

LIPID PEROXIDE AND ANTIOXIDANT STATUS OF BROILER CHICKENS AFTER ORAL ADMINISTRATION OF *FUSARIUM SPOROTRICHOIDES* FUNGAL CULTURE

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Introduction

Phytopathogenic *Fusarium* moulds infect the cereals in temperate climatic condition and the contamination has undesirable effects on the germination ability and the thousand-grain weight (Kerekes, 2004). Fungi have negative effect also on the development of young plants (Srobarova *et al.*, 2004), and last, but not least on the nutritive quality of grains (Györi *et al.*, 2005). *Fusarium* moulds produce trichothecene mycotoxins, which have great importance in animal nutrition, because of their widespread occurrence, diversity and diverse effects on livestock, such as teratogenic and genotoxic actions as well as toxicity (Leeson *et al.*, 1995). Animals can be commonly treated with antioxidant (e.g. vitamins E and C, selenium) supplementation against the chronic effects of mycotoxins (Hoechler and Marquardt, 1996; Surai *et al.*, 2002), because most of the trichothecene mycotoxins have marked effect on the antioxidant status of animals (Atroschi *et al.*, 2002), primarily due to their pro-oxidant effect (Rizzo *et al.*, 1994; Mézes *et al.*, 1998). It was also found that the deficiency of vitamins A and E is associated with significant increase in T-2 toxin toxicity (Tutelyan and Kravchenko, 1988).

The objective of the present study was to evaluate the effect of T-2 toxin load on the rate of lipid peroxidation, also on antioxidant status of broiler chickens.

Methods

A total of 35 ROSS 308 broiler cockerels (age: 21 days) divided into 2 groups of 15 birds each and 5 birds as absolute control were used in the experiment. In the control group 20 and the T-2 toxin loaded group 15 birds were used in the experiment. The diet of the chickens in the T-2 toxin loaded group was contaminated with T-2 toxin and was dosed for 14 days. The diet fed to the control group did not contain detectable amounts of mycotoxin. T-2 toxin was 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 Burmeister (1971). The contaminated feed contained 2.05 mg/kg T-2 toxin (as measured at the Central Veterinary Institute, Budapest by HPLC technique).

Samples were taken on days 0 (only absolute control samples), 3, 7 and 14 of the experiment (5 birds/groups). Chickens were sacrificed by cervical dislocation and blood as well as liver samples were taken. The samples were stored at –20 °C until analysis. Lipid peroxidation was determined by the quantification of malondialdehyde (MDA) levels with 2-thiobarbituric acid method in the blood plasma (Placer *et al.*, 1966) and liver (Mihara *et al.*, 1980). Among the small molecular weight antioxidants, the amount of reduced glutathione (Sedlak and Lindsay, 1968), vitamin E (McMur-ray *et al.* 1980) and ascorbic acid (Omaye *et al.*, 1979) was measured in the blood plasma and in the liver.

Statistical evaluation of the results was carried out by analysis of variance (ANOVA) and least significant difference (LSD) tests after calculating the means and standard deviations (S.D.) in all groups using MS Excel 7.0 (Microsoft, 2000) and Statistica™ 4.5 (Statsoft Inc., 1993) soft-ware.

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

Results

Vitamin E level in the liver of the toxin loaded group exceeded that of the control at each sampling time, and the difference was significant ($P<0.05$) on day 3. In the 10.000 g supernatant fraction of the liver homogenate the GSH content changed in the same way, the values in the control group it were always below those of the toxin loaded one. The difference has markedly decreased till day 14, thus it was significant only on day 3 ($P<0.01$) and 7 ($P<0.001$). The MDA content of the liver homogenate was outstandingly high on day 7 in both groups, otherwise notable decrease was found on day 14 in the toxin loaded group.

