 days of age. Five groups were set, with 20-20 birds per group. The feed of the treated groups was supplemented with sodium-selenite (Na_2SeO_3) (Sigma, St. Louis), so that it should contain 24.5 mg (Group 'I1') and 49.0 mg selenium per kilogram (Group 'I2'), which extremely exceed the actual requirement (0.3 mg Se/kg feed). The aim was that the average daily selenium-intake of the animals has to be 1 and 2 mg. To investigate the effect of the expected decrease in feed intake in consequence of selenium toxicosis, two 'pair-fed' control groups, with 20-20 animals were also used.

3.2. Methods of sampling

3.2.1. Sampling of feed samples

Samples were collected from every feed used in the experiments for the analysis of nutrient and selenium content. The analysis of nutrient content was done in the laboratory of the Department of Nutrition (Szent István University, Faculty of Agricultural and Environmental Sciences) according to the referring legislation (Hungarian Feed Codex, 2004).

3.2.2. Sampling of blood and liver samples

In the course of the experiments the first sampling was done after a 3 days long adaptation period. At that time 5 animals were exterminated as absolute control. (This sampling was done only in Experiments 3.1.3. and 3.1.5.) Afterwards further samples were taken every day (5 animals per group) for 4 days. At every sampling, animals were weighted one by one before extermination. During the bleeding blood samples were taken from cervical blood vessels (*aa. carotis ext. et int., v. jugularis*) of the birds. Sodium-EDTA was added in concentration of 0.2 M/l, 0.05 ml for each ml blood to inhibit clotting. After extermination *post mortem* liver samples were taken. All samplings were carried out with the allowance of the Animal Experimentation Ethics Committee of the Szent István University.

3.2.2.1. Preparation of blood samples for the biochemical analyses

Blood samples were stored at cooled place (+4 ^{o}C) then the plasma was separated from the blood cells with centrifugation. After collecting the blood plasma, red blood cells were lysed with deionized water (ratio 1:9). Blood plasma and red blood cells hemolysate samples were stored at -20 ^{o}C until the investigation.

3.2.2.2. Preparation of liver samples for the determination of selenium concentration

Livers were measured and regarding liver and body weight values relative liver weights (g/100 g body weight) were calculated. After weighting 2-2g samples were taken from the liver (*lobus dexter*) of each bird from each group, in order to determine their selenium content. A mixture-sample was made of the liver for every experimental group, and those were stored at -20 ^{o}C until the investigation.

3.2.2.3. Preparation of liver samples for the determination of stationary free radical level

During the experiment presented in Chapter 3.1.4. in which also stationary free radical level of liver was also determined, rolls of liver (diameter: 3mm, length: 1 cm) were formed from a small amount (approx. 100 mg) of tissue (*lobus dexter*) and were stored in liquid nitrogen (-196 ^{o}C) until further investigation.

3.2.2.4. Preparation of liver samples for the biochemical analyses

After sampling the livers for analyses of selenium content and stationary free radical level, the livers were stored at -20 ^{o}C. Before the biochemical analyses the livers were thawn and sampled at the *distalis* region of the right lobus. Liver samples (0.5 g) were homogenized in nine-fold volume of isotonic saline (0.65% w/v NaCl). Native homogenate was centrifuged and used for further analysis.

3.2.2.5. Preparation of blood plasma and liver samples for the determination of ascorbic acid concentration

After arriving the laboratory, samples were cured with trichloroacetic acid and centrifuged (10.000 g, 5 min., +4 ^{o}C). Upper layer was removed and stored at -20 ^{o}C until the investigation.

3.3. Studies carried out with different fish species

My experiments with African catfish and common carp were se tat the Department of Fish Management (Szent István University, Faculty of Agricultural and Environmental Sciences).

3.3.1. Experiments done with African catfish

In African catfish (*Clarias gariepinus*, Burchell) effects of acute selenium toxicosis was investigated in two experiments. In the first experiment lower water-borne selenium concentrations /sodium-selenite or sodium-selenate, (Sigma, St. Louis)/ (0,3; 1,5 and 3,0 mg Se/l), while in the second one, higher concentration (6,0 mg Se/l) of those selenocompounds was applied.

