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BIOCHEMICAL INDICES ARE MODULATED IN FISH EXPOSED TO CYANOBACTERIAL TOXINS (MICROCYSTINS)

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Abstract

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In this work were summarized changes of biochemical markers of fish under the thumb of cyanobacterial toxins (microcystins). Among the most studied biomarkers of the influence of cyanobacterial toxins on fish belong oxidative stress parameters – glutathione S-transferase (GST), non-enzymatic antioxidant glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), lipid peroxidation (LPO), malondialdehyde (MDA), glutatione reductase (GR), parameters of blood – values of haemoglobin (Hb), haematocrit (PCV), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), erythrocyte (RBC), leukocyte counts (WBC) and plasma – alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), cholinesterase (CHE), total serum protein (TP), glucose (GLU), lactate (LACT), iron (Fe), calcium (Ca), magnesium (Mg), total bilirubin (BIL), phosphorus (P) and protein phosphatase activities (PP1, PP2A).

fish, microcystins, biomarkers, oxidative stress

Eutrophication of aquatic ecosystem accompanied by cyanobacterial mass development represents serious environmental problem. Cyanobacteria as photosynthesizing organisms produce biologically active compounds that may affect growth and development of other water organisms and physical and chemical characteristics of water (CHORUS et al., 2000). Great attention has recently been paid to the impact of cyanobacterial toxins on fish. Symptoms of poisoning, pathological changes and influence on blood indices have been investigated as well (LANDSBERG et al., 2002).

The influence of cyanotoxins on fish following experimental intoxications or the impact of an environment containing cyanotoxins on fish have been studied by using clinical, morphological, histological, ultrastructural, haematological and biochemical methods. One of the most common genera (*Microcystis*) in cyanobacterial blooms produce to hepatotoxic microcystins that can occasionally occur in high concentrations at shallow waters where cyanobacteria can accumulate, and may induce injury to fish (MALBROUCK and KESTEMONT, 2006).

Toxins are synthesized during the growth phase of the cyanobacteria and large quantities of microcystins are released into the water during the collapse of the bloom or from actively growing cyanobacterial populations (MALBROUCK and KESTEMONT, 2006). CHORUS and BARTRAM (1999) showed that 100% of toxins are located in the cells of young populations of cyanobacteria whereas in decaying cells, toxin concentrations in water rose to values of 70–80%.

Most of toxins are absorbed into the fish organism through the gastrointestinal tract, whereas toxin penetration through the skin or gills is negligible (TENCALLA et al., 1994). It is supposed more affection of cyanobacterial toxins on fish organism at higher digestible of cyanobacterial water blooms. Fish exposed to media containing the dispersed microcystins demonstrated that toxic effects are time delayed due to limited penetration into the healthy fish. The toxic effect after oral administration is approximately 10 times weaker than after the intraperitoneal application (CARBIS et al., 1997).

Cyanotoxins are rarely ingested by man in amount high enough for a lethal acute dose, but the damage caused by chronic effect is particularly more probable if there is long term frequent exposure. The maximum allowable concentration for MCYST in drinking water was established in 1 $\mu g.l^{-1}$ (FALCONER et al., 1994). Based on this limit, WHO (World Health Organization) established 0.04 $\mu g.kg^{-1}$ of body weight day^{-1} as a tolerable daily intake (TDI) of cyanobacteria products content microcystins (CHORUS and BARTRAM, 1999).

CONCLUSIONS

Haematological parameters in blood and plasma

Liver enzymes (ALT, AST and LDH) are the most frequently tested enzymes in fish for the indication of cyanobacterial toxicity. RABERGH et al. (1991) reported that the activity of blood plasma enzymes (ALT, AST and LDH) raise in two hours after an intraperitoneal injection of toxin as a consequence of the hepatocyte necrosis. TENCALLA et al. (1994) observed a decrease in their activity after 48 h, and interpreted this fact as a result of damage of the majority of hepatocytes that were not able to release enzymes into circulatory system. Significant increase of the activities of ALT, AST and LDH after intraperitoneal or oral administration of microcystin-LR to the carp was observed (BURY et al., 1997; NAVRÁTIL et al., 1998; MALBROUCK et al., 2003; LI et al., 2004; 2007). KOPP and HETEŠA (2000) reported that the activity of blood plasma enzymes was increased after 96-hours of exposure of the carp to natural cyanobacterial population. CARBIS et al. (1996) noted a delay of toxic manifestation in fish exposed to water with dispersed microcystin. Serum activities of AST and ALT increased 7 days after the carps were exposed to water that contained microcystins. Feral carp from a lake, where toxic *Microcystis aeruginosa* was dominant, had higher activity of AST in serum (CARBIS et al., 1997). MALBROUCK et al. (2003) reported that activities of plasma enzymes (ALT, AST and LDH) completely recover after 21 days of intraperitoneal injection of microcystin-LR.

