Effects of feed-borne trichothecene mycotoxins on the lipid peroxidation processes and the glutathione redox system of common carps (*Cyprinus carpio*)

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Summary

The aim of this study was to investigate the long-term effects of T-2 toxin and deoxynivalenol on common carp (*Cyprinus carpio*). The long-term exposure of mycotoxins resulted in increased mortality at both toxin treated group, and the enzymatic antioxidant system was also activated. The parameters of the initial steps of the lipidperoxidation were elevated, which can be in association with the effects of increased oxidative stress, in spite there were no difference in the concentration of malondialdehyde in the mycotoxin treated groups, which means that the progress of lipidperoxidation has been successfully compensated by the antioxidant system.

1. Introduction and literature review

The primary aim of fish nutrition is to demand nutrient requirement with balanced mixture of ingredients to foster the maintenance, growth, reproduction, meat quality and health of the animals.

Due to economical and environmental reasons the need is increasing to replace animal-derived proteins in complete feeds, such as fish meal, with less expensive plant protein sources. This increases the impact of mycotoxin contamination in aquaculture feeds due to the higher susceptibility for mycotoxin contamination in ingredients of plant origin (eg. corn, wheat, barley, oats, soybean meal) (http_1).

Mycotoxins are secondary metabolites, produced by several moulds. The presence of mycotoxins in feed cause loss of production and toxic effects in animals in a dose dependent way (*Diaz,* 2005). These compounds are heat-stable, therefore they remain stable during conventional feed processes (*Szigeti,* 1997).

Fusarium species may produce a number of different mycotoxins with different chemical structures (*Wood,* 1992). The most frequently found mycotoxins in cereal grains worldwide is a 'type B' trichothecene mycotoxin, namely deoxynivalenol (DON). While 'type A' trichothecene mycotoxin, T-2 toxin is considered as one of the most toxic trichothecene (*Bamburg et al,* 1968).

T-2 toxin and also DON inhibit protein and DNA synthesis in eukaryotic cells (*Holladay,* 1995). Therefore T-2 toxin and DON are well-known immuno-depressive or immuno-suppressive compounds (*Kidd et al.,* 1995). Additionally, DON affects the serotoninergic system as a neurotoxic effect, resulting in its ability to have influence on feeding behavior and cause emetic response (*Fioramonti et al.,* 1993., *Prelusky and*

Trenholm, 1993) and also may be related to the effects on cell signaling processes (*Leathwood,* 1987). T-2 toxin and DON contain epoxy groups in their chemical structure, which may play role in dermatotoxic effects (*Szilágyi et al.,* 1994).

A maximum of 0.25 mg T-2 toxin/kg feed is recommended according to the EU proposal (2013/165/EU), while *Eriksen and Pettersson* (2004) suggests 0.5 mg T-2 toxin/kg feed concentration level as tolerable level. The recommendation for DON sets a limit of 5 mg/kg complete feed (2006/576/EC).

Nevertheless, the effects of T-2 and DON on fish species are not fully described in the literature. *Woodward et al.* (1983) and *Hooft et al.* (2011) showed reduced feed intake, growth and feed efficiency in parallel of the concentration of DON in diet rainbow trout (*Oncorhynchus mykiss*). Total feed refusal was observed at a concentration of 20 mg DON/kg feed (*Woodward et al.,* 1983). Similar results were found in these parameters in Atlantic salmon (*Salmo salar*) fed 3.7 mg DON/kg feed (*Döll et al.,* 2010). *Hooft et al.* (2011) also observed histopathological changes in liver and intestine in trout as an effect of DON.

In spite, feeding of diets containing DON up to 10 mg/kg feed had no effect on feed consumption, growth, hematocrit values and liver weights of channel catfish (*Ictalurus punctatus*). In particular mortality was lower at higher levels than 5.0 mg DON/kg feed. Indeed the consumption of DON-contaminated feed may resulted in a protective effect against the bacterium *Edwardsiella ictaluri* in channel catfish (*Manning et al.,* 2013). Also, in zebrafish (*Danio rerio*) DON increased fecundity and larvae swimming activity (*Sanden et al.* 2012).

Despite, channel catfish fed diets contaminated with T-2 toxin from 0.625-5.0 mg/kg feed reduced growth rate and increased mortality beyond 2.5 mg/kg feed (*Manning et al.,* 2003). Also in common carp (*Cyprinus carpio*) T-2 toxin in a dose of 2.45 mg/kg feed and 0.52 mg HT-2 toxin/kg feed toxin resulted similar responses in these parameters (*Balogh et al.,* 2009). In a study with T-2 toxin next to reduced feed consumption and growth, also lowered hematocrit and blood hemoglobin levels were observed in rainbow trout at higher levels than 2.5 mg/kg feed (*Poston,* 1983).

In addition, an isolated microbial community from catfish transformed DON to deepoxy DON. Also, the microbial culture's ability was proved to transform other trichothecene mycotoxins, where most of the toxins were transformed to deacetyl and/or deepoxy products, however, T-2 toxin was not examined (*Guan et al.,* 2009).