3.3.1.1. Experiments done with African catfish at lower selenium exposure

African catfish (n=145) were set into the experiment (10.45±0.63 cm body length, 25.02±5.42 g weight). Animals were placed into same sized aquaria (25 l each). Control group contained 25, while the treated groups 20-20 animals. At 24 h of the experiment all aquaria were sampled to determine water-borne selenium concentration. The experiment lasted 48 hour. No feeding was done during the experimental period.

3.3.1.2. Experiments done with African catfish at high selenium exposure

Because during the previous experiment selenium toxicosis with clinical signs did not emerge, in a new experiment I used sodium-selenite and sodium-selenate dissolved in water in higher (6 mg Se/L) concentration. The experimental protocol was the same a sin the previous experiment, except in the new experiment 65 African catfish were used, 25 fish were placed in the control, while 20-20 animals in the treated groups.

3.3.2. Experiments done with common carp

The effect of acute selenium exposure on common carp (*Cyprinus carpio morpha nobilis* L.) was investigated in two experiments. In the first one lower concentrations (0.3; 1.5 and 3.0 mg Se/L) of sodium-selenite (Sigma, St. Louis), while in the second one higher concentrations (3,0 and 6,0 mg Se/l) of the same inorganic selenocompound were used.

3.3.2.1. Experiments done with common carp at lower selenium exposure

Common carp (n=85) were set into the experiment (12.34±0.57 cm body length, 28.38±11.24 g weight). Animals were placed into same sized aquaria (25 l each). Control group contained 25, while the treated groups 20-20 animals. At 24 h of the experiment all aquaria were sampled to determine water-borne selenium concentration. The experiment lasted 48 hour. No feeding was done during the experimental period.

3.3.2.2. Experiments done with common carp at high selenium exposure

Common carp (n=65) (14.78±2.51 cm body length, 58.92±11.03 g weight). Animals were placed into same sized aquaria (350 l each). Control group contained 25, while the treated

groups 20-20 animals. The experimental protocol was the same as written in previous experiment.

3.4. Methods of sampling

3.4.1. Sampling of gill, muscle and liver tissues

During the experiments the first sampling was done from the control group before the beginning of the selenium exposure (n=5, absolute control).

Afterwards further samples were taken at 12 h, 24 h, 36 h and 48 h of the experiment. At every sampling 5 animals per group were exterminated by *cervicalis dislocatio*. After extermination *post mortem* muscle tissue (ca. 1 g) were cut off (from the ventral verge of the cut between the 3rd and 6th thoracic vertebra), then the gill, muscle and liver tissues were stored at -20 ^{o}C until the investigation. All samplings were carried out with the allowance of the Animal Experimentation Ethics Committee of the Szent István University.

Before the biochemical analyses, the samples were thawn and homogenized in nine-fold volume of isotonic saline (0.65% w/v NaCl). Native homogenate was centrifuged (10.000 g, 5 min., +4 ^{o}C) and used for further analysis.

3.5. Biochemical analyses

Concentration of ***thiobarbituric acid reactive substances (malondialdehyde)*** of the samples (blood plasma, red blood cell hemolysate, and liver, gill, and muscle crude homogenate) was measured with modified (Matkovics et al., 1988) colorimetric method of Placer et al. (1966). ***Reduced glutathione concentration*** of the same samples was determined with the method of Sedlak and Lindsay (1968), based on colour reaction of free sulfhydril groups with DTNB. ***Glutathione-peroxidase activity*** was measured with an end-point direct assay according to Matkovics et al. (1988). Reduced glutathione concentration and glutathione-peroxidase activity was referred to protein concentration of the samples. To measure ***protein concentration*** in case of blood plasma and red blood cell hemolysate the Biuret reaction (Weichselbaum, 1948) was applied, while in case of the 10.000 g supernatant fraction of tissue samples the Folin phenol reagent was used (Lowry et al., 1951).

