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EFFECTS OF HERBAL MIXTURE AND DON OR T-2 TOXIN EXPOSURE ON SOME GLUTATHIONE REDOX AND LIPID PEROXIDATION PARAMETER OF BLOOD AND LIVER IN BROILER CHICKENS

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Abstract

The purpose of present study was to investigate the short-term effect of DON and T-2 toxin exposure on some blood and liver lipid peroxide and glutathione redox parameters in broiler chickens. A total of 120 three-week old Cobb 540 broiler chickens were randomly assigned into five experimental groups of 24 chickens in each. The short-term trial lasted for 48 hours, after 12 hours of feed deprivation. The experimentally mycotoxin-contaminated diets contained (1 kg) 3.74 mg T-2 or 16.12 mg DON, respectively. Herbal mixture (Herbamix Basic Premix™, Herbamix Trade Ltd., Budapest) was added to the complete feed at the dose of 600 mg/kg. Six birds of each group were slaughtered at 12th, 24th, 36th and 48th hours of the experiment. Parameters of the lipid peroxidation (malondialdehyde) and the glutathione redox system (reduced glutathione content and glutathione peroxidase activity) were measured in blood plasma and liver homogenate. Malondialdehyde content did not change in blood plasma but it was significantly lower in liver homogenate in both mycotoxin loaded groups fed with herbal mixture supplemented feed at 24 hour as compared to the control. Reduced glutathione content did not change significantly in blood plasma, but in liver homogenate, at 24 hour sampling, T-2 toxin alone or in combination with herbal mixture showed significantly higher values as compared to the control. In conclusion, the investigated trichothecene mycotoxins at the dose applied, activated the glutathione redox system in liver of broiler chicken, while addition of herbal mixture has moderate effect against the mild oxidative stress as caused by DON or T-2 toxin.

Key words: *T-2 toxin, DON, lipid peroxidation, glutathione redox system, medicinal herb.*

INTRODUCTION

Moulds produce different mycotoxins that have importance in farm animal nutrition because of their widespread occurrence and diversity (Leeson et al. 1995). Among various trichothecene mycotoxins, those produced by *Fusarium* moulds, such as a 'type B' trichothecene deoxynivalenol (DON) is often found in feed ingredients even at high concentrations in different parts of the world under different environmental conditions (Jelinek et al. 1989).T-2 toxin, which is a 'type A' trichothecene is also important to the poultry industry because of their toxicity and co-occurrence in feeds (Devegowda and Murthy, 2005).

The maximum recommended concentration of T-2 toxin and its metabolite, the HT-2 toxin in feeds for broilers is 0.25 mg/kg complete feed (2013/165/EU) and in case of DON 5.0 mg/kg complete feed (2006/576/EC).

This relative high tolerance of poultry in case of DON is possibly due to the de-epoxidation in the gut before absorption (Awad et al., 2008).

Application of some herbal extracts of plant origin like turmeric (*Curcuma longa*), garlic (*Allium sativum*) and asafetida (*Ferula asafetida*) have shown to counteract mycotoxicosis in poultry through their antioxidant activity.

Several herbal products contain antioxidant substances capable of scavenging free radicals

and enhancing antioxidant enzymes (Nyandieka et al., 1990).
The purpose of present study was to investigate the short-term effect of T-2 or DON on lipid peroxidation processes and on the glutathione redox system in broiler chicken, in connection with the dietary addition of a medicinal herb mixture.