The absorption of common concentrations of microcystins in natural water through oral, dermal or brachial pathways may be limited in normal healthy fish. The acute toxicity of microcystins is unlikely to occur in feral carp and chronic injury will probably not be detected by changes of enzyme (AST, ALT, ALP and LDH) activity in the blood plasma. Other biochemical parameters of blood and plasma as Hb, PCV, RBC, MCV, MCH, MCHC, CHE, LACT, Ca, Mg, Fe, P, BIL and GLU, in particular, were influenced by the action of the natural population of cyanobacterial water bloom due to the participation of other active substances and changes in water chemistry.

Concentration of BIL rises eight hours after intraperitoneal injection of microcystins (CARBIS et al., 1996). Higher concentration of toxic cyanobacteria in a natural lake caused the increase of BIL concentration in serum of feral carp (CARBIS et al., 1997), on the second hand KOPP et al. (2005) was no-observed influence of toxic cyanobacteria on the values of BIL in silver carp. BURY et al (1996) (in brown trout) and ERNST et al. (2006) (in whitefish) observed slightly increased levels of GLU in fish exposed to cyanobacteria, but changes were not significant. KOPP et al. (2005) showed that serum activities of ALP and values of GLU, Ca and Mg significantly decreased and activities of CHE, LACT, Fe and P significantly increased in the silver carp exposed to the toxic cyanobacterial population.

The levels of blood cell components Hb, PCV, RBC, MCV, MCH and MCHC usually decrease after application of pure microcystins or toxic cyanobacterial biomass in consequence with patho-morphological changes. These comprise of extensive haemorrhage in the skin, eyes, hepatopancreas and swim bladder (NAVRÁTIL et al., 1998; VAJCOVÁ et al., 1998). A significant decrease of total leukocyte counts (WBC) was observed after intraperitoneal or oral administration of microcystin-LR in the carp (PALÍKOVÁ et al., 1998). Values of TP significantly decreased after intraperitoneal application of pure microcystin-LR into common carp (NAVRÁTIL et al., 1998), silver carp (VAJCOVÁ et al., 1998) and were not changed in common carp (CARBIS et al., 1996). Changes of TP under the influence of cyanobacterial populations were reduced in common carp (KOPP and HETEŠA, 2000) and were not changed in silver carp (KOPP et al., 2005).

Biochemical indices of blood and plasma in fish are affected by many endogenous and exogenous factors. Liver enzymes (ALT, AST and LDH) are the most suitable parameters in fish as indicators of the toxicity of cyanobacteria after intraperitoneal or per oral biomass application. The toxic effect of cyanobacteria on fish under natural environmental conditions is many times weaker than after the intraperitoneal or per-oral application. In case of chronic exposure, cyanobacteria would not be likely detected by enzyme activity changes (ALT, AST, ALP, ACP, CHE and LDH) in the blood plasma of fish.

Parameters of oxidative stress

Oxidative stress, i.e. pathological processes related to overproduction of reactive oxygen species (ROS) in tissues is one of important general toxicity mechanisms of many xenobiotics. Oxidative stress was shown to be induced by anthropogenic contaminants as persistent organic pollutants (POPs), heavy metals, and also by toxins produced during massive blooms of cyanobacteria (DING et al. 1998; VAN DER OOST et al., 2003).