Determination of ascorbic acid ***concentration*** in blood plasma and liver homogenate was based on the colorimetric method of Omaye et al. (1979). ***Selenium concentration*** of the feeds, the water of aquaria and liver samples were determined in the Central Laboratory of Szent István University, Faculty of Agricultural and Environmental Sciences. Measurement was based on flameless atomic absorption photometry following hydride generation.

Determination of ***stationary free radical level*** of liver samples by electron paramagnetic resonance spectroscopy (EPR) was done by the Biooxidation Group of the Chemical Research Institute of Hungarian Academy of Science.

In case of an experiment done with broilers the following parameters were analyzed from the blood plasma of the animals using reagent kits: ***aspartate-aminotransferase (AST)***, and ***alanine-aminotransferase (ALT) activity*** (Bergmeyer et al., 1978); ***lactate-dehydrogenase (LDH) activity*** (Howell et al., 1979), ***calcium concentration*** (Bauer, 1981); ***inorganic phosphor concentration*** (Daly and Ertingshausen, 1972); ***glucose concentration*** (Trinder, 1969); ***uric acid concentration*** (Barham and Trinder, 1972); ***total cholesterol concentration*** (Allain et al., 1974); ***triglyceride concentration*** (Young et al., 1975). ***VLDL- and LDL-level*** of blood plasma was analyzed by a turbidimetric assay of Griffin and Whitehead (1982).

3.6. Mathematical methods and statistics

Mean and standard deviation values were calculated for each group and each parameter. Statistical analyses of the data (analysis of variance, least significant difference test (LSD) and linear regression analysis) were done with STATISTICA for Windows 4.5 (StatSoft Inc., 1993).

4. DETAILED DISCUSSION

4.1. Investigation of the effect of selenium-dioxide dissolved in drinking water

Selenium treatment with selenium-dioxide in drinking water resulted no difference between the MDA content of tissues of control and treated group. According to the results, the conclusion is that the selenium-dioxide treatment exceeding the real requirements caused no important prooxidant stress in extensive breed during the experimental period, thus intensity of lipid peroxidation processes did not significantly change either. This result can partly be explained with former observations according to which selenium-dioxide is not a suitable compound to improve selenium status of the organism, since its absorption and biological effectiveness is quite low (Hill, 1974).

4.2. Investigation of the effect of sodium-selenite dissolved in drinking water

During the selenium exposition study with sodium-selenite in drinking water, neither in the blood (red blood cells and blood plasma) nor in the liver did find higher MDA content (a sign of increased lipid peroxidation processes).

This result shows that – on contrary of *in vitro* results of Seko et al. (1989) - sodium-selenite applied in this dose in the drinking water did not increase the intensity of lipid peroxidation processes significantly in any tissues in case of extensive breed kept on feedstuff below the actual nutritional requirement (also poor in selenium) the so predicted prooxidant effect of selenite did not show up.

4.3. Investigation of the effect of sodium-selenite and sodium-selenate dissolved in drinking water

On the basis of my results it can be declared that depression of feed intake (which meant 40% feed intake compared to control on Day 3 and 4) had no significant effect on MDA content of blood plasma. It means, that in the present study depression of feed intake probably caused no hyperlipidemia in blood plasma, which may – because of increased total lipid content – also influence the actual MDA content of plasma (Dworschák et al., 1988). MDA content of liver of treated animals was somewhat higher than control ones on the first and 4th days of sampling, but it seems that moderate peroxidative effect was effectively blocked by the active antioxidant system of liver. GSH concentration in blood plasma showed an interesting change. In avian species inhibitory effect of fasting on glutathione synthesis is well-known (Mézes and Oppel, 1995). According to the present study the glutathione depletory effect of fasting showed up later in time. In significant depression of GSHPx activity of blood plasma one day of difference appeared between selenite and selenate treatments. This may be due to the different effects of different selenocompounds on depression of GSH level. Significant