			Control group			Toxin loaded group		
			Day					
		0	3	7	14	3	7	14
Liver homogenate								
Vitamin C	mean	27,15	32,62	25,43 ^a	27,52	27,88	29,85 ^c	31,14
	SD	±3,490	±5,036	±2,725	±2,566	±4,205	±0,868	±7,941
Vitamin E	mean	4,63	4,78 ^a	2,60	3,31	7,67 ^b	3,06	5,17
	SD	±1,303	±1,813	±0,121	±0,972	±1,130	±1,123	±1,580
GSH	mean	1,72	1,78 ^a	2,08 ^a	3,84	4,48 ^d	4,41 ^d	4,07
	SD	±0,456	±0,620	±0,697	±0,091	±0,956	±0,551	±0,927
MDA	mean	1,42	1,40	2,26	1,96	1,89	2,66	1,83
	SD	±0,360	±0,392	±0,414	±0,360	±0,297	±0,889	±0,300
Blood plasma								
Vitamin C	mean	1,34	1,38	1,21	1,97	1,26	0,76	1,93
	SD	±0,663	±0,801	±0,910	±0,690	±1,030	±0,471	±1,520
Vitamin E	mean	3,49	6,83 ^a	5,81 ^a	4,81	5,06 ^b	3,78 ^b	4,44
	SD	±0,856	±1,091	±1,306	±1,201	±1,289	±1,252	±2,627
GSH	mean	5,35	5,46	5,18	5,28	6,29	6,35	6,35
	SD	±0,637	±0,237	±0,697	±0,483	±0,987	±0,910	±1,718
MDA	mean	0,55	0,53	0,52	0,57	0,54	0,54	0,63
	SD	±0,018	±0,075	±0,039	±0,094	±0,130	±0,057	±0,033

Table 1 Biochemical parameters of the liver homogenate and the blood plasma (significance levels are between groups on the same day: a-b: $P<0,05$; a-c: $P<0,01$; a-d: $P<0,001$)

		Liver homogenate	Blood plasma
Vitamin C	day 3	0,1448	0,8428
	day 7	0,0087	0,2374
	day 14	0,5727	0,9704
Vitamin E	day 3	0,0162	0,0472
	day 7	0,3852	0,0366
	day 14	0,0760	0,8041
GSH	day 3	0,0007	0,1048
	day 7	0,0004	0,0526
	day 14	0,6892	0,3955
MDA	day 3	0,2656	0,4679
	day 7	0,3928	0,5669
	day 14	0,7113	0,2376

Table 2 Parameters of the treated group (as compared to the control group) evaluated by ANOVA

In the control group the ascorbic acid content of the blood plasma at every sampling time was higher, than in the toxin loaded group; however the difference was not significant. The vitamin E content of the blood plasma, contrary to the liver, was higher than the values in the control group each time. The difference was significant ($P < 0.05$) between the groups on the 3rd and 7th day. Similarly to the liver homogenates, the GSH content of the blood plasma was always higher in the toxin loaded group; however the difference was not significant. Due to the low values measured in both groups, the findings did not reveal any significant difference in case of the MDA level of blood plasma; however, its level was much higher in the toxin loaded group. It might possibly be attributed to the moderately higher intensity of the lipid peroxidation processes.

Discussion

Although in the present study neither clinical signs of toxicity; nor significant increase in the MDA content was experienced, some parts of the antioxidant defence system measured in the blood plasma and liver homogenates confirm oxidative stress caused by the T-2 toxin. It is well known, that the feed containing high levels of trichothecene mycotoxins activates the lipid peroxidation processes in the case of high amount of T-2 toxin in the feed (Surai et al., 2002) and reduces the functioning of the antioxidant system (Rizzo et al., 1994). In the present study the mentioned effect could be determined only in the vitamin C and E level of the blood plasma, where the T-2 toxin load has reduced the levels of the two synergetic vitamins. It must be taken into consideration that the level of vitamin C, measured in birds, is not only affected by the nutritional level and its oxidation in the organism, but it is also influenced by the ascorbic acid synthesis of the organism. Accordingly, the activation of the ascorbic acid synthesis caused by the toxin load as environmental stressor could be a possible explanation of the change in the vitamin C content of the liver homogenate. This hypothesis is supported by the results of selenium toxicosis (Balogh et al., 2004). The insignificantly enhanced level of the MDA values in the blood plasma could presumably be due to the consequence of the increased lipid peroxidation, though such tendency was not experienced in the liver during the 14 days period of investigation. Examination of the longer feeding period is also needed to clarify the role of this factor.

We can conclude that the consumption of feeds containing relatively high levels of T-2 toxin over $\mu\text{mol/g}$ for two weeks does not induce significant differences in the vitamin C and GSH content of the blood plasma, but their amount is reduced in the liver homogenate. In the present study the amount of T-2 toxin used in the feed did not significantly affect the lipid peroxidation processes.

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