Glutathione S-transferase (GST)

Changes in activity of detoxification enzyme glutathione-S-transferase (GST) have been used as a biomarker of chronic cyanobacterial toxicity in fish. However, the responses in GST might be highly variable (BLÁHA et al. 2004). The study with carp hepatocytes has shown significant increases in production of reactive oxygen species (ROS), elevation in activities of detoxication enzymes SOD, CAT, GPX, but the authors observed no significant changes in reduced glutathione (GSH) levels and no modulations of GST activity (LI et al., 2003). Similar weak responses of GST to microcystin-LR exposure were also observed with early life-stages of zebra fish (*Danio rerio*) (WIEGAND et al., 1999). PIETSCH et al. (2001) reported significant suppression of GST in zebra fish (*Danio rerio*) after 24h exposure of fish eggs to cyanobacterial extract, similar inhibition of GST activity reported CAZENAVE et al. (2006a) in *Corydoras paleatus* after exposure to different doses of microcystin-RR. Statistically significant decrease in GST activities was also observed after co-exposure of zebra fish to microcystin-LR and cyanobacterial lipopolysacharides (LPS) (BEST et al., 2002) and in *Jenynsia multidentata* fed pellets with microcystin-RR (CAZENAVE et al., 2008). About the juvenile goldfish (*Carassius aureus*) after intraperitoneal injection of microcystin-LR was observed decrease of hepatic glutathione-S-transferase (MALBROUCK et al., 2003). Activity of GST in the embryos of common carp was determined after exposure to four cyanobacterial biomasses. Biomasses with coccal cyanobacteria caused variable modulations of the GST enzyme activities (either increase or decrease), but exposures to biomass with filamentous cyanobacteria caused significant decrease of GST activity (PALÍKOVÁ et al., 2007b).

GST activity was significantly increased in *Danio rerio* embryos exposed to pure MC-RR and MC-LF (CAZENAVE et al., 2006b). Significant increase of GST was observed after exposure of silver carp (*Hypophthalmichthys molitrix*) to purified MC-LR and MC-RR (LI et al., 2007). Silver carp (*Hypophthalmichthys molitrix*) exposed to cyanobacterial bloom had significantly elevated hepatopankreas concentrations of GSH (BLÁHA et al., 2004). Effects of complex cyanobacterial biomass and aqueous extract were tested on embryo of common carp. GST activity was increased in treatments but the changes were not always significant (PALÍKOVÁ et al., 2007a). Biochemical changes in common carp (*Cyprinus carpio*) and silver carp (*Hypophthalmichthys molitrix*) exposed to toxic cyanobacterial blooms in natural environment was described. Activity of GST was elevated in a majority of experimental variants in both of kinds, but only in silver carp significantly (ADAMOVSKÝ et al., 2007).

Non-enzymatic antioxidant glutathione (GSH)

Elevation of GSH levels reflects stimulation of detoxication metabolism followed by increased GSH demand (e.g. stimulation of glutathione-S-transferases) as also previously reported in other aquatic organisms (BEST et al., 2002). Inductions of GST seem to correspond to detoxification of MCs by GST-mediated conjugation with GSH (WIEGAND et al., 1999; PFLUGMACHER et al.; 1998, PIETSCH et al., 2001). Elevated GSH concentrations and activities of the GR (the enzyme regenerating GSH from its oxidized form) further reveal increased demands for reduced GSH because of enhanced detoxification and/or oxidative stress induced by toxic cyanobacteria (LI et al., 2003; JOS et al., 2005; BLÁHA et al., 2004).

Decrease trend in glutatione (GSH) concentration was observed in the liver of silver carp after injection microcystins (LI et al., 2007), similarly decrease of GSH levels in the hepatocytes of common carp (*Cyprinus carpio*) exposed to microcystins-LR reported (LI et al., 2003). Biochemical responses in common carp (*Cyprinus carpio*) and silver carp (*Hypophthalmichthys molitrix*) exposed to toxic cyanobacterial blooms in natural environment was monitored. Activity of GSH was significantly increased after 4 weeks and significantly decreased after 9 weeks of exposure to in silver carp (no significantly changed on common carp) (ADAMOVSKÝ et al., 2007).