depression of GSHPx activity was probably due to the lack of co-substrate (namely the GSH). Reduced activity and quantity of glutathione redox system resulted less capacity to eliminate harmful free radicals in red blood cells, which is also confirmed by significantly elevated MDA concentration on 4th day of experiment of sodium-selenite treatment. Sodium-selenate treatment had no such effect. Results of ascorbic acid measurement lead to the conclusion that excess application of inorganic selenocompounds in drinking water resulted ascorbic acid depletion and/or inhibited its synthesis in liver in a quite short time. However later ascorbic acid content of liver was normalized, which is in connection with an active compensation mechanism. The reaction given at different times in case of selenium treatments may be caused by different rate and intensity of absorption of selenite and selenate forms and their different way of transport in the organism. It was also observed that blood plasma and red blood cell hemolysate reacts quicker to selenium supplementation in drinking water than liver does. The applied selenium concentration however, according to the results, temporarily enhanced the activity of the glutathione redox system in liver, while at the same time disfavourable effects were visible in blood plasma and red blood cell hemolysate. Applied selenocompounds and doses induced a more intensive response in intensive broiler chicken, than in the extensive ones. Significant difference was found between the effect of the two selenocompounds, which is probably due to their different rate of absorption and utilization in the body.

4.4. Investigation of the effect of sodium-selenite and sodium-selenate dissolved in drinking water on the stationary free radical level of liver

Overdose of sodium-selenite in drinking water resulted similar changes in feed intake of broilers as Gowdy and Edens (2005) reported. On the 3rd and 4th day of the experiment the feed intake of the treated groups was 30-40% of the control. Body weight of treated animals was significantly reduced, while relative liver weight was increased. Significant depletion of ascorbic acid concentration in liver as a response to treatment with different selenocompounds was observed here, as well as in the experiment with the same selenocompounds, doses and experimental period presented in Chapter 4.3. Reduced glutathione content of liver exceeded that of the control ones at most samplings, both in case of sodium-selenite and sodium-selenate treatments. This result is particularly important, because several data prove that as an effect of fasting glutathione depoes of liver run out rather quickly. But it seems, that if fasting is connected with improved selenium exposure and with the accompanying stress, this depletion does not turn up.

During the 96-hour long experiment none of the treatments with different selenocompounds enhanced the stationary free radical level of liver, which could have been though expected, based on the results of the connecting literature about mostly *in vitro* studies (Seko et al. (1989), Spallholz (1998)). I think it can be led back to the fact that selenium exposure exceeding the actual requirement induced stress in the liver, which improved the antioxidant defense system, so quantity (GSH) and activity (GSHPx) of the glutathione redox system, thus free radicals were effectively eliminated (stationary free radical level not differing significantly from control group) and reduced the potentially occurring peroxidative effects (MDA content not exceeding that of the control group significantly).

4.5. Investigation of the effect of selenomethionine (selenium-enriched yeast) added in feed in higher concentrations

As an effect of selenium overdose mixed in feed in form of selenomethionine body weight of the treated animals decreased dramatically. The reason of this was, that in the two treated groups the average daily feed intake was significantly lower from the 2nd day of experiment (depending on selenium concentration) than that in the control one. Confirming results of Hoffman et al. (1989) and O'Toole and Raisbeck (1997) with mallard ducklings I observed increased selenium concentration in liver of treated animals (3,84 and 4,03 times higher than in the control). Dose-dependent difference between the treated groups was not observed because on and after the 2nd day the selenium intake of the animals treated with the higher selenomethionine dose decreased to the selenium intake of the other group originally treated with the lower selenium dose, because of the great reduction of the feed intake of the above mentioned group. Examining MDA content of different tissues, it can be declared that though the applied dose of selenium caused toxicosis with clinical symptoms, no important peroxidative effect occurs in case of the blood plasma and red blood cells, or this effect is eliminated by the antioxidant defense system effectively. However, contrary of the results with inorganic selenium forms, organic selenium exposure induced important and significant peroxidative processes in liver. Regarding connection between GSHPx activity and GSH content, my results were similar, but not totally the same as in the former experiments (Chapter 4.3. and 4.4.). Cause of the latest statement may be the delay of time in the changes of mechanism of glutathione redox system. Thus the improved GSHPx activity gradually tires out the GSH depoes of cells, which is decreasing because of the lack of proper supply and the reduced activity of repair enzymes (e.g. glutathione-reductase in this case). (As a result of reduced feed intake, the quantity of NADPH co-substrate might have decreased as well.) At the beginning of the process, this effect was not obvious by the sensitivity level of the