Previously, it was found that the intensity of lipid peroxidation processes increased in farm animals as a result of long-term exposition of trichothecenes in context of biochemical changes in cells which affect the activity of the biological antioxidant system as well (*Mézes et al.,* 1998; *Surai et al.,* 2002). *Sanden et al.* (2012) showed elevated levels in zebrafish liver of CYP1A mRNA levels and gene transcripts of CuZn SOD and Cyclin G1 with increasing content of dietary DON. Also, *Kravchenko et al.* (1989) investigated the effect of T-2 toxin on the enzymes of xenobiotic transformation in common carp. They found moderately increase in the activity of glutathione S-transferase (GST). A long-term feeding trial with T-2 toxin in a dose of 2.45 mg/kg feed and 0.52 mg HT-2 toxin/kg feed resulted elevated levels of reduced glutathione (GSH) and affected glutathione-peroxidase (GPx) activity, while malondialdehyde (MDA) content did not change in a significant way (*Balogh et al.,* 2009).

2. Material and methods

During this long-term experiment the effect of T-2 toxin (5.00 mg/kg feed) and deoxynivalenol (DON) (6.00 mg/kg feed) treatments was investigated in common carp for 5

weeks. A total of 144 one-year old common carps (Szarvasi P34 hybrid) were obtained from a commercial fish farm (ÖKO 2000, Akasztó) and after a one-week acclimatization period were divided randomly into three treatment groups (control, T-2 toxin treated and DON treated) into 6 aquaria (150 L each). All aquaria were filled up with aerated de-chlorinated tap water and were connected to a re-circulating system. Light regimen was maintained at a 12:12 h light:dark schedule.

Six carps were weighed and exterminated from each group weekly. Liver samples were taken and stored at -18 °C until analysis. Reduced glutathione concentration, and activities of glutathione-peroxidase and glutathione-S-transferase were measured to observe alterations in the antioxidant system. To investigate the lipidperoxidation processes, the amount of conjugated dienes (CD) and trienes (CT) were measured, as biomarkers for the initiation phase of the progress, while malondialdehyde concentration, a meta-stable end product of lipid peroxidation processes were determined.

Determination of malondialdehyde (MDA) content was carried out in the native homogenate, while the other parameters were determined in the 10,000 g supernatant fraction of the homogenate.

Conjugated diene (CD) and -triene (CT) content of the liver samples were measured according to the *AOAC* (1984) after extraction of the sample with trimethyl-pentane and measuring the absorption at 232 nm and 268 nm, respectively.

MDA concentration 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). Reduced glutathione (GSH) content of liver homogenate was measured as described by *Rahman et al.* (2007). Glutathione peroxidase (GPx) activity was determined according to *Lawrence and Burk* (1976), where 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, and it 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).

Glutathione-S-transferase (GST) activity in the 10,000 g supernatant fraction of liver homogenate was measured by an assay kit (Sigma, St.Louis, USA) according to the method as described by *Habig et al.* (1974). GST catalyzes the conjugation of glutathione to CDNB. The product, GSH-DNB conjugate, absorbs at 340 nm. The increase in the absorption is directly proportional to the GST activity in the sample.

Statistical evaluation of the data, treatment groups vs. control was performed using Fisher's Least Significance Difference post-hoc test of the one-way ANOVA after calculating the means and standard deviation with Statistica for Windows 4.5 (Statsoft Inc., Tulsa, OK, USA) software.

3. Results and discussion

The applied doses of mycotoxins increased the mortality in the treated groups compared to control (8.3%) during the experiment. The highest mortality rate (29.2%) was measured in the T-2 toxin treated group, but the mortality rate of DON treatment also was twice as big as of control (16.7%).

Feeding DON toxin contaminated diet caused significantly elevated GSH concentration at 4th week of mycotoxin exposition (Table 1.), and the glutathione-peroxidase activity was also higher than the control (Table 2.). In addition GST activity was significantly lower than

the other groups at the 5th week of experiment (Table 3.). There were no significant changes in other sampling times and other measured endpoints in DON treated group.

The T-2 toxin treatment increased the GSH concentration in liver at 2nd week of mycotoxin exposition, but later, at the 3rd week, there was a significant decrease, then it increased again during the following weeks of the trial (Table 1.). Although at the 2nd week T-2 toxin exposition elevated the GPx activity as compared to the control, at the 3rd week it resulted significant decrease as compared to the control (Table 2.). The activity of glutathione-S-transferase (GST) in liver was significantly lower than the control at 2nd and 3rd weeks of mycotoxin exposition (Table 3.)

The amount of CD and CT was significant higher in T-2 toxin treated group at 5th week of mycotoxin exposition (Table 4. and 5.). Also, no significant changes were found in the MDA concentration of liver (Table 6.) as compared to the control.