Superoxide dismutase (SOD)

The results, when tilapia fish (*Oreochromis niloticus*) were injected intraperitoneally with a single dose of MC-LR or MC-RR showed a different response pattern of both MC analogs in the different organs. Thus, MC-LR induced the activity of SOD in the three organs (liver, kidney, gills), MC-RR on the other hand, induced SOD activity only in the liver (PRIETO et al., 2006). Activity of SOD was increased in liver of loach (*Misgurnus mizolepis*) after orally exposure to low dose of *Microcystis* cells (LI et al., 2005). The activity of SOD was significantly increased after exposure of common carp to microcystins-LR (LI et al., 2003). On the second hand, the activity of SOD was significantly decreased about the tilapia fish (*Oreochromis niloticus*) were exposed to a single dose of cyanobacterial cells containing MC-LR (PRIETO et al., 2007). The effects of microcystins from cyanobacterial cells on various oxidative stress biomarkers in liver, kidney and gill tissues in freshwater tilapia fish (*Oreochromis* sp.) were investigated under laboratory conditions. SOD activity did not change significantly in the liver, kidney or gills of fish that had been exposed to crushed cyanobacteria for 14 days, but the longer exposure (21 days) resulted in a significant increase in the SOD activity in liver and in gills (JOS et al., 2005).

Catalase (CAT)

Tilapia fish (*Oreochromis niloticus*) after intraperitoneally injection a single dose of MC-LR or MC-RR showed increased of the activity of CAT in the three organs (liver, kidney, gills) (PRIETO et al., 2006). Activity of CAT was enhanced in liver *Corydoras paleatus* after exposure to different doses of microcystin-RR (CAZENAVE et al., 2006a). The activity of CAT was significantly increased after exposure of common carp to microcystins-LR (LI et al., 2003). Activity of CAT was increased in liver of loach (*Misgurnus mizolepis*) after orally exposure to low dose of *Microcystis* cells (LI et al., 2005). CAT activity was significantly increased in *Danio rerio* embryos exposed to pure MC-RR and MC-LF (CAZENAVE et al., 2006b). The effects of microcystins from cyanobacterial cells on various oxidative stress biomarkers in liver, kidney and gill tissues in freshwater tilapia fish (*Oreochromis* sp.) were investigated under laboratory conditions. No discernible effects were observed in CAT activity of liver or kidney after 14 days of exposure, but activity increased in liver and kidney after 21 days of treatment (JOS et al., 2005).

The activity of CAT was significantly decreased about the tilapia fish (*Oreochromis niloticus*) were exposed to a single dose of cyanobacterial cells containing MC-LR (PRIETO et al., 2007).

Glutatione reductase (GR)

Intraperitoneally injection a single dose of MC-LR or MC-RR showed a different response pattern of both MC analogs in the different organs of tilapia fish (*Oreochromis niloticus*). Thus, MC-LR induced the activity of GR only in the liver (not in kidney and gills), GR were not influenced by MC-RR (PRIETO et al., 2006). Activity of GR was enhanced in liver and inhibited in gills *Corydoras paleatus* after exposure to different doses of microcystin-RR (CAZENAVE et al., 2006a). The effects of microcystins from cyanobacterial cells on various oxidative stress biomarkers in liver, kidney and gill tissues in freshwater tilapia fish (*Oreochromis* sp.) were investigated under laboratory conditions. GR activity was significantly induced after 21 days of exposure in liver and kidney and showed no significant changes in gills (JOS et al., 2005). Biochemical responses in common carp (*Cyprinus carpio*) and silver carp (*Hypophthalmichthys molitrix*) exposed to toxic cyanobacterial blooms in natural environment was monitored. Activity of GR was elevated in a majority of experimental variants in both of kinds, but only in common carp significantly (ADAMOVSKÝ et al., 2007).

Impact of pure microcystins (-RR and -LF) on zebra fish (*Danio rerio*) embryos were monitored. Embryos did not show clear changes in activities of GR (CAZENAVE et al., 2006b). The activity of GR was significantly decreased about the tilapia fish (*Oreochromis niloticus*) were exposed to a single dose of cyanobacterial cells containing MC-LR (PRIETO et al., 2007).