methods applied, maybe due to the above mentioned reasons. Similarly to my results Hoffman et al. (1989) had similar observations regarding significant increase of GSHPx activity in blood plasma, when they fed mallards with feed high in selenium. Changes of GSH concentration in liver are especially interesting, because several data prove that as the effect of fasting the glutathione stores of liver run out very quickly (Comporti, 1987). But it seems, that if reduced feed intake is accompanied by selenium burden or with its stress effect, this decrease in glutathione concentration does not show up. On the other hand significantly increased GSH content in liver resulted significant increase of GSHPx activity at the same time. Hoffman et al. (1989) observed significant decrease of GSH content in liver (depending on selenium dose) and the activity of GSHPx showed no change, when they fed mallard ducklings with selenomethionine at a dose of 20 and 40 mg Se/kg feed for 6 weeks. Results of cited authors thus can not be the base for evaluating the short term effect examined by me. Great importance of elevated ascorbic acid content of blood plasma of treated group is, that animals belonging to the selenomethionine treated groups (especially to the group fed with higher

dose of selenomethionine) had practically no feed-intake from the 2nd day of experiment, thus elevated ascorbic acid concentration in plasma may have resulted exhaustment of tissue stores. It is known, that different tissues contain different amount of ascorbic acid, out of which liver and adrenal gland is a well-known store organ (Staber and Kraus, 2003). In case of stress – as Mahan et al. (2004) reported – ascorbic acid content of adrenal gland decreases drastically. Kutlu and Forbes (1993) reported elevated ascorbic acid concentration in blood plasma as an effect of heat-stress in broilers. In my experiment elevated ascorbic acid content of blood plasma of the treated groups was probably caused by stress-effect of selenium burden and by the response of the organism to it.

4.6. Investigation of the effect of selenomethionine (selenium-enriched yeast) added in feed in lower concentration

In this study I examined the effect of dietary selenium on broilers at a lower concentration (12.25 mg Se/kg feed) than in the previous experiment (Chapter 4.5.). The applied concentration was similar to the one described by Latshaw et al. (2004) occurring due to feeding failure (9.3 mg Se/kg feed). Though selenium intake per kilogram body weight was nearly 82% of the intake of animals treated with 24.5 mg Se/kg feed, because of the lower depression of total feed intake, during the whole experimental period I obtained a remarkably lower level of reduction of average daily feed intake. As a result of this neither the selenomethionine treated group, nor in the pair-fed control group showed significantly lower

body weight than the control one. Excess selenium intake resulted 3.2 fold increase in the selenium content of liver compared to control group. In the examined tissues MDA concentration – which is a marker of intensity of lipid peroxidation processes – did not show significant increase, which – on my opinion – can be led back to the improved activity of antioxidant defense system. For example in my previous experiments it was also concluded that excess selenium caused elevated GSH content in liver, which was also experienced during the whole period of the present study. As an effect of co-substrate surplus, similarly to the former results elevated GSHPx activity was also proved. Among the clinical-chemical parameters examined, activity of enzymes signaling serious tissue damage (AST, ALT, LDH) – in contrast with my hypothesis based on literature data – no significant increase was observed during the whole experimental period compared to control group. The reason of this may be that selenium burden and this study lasted for a short time (96 hour), in contrast with the most literature data. On the basis of my examination the conclusion is that if 3 weeks old broilers are treated with a selenium concentration applied by me (12.25 mg Se/kg feed), during the experimental period (96 hour) there are no such level of damage in liver tissue which can be proved by examination of these biochemical parameters.