MATERIALS AND METHODS

A total of 120 three-week old Cobb 540 broiler chickens (body weight: 749.60±90.98 g) was randomly assigned into five experimental groups of 24 chickens in each. The short-term trial lasted for 48 hours, after 12 hours of feed deprivation. The basal diet was a commercial broiler feed (13.4 MJ/kg AME, 20% crude protein, 10% crude fat, 3.5% crude fibre, 35mg/kg vitamin E and 0.25mg/kg selenium). The nutrient content of the diet met the requirements for broiler chickens (Hungarian Feed Code, 2004). Measured mycotoxin concentrations of the commercial diet (1 kg) were: T-2: <0.10 mg; DON: 0.25 mg. The experimentally contaminated diets contained (1 kg) 3.74 mg T-2 and 1.26 mg HT-2 or 16.12 mg DON, respectively. Herbal mixture (Herbamix Basic Premix™, Herbamix Trade Ltd., Budapest) was added to the complete feed in powder form at the dose of 600 mg/kg. The main components of the applied herbal mixture are rosemary (*Rosmarinus officinalis*), oregano (*Origanum vulgare*) thyme oil (*Thymus vulgaris*) and Mary thistle (*Silybum marianum*).
DON was produced by *Fusarium graminearum* (NRRL 5883) and T-2 by *Fusarium sporotrichioides* (NRRL 3299) strains on corn substrate according to Fodor et al. (2006).DON content of feed was determined according to Pussemier et al. (2006), and T-2 and HT-2 concentration were measured based on the method of Trebstein et al. (2008) with HPLC after immunoaffinity cleanup.
Six birds were exterminated from each group at 12th, 24th, 36th and 48th hours of the experiment.
After cervical dislocation, blood samples were collected into EDTA-Na_2 containing tubes. The whole blood was separated by centrifugation (2,500×g, 20 min) and the blood plasma was stored at −70°C until analysis.
Post mortem liver samples were taken for biochemical analyses and stored at -70°C until analysis. Before the biochemical analysis liver homogenates were made with 9-fold cold (4°C) physiological saline (0.65% w/v NaCl).
Initial phase products of lipid peroxidation, conjugated dienes (CD) and trienes (CT) were measured by spectrometry at 232 nm (dienes) and 268 nm (trienes) according to AOAC (1984) in the liver. Determination of malondialdehde (MDA) concentration was carried out in the native liver homogenates, while the other parameters were determined in the 10,000×g supernatant fraction of the homogenate. MDA content of blood plasma was determined using the 2-thiobarbituric acid method according to Placer et al. (1966), in liver homogenates according to Botsoglou et al. (1994). The concentration of MDA was calculated using standard curves of increasing 1,1,3,3 tetraethoxypropane (Fluka, Buchs). Reduced glutathione (GSH) content of blood plasma, and a 10,000×g supernatant fraction of liver homogenates was measured as described by Sedlak and Lindsay (1968). Glutathione peroxidase (GPx) activity was determined according to Lawrence and Burk (1976). GSH content and GPx activity were expressed in protein content, which was determined in blood plasma by the biuret method (Weichselbaum, 1948) or with Folin-phenol reagent in a 10,000×g supernatant fraction of liver homogenates (Lowry et al., 1951).
Statistical analyses Statistical analysis of data (calculation of means and standard deviations, one-way analysis of variance with Tukey's post-hoc test) was performed with GraphPad Prism 5.04 for Windows (GraphPad Software, San Diego, CA, USA).

RESULTS AND DISCUSSIONS

There was no mortality during the trial, and no clinical signs of toxicity were observed. Calculated mycotoxin intake, which was calculated from feed intake and measured mycotoxin content of the particular complete feed, was almost the same between the groups fed with mycotoxin contaminated and mycotoxin contaminated and herbal mixture supplemented groups (Table 1).

Table 1. Calculated mycotoxin intake of broiler chickens

Group	Calculated mycotoxin intake (mg/bird)							
	0-12 h		12-24 h		24-36 h		36-48 h	
	DON	T-2	DON	T-2	DON	T-2	DON	T-2
Control	0.017		0.011		0.019		0.022	
T-2 toxin		0.245		0.174		0.179		0.390
DON	1.139		0.811		0.960		2.163	
Herbamix™	0.017		0.013		0.013		0.031	
T-2 toxin + Herbamix™		0.256		0.178		0.178		0.430
DON + Herbamix™	1.102		0.833		0.907		1.934	

Early markers of lipid peroxidation, the level of CD and CT did not change significantly as effect of T-2 toxin or DON, and it was not modified by the supplementation of herbal mixture in liver (data not shown).

End product of lipid peroxidation, the MDA concentration did not change in blood plasma (data not shown), but it was significantly lower in the liver homogenate in both mycotoxin loaded groups fed with herbal mixture supplemented feed at 24 hour sampling as compared to the control (Table 3).

GSH concentration did not change significantly in blood plasma (data not shown), but in liver homogenate showed significant differences at 24 hour sampling, when T-2 toxin alone ($p<0.05$) or in combination with herbal mixture resulted in higher values as compared to the control, while significantly ($p<0.05$) higher GSH concentration was found in case of DON only when it was combined with herbal mixture supplementation (Table 3).