Glutathione peroxidase (GPx)

The results, when tilapia fish (*Oreochromis niloticus*) were injected intraperitoneally with a single dose of MC-LR or MC-RR showed a different response pattern of both MC analogs in the different organs. Thus, MC-LR induced the activity of GPx only in the kidney (not in liver and gills), GPx were not influenced by MC-RR (PRIETO et al., 2006). The activity of GPx was significantly increased after exposure of common carp to microcystins-LR (LI et al., 2003). Activity of GPx was enhanced in liver and inhibited in gills *Corydoras paleatus* after exposure to different doses of microcystin-RR (CAZENAVE et al., 2006a). Activity of GPx was increased in liver of loach (*Misgurnus mizolepis*) after orally exposure to low dose of *Microcystis* cells (LI et al., 2005). The effects of microcystins from cyanobacterial cells on various oxidative stress biomarkers in liver, kidney and gill tissues in freshwater tilapia fish (*Oreochromis* sp.) were investigated under laboratory conditions. After the longer exposure there was a significant induction of GPx activity in liver and kidney, however GPx activity showed a significant decrease in gills (JOS et al., 2005).

The activity of GPx was significantly decreased about the tilapia fish (*Oreochromis niloticus*) were exposed to a single dose of cyanobacterial cells containing MC-LR (PRIETO et al., 2007). Impact of pure microcystins (-RR and -LF) on zebra fish (*Danio rerio*) embryos were monitored. Embryos did not show clear changes in activities of GPx (CAZENAVE et al., 2006b). Biochemical changes in common carp (*Cyprinus carpio*) and silver carp (*Hypophthalmichthys molitrix*) exposed to toxic cyanobacterial blooms in natural environment was described. Activity of GR was no significantly changed in a majority of experimental variants in both of kinds (ADAMOVSKÝ et al., 2007).

Lipid peroxidation (LPO)

Many studies have demonstrated that lipid peroxidation and oxidative stress increases in tissues of different species of aquatic organisms, as a result of being exposed to environmental stressors (WINSTON and DIGIULIO, 1991).

All three organs studied from tilapia fish i.p. injected with MC-LR showed a significantly increased level of lipid peroxidation. The liver was the most affected organ. MC-RR also increased LPO values in kidney and gills, while the liver maintained its basal value. Also a differential response to both MC variants was observed in the liver and kidney of treated fish (PRIETO et al., 2006). Microcystin-RR induced LPO in brain of exposed fish (*Corydoras paleatus*), but non in other organs (CAZENAVE et al., 2006a). The activity of LPO was significantly increased when the tilapia fish (*Oreochromis niloticus*) were exposed to a single dose of cyanobacterial cells containing MC-LR (PRIETO et al., 2007).

Activity of LPO did not change in liver of loach (*Misgurnus mizolepis*) after orally exposure to low dose of *Microcystis* cells (LI et al., 2005).

Malondialdehyde (MDA)

The effects of microcystins from cyanobacterial cells on various oxidative stress biomarkers in liver, kidney and gill tissues in freshwater tilapia fish (*Oreochromis* sp.) were investigated under laboratory conditions. After 14 days, were observed significantly increased of MDA in liver, kidney and gills in fish exposed to the crushed cyanobacteria (JOS et al. 2005). Silver carp (*Hypophthalmichthys molitrix*) exposed to cyanobacterial bloom had increasing trend (but no significant) concentrations of MDA (BLÁHA et al., 2004).

Changes in activity of detoxification enzymes and lipid peroxidation in tissues have been used as a biomarker of chronic cyanobacterial toxicity in fish. However, the responses in these biomarkers could be highly variable. The main factors in activity of biomarkers are time of exposure to toxins, kinds of microcystins (MC-LR, MC-RR or another variant), kinds of fish and differences in detoxification potency among diverse organs of fish.

Protein phosphatases assay

Microcystins in hepatocytes binds covalently with protein phosphatases 1 and 2A in the cytosol and nuclei (FUKUJI and SUGANUMA, 1993). Inhibition of the enzyme activity results from an initial non-covalent interaction, which is mediated by MCs hydrofobic Adda side chain and the glutamyl carboxyl (RUNNEGAR et al., 1995). Pathological changes connected with PP inhibition were also observed in fish treated with purified MC or cyanobacterial material. Inhibitory effect of MCs on protein phosphatase in the liver of carp (*Cyprinus carpio*) investigated TENCALLA and DIETRICH (1997), FISHER and DIETRICH (2000), in grass carp XU et al. (2000) in rainbow trout (*Oncorhynchus mykiss*) SAHIN et al. (1995), FISHER et al. (2000) in medaka fish (*Oryzias latipes*) HUYNH-DELERME et al. (2005) and MEZHOUD et al. (2008). On the other hand the effect of MC-LR in embryonic development of zebrafish (*Danio rerio*) on PP inhibition was very low. The causes of difference may be due to membrane impermeability that impaired the delivery of MC-LR into cytoplasm of zebrafish (WANG et al., 2005).