4.7. Investigation of the effect of sodium-selenite added in feed

On occasion of feeding sodium-selenite mixed in the feed at a great overdose, liver samples of the treated animals contained 2.6^{th} and 2.97^{th} as much selenium as the control. Sodium-selenite treatment caused significant decrease in feed intake in a short time, thus average body weight of the treated groups was significantly lower than that of control at the end of the study. My observations are similar to the results of Gowdy and Edens (2005), who treated broiler with various concentrations (0.3; 0.6; 1.2; 5; 10 and 15 mg Se/kg feed) of sodium-selenite and selenium-enriched yeast. In case of sodium-selenite treatment from hatching to 21^{st} day, above 6 mg Se/kg feed the body weight and the weight of lymphoid organs decreased, while relative liver weight increased significantly. In case of inorganic selenium supplementation though MDA concentration of liver in treated groups was constantly higher than that of control one, no significant difference was measured (in contrast with selenomethionine treatment, Chapter 4.5). As the same time MDA content of liver of pair-fed control groups, perhaps due to a reduced quantity and activity of glutathione redox system, exceeded that of control and treated groups significantly at most sampling periods. Glutathione depletion appearing due to fasting was well visible in pair-fed control group, as also reported by Mézes and Oppel (1995) since GSH concentration was lower than that of

control one at 48th hour, and this difference become significant at 72nd hour of experiment. GSH concentration of liver homogenate of sodium-selenite treated group – similarly to the results of Hoffman et al. (1989) – exceeded the values measured in control group during the whole period. According to the results, it seems that if the reduced feed intake is accompanied by high selenium intake, the glutathione depletory effect of fasting did not occur, which may indicate changes in GSH synthesis and/or oxidation, and in reduction of GSSG as an effect of selenium toxicosis. GSHPx activity of blood plasma and red blood cell hemolysate showed strong correlation with changes of GSH content. In cases when GSH content of pair-fed control group was lower than that of treated groups, the GSHPx activity was also lower. Similarly to results of blood plasma and red blood cell hemolysate, GSH concentration and enzyme activity showed strong correlation in liver, too. So elevated GSH content of treated groups (compared to control) was accompanied by elevated enzyme activity, probable due to improved quantity of co-substrate.

4.8. Experiments done with African catfish

The results of this short term study (48 hours) showed that increasing the dissolved selenium content of water either in form of selenite or selenate does not induce toxicosis with clinical symptoms in juvenile (approx. 10 cm body length) African catfish. In the same time, considering biochemical parameters high amount of water-borne selenium, depending on its chemical form and quantity, was found to affect lipid peroxidation processes and to burden glutathione redox system.

A feasible explanation of the statistically significant and occasionally divergent changes found with selenate and selenate treatment is the different absorption and utilization properties of the two inorganic selenium forms. This theory is supported by several observations (Hodson and Hilton, 1983; Mézes et al., 1999), describing selenite can be absorbed more efficiently and stored for a long time in selenoproteins, while absorption of selenate is even better than that of selenite, however its excretion is rather quickly. Results of the present study showed that as an effect of selenium exposure – especially of selenite – fish have to face with the appearance of lipid peroxidation processes in the gill as well as in the liver. Beside this, after a certain shift of time – which is explained by different intensity and effectiveness of absorption and transport of selenocompounds – oxidative stress could be observed in muscular tissues as well. However it was strongly dose-dependent and was observed only at the greatest doses and also because of different effectiveness of absorption of the two compounds, it showed up with a slip of time. Presence of oxidative stress caused by

overdosed selenium in the distinct tissues is also confirmed by the decreasing quantity of reduced glutathione, which is an important element of the antioxidant system. Remarkable differences were detected between the processes caused by the two selenocompounds in the studied tissues. Considering gill, which is probably the place of selenium absorption, effect of selenite – considered to be the more toxic compound – was observed earlier than that of the selenate. Vica versa dynamics were found in the liver – where effective transport is assumed following effective absorption – thus the effect of selenate preceded that of the selenite.

