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ARTICLE

Effect of long term feeding of T-2 and HT-2 toxin contaminated diet on the glutathione redox status and lipid peroxidation processes in common carp (Cyprinus carpio L.)

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ABSTRACT Effect of sublethal T-2 (2.45 mg kg^{-1} feed) and HT-2 (0.52 mg kg^{-1} feed) toxin treatment for 4 weeks was investigated in common carp. Two groups, a control and a T-2+HT-2 toxin treated were formed. Six carps were exterminated from each group weekly. Liver samples were taken, in which reduced glutathione concentration and glutathione-peroxidase activity were measured. Free radical formation was measured by a direct reactive oxygen metabolites test, and also malondialdehyde concentration was determined. From the first week lower feed consumption and weight gain was recorded in T-2 toxin treated group, which resulted significantly lower live weight at the end of experiment. Feeding the T-2 toxin contaminated diet caused, significantly elevated glutathione concentration and glutathione-peroxidase activity during the first week. At second week the T-2 toxin loading resulted decrease in glutathione concentration and glutathione-peroxidase activity of liver as compared to the control. Although the reactive oxygen metabolite concentration was elevated in T-2 toxin treated group during the first two weeks, no significant changes were found in the MDA concentration. The results show that the biological antioxidant system was able to eliminate the harmful peroxidative effect of T-2 toxin in common carp. **Acta Biol Szeged 53(Suppl.1): (2009)**

KEY WORDS

common carp
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Healthy environment (also healthy feed) is essential to maintain fish harvest level in the face of increasing demand. Moulds, and also their secondary metabolites, the mycotoxins are responsible for the impairment/mildew of feedstuffs either in field, or during transportation and/or storage (Lawlor and Lynch 2005). The occurrence of mycotoxins in cereal grains is a great concern, because their presence in feeds is often associated with chronic of acute mycotoxicoses in animals. Mycotoxins cause a wide variety of adverse effect (decrease of immune response, clinical signs) depending on the nature and concentration of mycotoxins present, the duration of exposure, the animal species, its age, health and nutritional status during the exposure to mycotoxin contaminated feed (Diaz 2005).

In fish feeds and feedstuffs several moulds are producing mycotoxins (e.g. Aspergillus, Penicillium and Fusarium). Moreover any mycotoxin could be produced from different mould species, and different moulds can produce the same mycotoxins (Jouany 2007).

With the environmental pressure on the aquaculture industry to reduce the level of fish meal in the diet with plant based proteins, the occurrence of mycotoxin contamination in fish feeds may increase. Although the presence the mycotoxin contaminated feeds in fish nutrition in tropical and subtropical areas of the world is relative high, few data are available about its toxic effect on fish species, their harmful effect on the biological antioxidant system and thereby the immune response and health status of the animals (Manning 2005).

In Hungary, the continental climate is preferable for Fusarium species, which produce a variety of thichotecene mycotoxins. Fusarium sporotrichoides is a widespread mould on plant and in the soils of the temperate climate of the world, producing 'type A' trichothecene mycotoxins, e.g. T-2 toxin, HT-2 toxin, T-2 triol, T-2 tetraol, scirpentriol, and diacetoxyscirpenol (Ueno 1983)..

The first report on toxicity of Fusarium mycotoxins in fish was published by Marasas et al. (1969). They found that low doses (0.2 or 0.4 mg kg^{-1}) of T-2 toxin derived from Fusarium tricinctum in the feed of rainbow trout (Salmo gairdneri) for 12 months failed to induce hepatoma and actually had a growth promoting effect. However, an acute dose (6.1 mg kg^{-1}) of T-2 toxin was more toxic to fingerlings than adults, although dose of 8 mg kg^{-1} severely damaged the intestinal tract of the fishes.

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Table 1. Measured nutrient content of diet.