In summary, the data suggest that the IC_{50} of protein phosphatase inhibition by MCs is in similar range (0.1–0.25 nM) throughout a wide selection of organisms, including mammals, bivalves, zooplankton and plant (MACKINTOSH et al., 1990; DEMOTT and DHAWALE, 1995), i. e., the acute symptom of intoxication are associated with the reversible interaction of MC with hepatic PP. Hepatocyte necrosis appears to be primarily associated with the reversible and irreversible inhibition of PP1, whereas apoptosis, a late event, is associated with the irreversible inhibition of PP-2A (FISHER et al., 2000). The ability of MCs to inhibit PP is used in a colorimetric protein phosphatase inhibition assay (PPI assay) for detection of microcystins (RAPALA et al., 2002).

SOUHRN

Vliv microcystinů na změny biochemických parametrů u ryb

V uvedené práci byla sledována odezva biochemických markerů u ryb na toxické působení cyanobakteriálních toxinů (microcystinů). Mezi nejsledovanější biomarkery vlivu cyanobacteriálních toxinů paří parametry oxidativního stresu – glutation S-transferáza (GST), neenzymatický antioxidant glutation (GSH), superoxid dismutáza (SOD), kataláza (CAT), glutation peroxidáza (GPx), lipidová peroxidace (LPO), malondialdehyd (MDA), glutation reduktáza (GR), parametry krve – hodnota hemoglobinu (Hb), hematokryt (PCV), střední barevná koncentrace (MCHC), střední objem erytrocytu (MCV), střední obsah hemoglobinu erytrocytu (MCH), počet erytrocytů (RBC) počet leukocytů (WBC), parametry krevní plazmy – alanin aminotransferáza (ALT), aspartát aminotransferáza (AST), laktát dehydrogenáza (LDH), alkalická fosfatáza (ALP), cholinesteráza (CHE), celkové bílkoviny (TP), glukóza (GLU), laktát (LACT), železo (Fe), vápník (Ca), hořčík (Mg), celkový bilirubin (BIL), fosfor (P) a aktivita protein fosfatáz (PP1, PP2A).

Biochemické parametry krve a plazmy u ryb jsou ovlivněny mnoha endogenními i exogenními faktory. Mezi nejsledovanější parametry patří enzymy krevní plazmy (ALT, AST and LDH), které výrazně zvyšují svoji aktivitu po intraperitoneální nebo perorální aplikaci microcystinů rybám. Toxický účinek sinic na ryby v přírodním prostředí je výrazně nižší než po přímé aplikaci toxinu do rybího organismu a změny v aktivitě enzymů u těchto přírodních experimentů jsou většinou neprůkazné. Na významu pak nabývají další parametry krevní plazmy indikující negativní působení sinic (elektrolyty, laktát, glukóza, albumin, cholesterol aj.).

Toxické metabolity sinic vyvolávají oxidativní stres a sledování změn vhodných biomarkerů lze dobře využít také u ryb k časné indikaci poškození organismu v důsledku expozice toxickým sinicím. Je zřejmá silná časová závislost modulace detoxikačních pochodů při vystavení ryb vlivu microcystinů. V závislosti na délce expozice tak lze vysledovat nárůst i pokles aktivity jednotlivých biomarkerů oxidativního stresu způsobený stimulací nebo inhibicí protektivních procesů. Výrazné rozdíly lze v závislosti na intenzitě detoxifikačních pochodů vysledovat i u různých orgánů ryb, rovněž tak jsou nalézány výrazné rozdíly mezi jednotlivými druhy ryb.

ryby, microcystiny, biomarkery, oxidativní stres

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