Changes of GSH concentration might result from enhanced oxidation caused by increased activity of glutathione peroxidase in oxidative stress. In the presented study expressive decrease of enzyme activity was observed due to selenium exposure. These results indicate that decreased glutathione content, the co-substrate of the enzyme, results reduced activity due to the lack of the co-substrate. Opposite changes of GSH content in liver in case of greater doses of the two inorganic selenocompounds are due to the phenomena that xenobiotic exposition is activating the antioxidant defense system – at least at the beginning of the exposition – in order to keep lipid peroxidation processes at physiological level. Xenobiotic exposition also caused an elevated GSHPx activity, especially in liver, and increased GSH concentration, too. According to results of Ali et al. (2000) toxic components from the environment have the potential to induce lipid peroxidation in organ of fish, out of which the most sensitive organs regarding lipid peroxidation are the gills. My experiments confirmed these results in case of water-borne selenium as well.

4.9. Experiments done with common carp

According to the results, it can be conclude that the applied water selenium concentrations, in case of selenite form, did not cause clinical symptoms in young (approx. 10 cm body length) carp in a short period of treatment (48 hours).

Results of biochemical examinations indicated that great quantity of water-borne selenium, depending on its amount, has effect on lipid peroxidation processes in the body, and also burdens the biological antioxidant system, namely glutathione redox system. Selenium of dissolved sodium-selenite is absorbed in the gills, where the first effect has appeared. Similarly to my experiment with African catfish, it can be declared that common carp is also able to take up great quantity of water-borne selenite through the gill lamellae, which caused statistically proven changes (elevated intensity of lipid peroxidation processes) especially in gill and liver. Oxidative stress following selenium exposure was also detected as a decrease in the reduced glutathione concentration. Decrease of reduced glutathione concentration had an

impact on glutathione peroxidase activity, as presence of decreasing amount of co-substrate results in reduced enzyme activity. The results lead to conclusion that though common carp reacts sensitively to the environmental damages, thus to the elevated water-borne selenium content, which can also induce lipid peroxidation processes in the organs, but at the same time it can also overcome these effects, partly through biological antioxidant system, particularly through the glutathione redox system.

5. NEW SCIENTIFIC RESULTS

1. I observed that as an effect of sodium-selenite burden, relative weight of liver of treated broilers significantly increased, even comparing to pair-fed group – which was fed with the same amount of feed without selenium overdose –, so it is obviously the result of selenium toxicosis.
2. I observed that as effect of sublethal dose of selenium burden – even in inorganic or organic selenium form – feed intake and this living weight of broilers significantly decreased in a short time.
3. In my studies with broilers I experienced, that if fasting is accompanied by sublethal dose of selenium burden, the well known phenomena of glutathione depletion did not occur, which effect was also visible compared to the pair-fed control group, consuming the same amount of feed without selenium overdose.
4. Examining prooxidant effect of sublethal doses of inorganic selenocompounds I observed that even by EPR spectroscopy no change was detected in the stationary free radical content of liver during the short term (96 hours) of exposition period, thus selenium toxicosis is not primarily caused by production of reactive oxygen species
5. I was the first to describe the effect of water-borne inorganic selenocompounds highly exceeding, selenium concentration of living waters (but still below the lethal dose) on the glutathione redox system and the lipid peroxidation processes of different organs in African catfish and common carp.
6. Comparing response of African catfish and common carp to selenium burden in form of sodium-selenite, I observed that in carp MDA content showed a more marked increase in all three tissues examined, while GSH content showed the same changes in both species, except in case of gills. In gills more marked and quicker changes were measured in case of common carp. GSHPx activity showed marked changes in gills and muscles of carp, and in liver of African catfish in case of the same amount and period of selenium burden.

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