Nutrient content (% dry matter)		
Crude protein		33.43 %
Crude fat		6.66 %
In which	saturated fatty acids	23.00 %
	monounsaturated fatty acids	35.70 %
	polyunsaturated fatty acids	40.90 %
Crude fiber		0.57 %
Nitrogen free extract		55.26 %
Ash		4.08 %

Woodward et al. (1983) found that deoxynivalenol (1 to 13 mg kg^{-1}) produced by Fusarium moulds in diet of rainbow trout led to progressively greater depression of weight gain ranged (from 12% to 92% of the control) and resulted from an adverse effect on both feed intake and feed conversion efficiency. Complete feed refusal occurred at 20 mg kg^{-1} deoxynivalenol. The T-2 toxin in different dietary concentration (0.625, 1.25, 2.5 and 5.0 mg kg^{-1} of diet) according to Manning et al. (2003) is responsible for significant reduction of growth (in all treatments), poor feed conversion (only at 5.0 mg kg^{-1} T-2 toxin level), adversely affected hematocrit value (at 1.25, 2.5 and 5.0 mg kg^{-1} T-2 toxin level), low survivability and histopathological anomalies of stomach and kidney in juvenile channel catfish.

There are few data about the effect of T-2 toxin on the xenobiotic transforming enzymes in fishes. Kravchenko et al. (1989) found that 0.46 mg kg^{-1} body weight T-2 toxin moderately increased glutathione-S-transferase (GST) activity in carp, whereas significantly increased (2-11 fold) activity of lysosomal enzymes and elevated (2-fold) alkaline phosphatase activity was also measured.

Materials and Methods

A total of 72 common carps (Cyprinus carpio L.) were obtained from a commercial fish farm and divided into 6 aquaria (150 L each), at a stocking density of 12 fish per aquaria. All aquaria were filled up with aerated dechlorinated tap water and were connected to a re-circulating system. Light regimen was maintained at a 12:12 h light:dark schedule. The aquaria were cleaned every day by syphoning out the debris and faeces. After 3 weeks of adaptation period, at the initial of the experiment two groups were formed, a control (3 aquaria) and a treated one (3 aquaria).

The diet of the control group (carp grower complete feed) did not contain detectable amounts of trichothence mycotoxins, while the feed of the treated group was contaminated by T-2 (2.45 mg kg^{-1}) and HT-2 (0.52 mg kg^{-1}) toxin. Mycotoxin analyses were carried out by HPLC method according to the standard method (Hungarian Feed Code 2004). The nutrient content of diet was determined according to the standard methods (Hungarian Feed Code 2004) and it is shown in Table 1.

T-2 and HT-2 toxin were produced experimentally on maize by Fusarium sporotrichioides strain NRRL 3299 (Agricultural Research Service Culture Collection, National Centre for Agricultural Utilization Research, Peoria, IL). Extraction and purification of toxin was carried out according to the method of Fodor et al. (2006).

The experiment lasted four weeks. At the 7^{th}, 14^{th}, 21^{st} and 28^{th} days of experiment 6-6 fish were sampled both from the control and the T-2 toxin treated group. They were weighed and exterminated. Liver samples were taken and were stored at -20°C (2 days), then at -70°C for further analysis.

The experiment was approved by the Animal Experimental Committee of the Faculty of Agricultural and Environmental Sciences of the Szent István University.

Biochemical analyses

For the biochemical analyses small amount (0.5 g) of the thawn liver samples were homogenized in nine-fold volume of isotonic saline (0.9% w/v NaCl).

Glutathione peroxidase (E.C.1.11.1.9) activity was measured in the 10,000 g supernatant fraction of liver homogenate, using reduced glutathione (GSH) and cumene hydroperoxide as co-substrates in an end-point direct assay following the system of Lawrence and Burk (1976). 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°C. The enzyme activity was calculated to protein content of the 10,000 g supernatant fraction of tissue homogenate, which was measured using Folin-phenol reagent (Lowry et al. 1951).

Table 2. Live weight of the sampled animals during the experiment.