Table 1.:Changes in reduced glutathione concentration in carp liver

GSH (umol/g prot.)				
		Control	DON	T-2 toxin
0. week	mean	1.67		
	s.d.	0.36		
1. week	mean	1.80	1.62	1.43
	s.d.	0.65	0.22	0.23
2. week	mean	1.47	1.82	1.48
	s.d.	0.33	0.30	0.59
3. week	mean	1.60	1.53	1.42
	s.d.	0.49	0.28	0.08
4. week	mean	1.41	**1.94***	1.67
	s.d.	0.28	0.36	0.23
5. week	mean	1.42	1.32	1.51
	s.d.	0.06	0.15	0.22

*P<0.05; compared to the control group

Table 2.: Changes in glutathione peroxidase (GPx) activity in carp liver

GPx (E/g prot.)				
		Control	DON	T-2 toxin
0. week	mean	1.68		
	s.d.	0.48		
1. week	mean	1.97	1.64	1.51
	s.d.	0.61	0.30	0.27
2. week	mean	1.37	1.91	1.80
	s.d.	0.76	0.36	0.67
3. week	mean	1.91	1.69	**1.49***
	s.d.	0.36	0.16	0.20
4. week	mean	1.86	2.17	2.04
	s.d.	0.41	0.48	0.54
5. week	mean	1.74	1.71	1.74
	s.d.	0.12	0.10	0.28

*P<0.05; compared to the control group

Table 3.: Changes in glutathione S-transferase (GST) activity in carp liver

GST (nmol/min/mg prot.)				
		Control	DON	T-2 toxin
0. week	mean	12.93		
	s.d.	2.39		
1. week	mean	11.02	12.07	11.78
	s.d.	1.89	0.73	0.79
2. week	mean	12.99	11.88	**10.36***
	s.d.	1.48	1.69	1.93
3. week	mean	13.04	11.37	**10.93***
	s.d.	1.73	2.11	1.01
4. week	mean	14.78	16.52	15.88
	s.d.	2.29	2.84	3.22
5. week	mean	15.09	**10.80***	13.90
	s.d.	1.27	0.60	0.78

*P<0.05; compared to the control group

Table 4.: Changes in malondialdehyde (MDA) content in carp liver

MDA (nmol/g)				
		Control	DON	T-2 toxin
0. week	mean	8.66		
	s.d.	2.84		
1. week	mean	5.47	5.67	5.48
	s.d.	1.74	0.90	0.81
2. week	mean	6.58	5.49	8.10
	s.d.	0.96	1.39	2.98
3. week	mean	10.59	8.51	8.88
	s.d.	2.98	3.14	2.23
4. week	mean	13.04	13.40	12.25
	s.d.	3.94	4.98	3.44
5. week	mean	10.11	11.29	11.46
	s.d.	1.56	0.77	0.98

*P<0.05; compared to the control group

1. table: Changes in conjugated dienes in carp liver

Conjugated dienes OD 232nm				
		Control	DON	T-2 toxin
0. week	mean	0.21		
	s.d.	0.10		
1. week	mean	0.25	0.26	0.23
	s.d.	0.14	0.09	0.08
2. week	mean	0.30	0.30	0.34
	s.d.	0.15	0.14	0.13
3. week	mean	0.33	0.29	0.23
	s.d.	0.10	0.10	0.08
4. week	mean	0.33	0.31	0.43
	s.d.	0.13	0.14	0.19
5. week	mean	0.26	**0.27***	**0.44***
	s.d.	0.04	0.05	0.09

*P<0.05; compared to the control group

2. table: Changes in conjugated trienes in carp liver

Conjugated trienes OD 268nm				
		Control	DON	T-2 toxin
0. week	mean	0.15		
	s.d.	0.07		
1. week	mean	0.16	0.16	0.14
	s.d.	0.08	0.05	0.04
2. week	mean	0.19	0.15	0.19
	s.d.	0.09	0.04	0.07
3. week	mean	0.20	0.17	0.15
	s.d.	0.07	0.06	0.07
4. week	mean	0.20	0.20	0.26
	s.d.	0.07	0.07	0.12
5. week	mean	0.16	**0.17***	**0.25***
	s.d.	0.03	0.02	0.04

*P<0.05; compared to the control group

4. Conclusions

To summarize the results, long term exposure of T-2 toxin or DON resulted in higher mortality as compared to control group, which may be related to the inhibitory effect of these mycotoxins on protein synthesis and also other complex effects of T-2 toxin and DON, which may sensitize the fishes for diseases as well results in weaker survivability of the carps. Also, GSH, GPx and GST levels were affected by the mycotoxins and the parameters about the initial steps of the lipidperoxidation were elevated, which can be in association with the effects of oxidative stress, in spite there were no difference in the concentration of malondialdehyde in the mycotoxin treated groups, which means that the progress of lipidperoxidation has been successfully terminated.

These results suggest that, the activation of the biological antioxidant system against oxidative stress caused by T-2 toxin and deoxynivalenol exposure was successful and the biological antioxidant system was able to eliminate the harmful peroxidative effect of T-2 toxin and DON in common carp in the current doses and the current length of exposure.

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