	Live weight (g)			
	7th day	14th day	21st day	28th day
Control	53.45 ± 5.20	55.55 ± 8.53	56.60 ± 3.73	62.45b ± 6.48
T-2 and HT-2 toxin treated	52.35 ± 3.88	53.55 ± 6.26	54.52 ± 10.33	53.68a ± 5.61

All values are given as the mean ± SD; $n = 6$. Values in the same column with different superscripts significantly differ at $p < 0.05$.

Table 3. Effect of long-term feeding of T-2 and HT-2 toxin contaminated diet on the glutathione redox system of carps.

	GSH concentration (umol/g)			
	7th day	14th day	21st day	28th day
Control	1.68aA ± 0.20	1.74AB ± 0.28	2.15B ± 0.53	1.55aA ± 0.39
T-2 and HT-2 toxin treated	2.36bB ± 0.52	1.60A ± 0.21	2.44B ± 0.65	2.24bB ± 0.66
		GSHPx activity (U/g protein)		
	7th day	14th day	21st day	28th day
Control	1.87a ± 0.40	2.09 ± 0.37	2.36 ± 0.50	1.96 ± 0.51
T-2 and HT-2 toxin treated	2.60bAB ± 0.32	1.89A ± 0.42	2.84B ± 1.04	2.61AB ± 0.76

All values are given as the mean ± SD; $n = 6$. Values in the same column with different superscripts (a, b) significantly differ at $p < 0.05$. Values in the same row with different superscripts (A, B) significantly differ at $p < 0.05$.

GSH content of the 10,000 g supernatant fraction of liver homogenate was determined based on the colour complex formation of glutathione with Ellmann's reagent (Sedlak and Lindsay 1968).

Reactive oxygen metabolites (ROMs) of the 10,000 g supernatant fraction of liver homogenate were measured by a colorimetric determination kit (Diacron, Grosetto, Italy). In the test hydroperoxides in presence of iron are able to generate alkoxyl and peroxyl radicals. These radicals are able to oxidize an alkyl-substituted aromatic amine transforming them in a pink-colored derivative, which can be photometrically quantified (Cornelli et al. 1999).

Malondialdehyde (MDA) content of the liver 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 temperature (Placer et al. 1966). The standard was 1,1,3,3-tetraethoxypropane (Fluka, Buchs, Switzerland).

Statistical analysis

After calculating the means and standard deviations (S.D.) statistical evaluation (paired t-tests, analysis of variance, linear regression analysis) of the results was carried out with Statistica™ for Windows 4.5 (Statsoft Inc., 1993) software.

Results

During the experiment no clinical signs of mycotoxicosis or mortality emerged. Live weight of fishes in the T-2 toxin treated group was significantly lower from the 1st week of the trial up to the end of experiment (Table 2).

Feeding the T-2 and HT-2 toxin contaminated diet increased the amount/activity of glutathione redox system during the 1st week, which is shown by the significantly elevated GSH concentration and glutathione peroxidase (GSHPx) activity of the 10,000 g supernatant fraction of liver homogenate (Table 3). At 2nd week of the trial the T-2 and HT-2 toxin treatment resulted decrease in GSH concentration and GSHPx activity of liver compared to control, but at the end of the experiment both parameters – GSH concentration even at $p<0.05$ level of significance – exceeded the values measured in control fishes (Table 3). In the T-2 and HT-2 toxin treated group the lowest GSH concentration and GSHPx activity was measured at 14th day of experiment which was significantly lower – in case of GSH concentration – than the values at the

Table 4. Effect of long-term feeding of T-2 and HT-2 toxin contaminated diet on the free radical generation and lipid peroxidation processes of carps.

	dROMs concentration (mg H_2O_2/dl)			
	7th day	14th day	21st day	28th day
Control	0.61 ± 0.34	0.81±0.46	2.05±0.36	1.52±1.21
T-2 and HT-2 toxin treated	1.52 ± 1.09	1.88±1.36	1.98±0.67	1.28±1.03
		MDA concentration (µmol/g)		
	7th day	14th day	21st day	28th day
Control	39.78BC ± 10.56	30.35B ± 2.86	16.29A ± 6.57	24.59AB ± 12.97
T-2 and HT-2 toxin treated	36.41C ± 5.47	34.70C ± 4.55	15.30A ± 1.94	24.54B ± 12.95

All values are given as the mean ± SD; $n = 6$. Values in the same column with different superscripts (a, b) significantly differ at $p < 0.05$. Values in the same row with different superscripts (A, B, C) significantly differ at $p < 0.05$.

Table 5. Summary of the linear regression analysis (linear regression coefficients) between the measured parameters of glutation redox system and lipid peroxidation processes in liver of carps. n.s. – non significant.

		GSHPx	MDA	dROMs
GSH control	r =	0.765	- 0.275	0.370
		p < 0.001	n.s.	n.s.
T-2 and HT-2 toxin treated	r =	0.913	- 0.019	- 0.087
		p < 0.001	n.s.	n.s.
GSHPx control	r =		- 0.258	0.191
			n.s.	n.s.
T-2 and HT-2 toxin treated	r =		- 0.106	- 0.063
			n.s.	n.s.
MDA control	r =			- 0.804
				P<0.001
T-2 and HT-2 toxin treated	r =			- 0.326
				n.s.

other samplings, and in case of GSHPx activity than at 21st day of experiment. (Table 3).

Although the reactive oxygen metabolite concentration was elevated in T-2 and HT-2 toxin treated group during the first 2 weeks of the trial, no significant changes were found in the MDA concentration between the T-2 and HT-2 toxin treated and the control carps (Table 4). Both in control and T-2 + HT-2 toxin treated groups significantly lower MDA concentration was measured at 21st day of experiment than at the other days of sampling (Table 4).

Linear regression analysis of the measured parameters showed close positive correlation between the GSH concentration and GSHPx activity of liver both in control ($r=0.765$; $p<0.001$) and T-2 and HT-2 toxin treated groups ($r=0.913$; $p<0.001$). Negative, although weak, correlation was found between the measured parameters of glutathione redox system (GSH concentration or GSHPx activity) and the MDA concentration in both experimental groups (Table 5). Negative linear correlation was found between the concentration of reactive oxygen metabolites (ROMs) and the MDA concentration in both experimental groups, which was strong in the control group ($r=-0.804$; $p<0.001$) (Table 5).

Discussion

The observed reduced live weight of the T-2 toxin treated group at the end of the 4 weeks long experiment are in line with the findings of Manning et al. (2003) in channel catfish, who used similar T-2 toxin concentrations to this experiment. Marasas et al. (1969) also observed this effect of T-2 toxin exposure in rainbow trout but in a long-term (12 month) feeding trial.

The changes of GSH concentration in liver of T-2 toxin treated group are slightly controversial to the findings of Kravchenko et al. (1989). In their study with common carp they found no effect of T-2 toxin (0.46 mg kg^{-1} body weight) on the GSH level of liver. Our result may show that T-2 toxin at the applied dose (3.0 mg kg^{-1}) at the beginning of exposure increases the amount (GSH) and activity (GSHPx) of glutathione redox system, which play important role in elimination of harmful peroxides. Later, at 14th day there were a small decrease both in the amount and activity of glutathione redox system in the T-2 toxin treated group compared to the values a week before, but increased again during the following weeks of the trial. These results suggest continuous activation of the glutathione redox system as effect against oxidative stress caused by T-2 and HT-2 toxin exposure.

Linear regression analysis showed close positive correlation between the GSH concentration and GSHPx activity of liver both in control and T-2 toxin treated groups, which are in line with our previous findings in broiler chickens (Balogh et al 2007). The co-substrate (GSH) surplus caused elevated GSHPx activity both at 7th and 28th day of experiment, but at 14th day it has resulted lower GSHPx activity than the control.

Although the reactive oxygen metabolite concentration was slightly elevated in T-2 toxin treated group during the first two weeks of T-2 and HT-2 toxin load, no significant changes were found in the MDA concentration, which shows that the biological antioxidant system was able to eliminatethe harmful peroxidative effec of T-2 toxin n commo capAcknowledgment

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