GPx activity in blood plasma showed moderate changes. At 12th hour it was lower in DON and herbal mixture treated group as compared to T-2 toxin and herbal mixture group, and at 48th hour herbal mixture alone caused significantly lower enzyme activity in blood plasma than the control (Table 2).

In liver homogenate GPx activity changed significantly at 24 hour sampling, when higher values were found as effect of T-2 toxin, also in combination with herbal mixture, and in DON + herbal mixture group, as compared to control group and to the group fed with DON contaminated diet alone (Table 3).

Reduced glutathione (GSH) content in liver homogenate showed higher values when T-2 toxin contaminated feed was fed, alone or in combination with herbal mixture.

It means that the moderate oxidative stress in the liver as effect of T-2 toxin activates the glutathione synthesis, as part of the antioxidant response (Zimniak et al., 1997).

The results revealed that trichothecene mycotoxins, DON or T-2 toxin, have an effect on oxygen free radical formation, and it activates the glutathione redox system in liver of broiler chicken.

Table 2. Individual and combined effect of T-2 toxin, DON and herbal mixture on glutathione peroxidase activity in blood plasma (mean±SD; n=6)

Time	Control	T-2 toxin	DON	Herbamix	T-2 toxin + Herbamix	DON + Herbamix
GPx (U/g protein content)						
12th hour	10.0^{ab}± 1.42	9.88^{ab}± 0.74	8.16^{ab}± 1.94	8.45^{ab}± 1.13	10.27^{b}± 1.88	8.06^{a}± 1.85
24th hour	9.80± 0.83	11.45± 2.72	9.52± 2.94	11.92± 2.74	10.53± 0.55	10.82± 0.64
36th hour	7.41± 1.47	9.61± 2.60	6.96± 1.33	7.56± 2.06	8.53± 1.60	8.39± 1.83
48th hour	11.00^{b}± 2.86	10.37^{ab}± 2.81	8.35^{ab}± 1.64	7.88^{a}± 0.98	8.38^{ab}± 0.56	8.30^{ab}± 1.40

^{a,b} Means designated with different letters within the same rows mean significant difference ($p<0.05$)

Table 3. Individual and combined effect of T-2 toxin, DON and herbal mixture on lipid peroxidation and glutathione redox system of liver homogenates (mean±SD; n=6)

	Control	T-2 toxin	DON	Herbamix™	T-2 toxin + Herbamix™	DON + Herbamix™
	MDA (µmol/)					
12th hour	10.99± 2.15	10.12± 3.02	9.36± 0.54	9.74± 2.10	9.15± 2.40	9.88± 4.47
24th hour	17.95^{b}± 3.69	12.62^{ab}± 1.88	15.00^{ab}± 4.94	14.42^{ab}± 3.51	11.99^{a}± 2.60	11.72^{a}± 1.61
36th hour	10.05± 1.37	11.37± 2.31	10.04± 2.12	10.15± 2.33	10.69± 0.90	9.16± 2.49
48th hour	12.57± 3.47	15.51± 2.68	12.03± 3.24	11.81± 3.64	12.46± 2.31	14.54± 6.23
	GSH (µmol/g protein content)					
12th hour	3.03± 0.75	4.03± 1.12	3.55± 0.89	3.15± 0.27	3.22± 0.51	3.19± 0.77
24th hour	2.84^{a}± 1.07	4.56^{bc}± 0.74	3.00^{ab}± 1.17	3.55^{abc}± 0.70	4.17^{abc} ± 0.86	4.51^{b}± 0.50
36th hour	3.46± 0.83	3.60± 0.58	3.08± 0.60	2.93± 0.39	3.42± 0.32	3.19± 0.48
48th hour	2.62± 0.40	2.92± 0.51	3.15± 0.58	3.03± 0.66	2.82± 0.30	2.74± 0.29
	GPx (U/g protein content)					
12th hour	3.01± 0.66	3.99± 0.91	3.48± 0.68	3.14± 0.21	3.21± 0.53	3.07± 1.35
24th hour	3.10^{a}± 1.24	4.78^{b}± 0.67	2.89^{a}± 0.96	3.51^{ab}± 0.94	4.69^{b}± 0.74	4.70^{b}± 0.44
36th hour	3.23± 0.88	3.44± 0.61	3.16± 0.73	3.06± 0.63	3.40± 0.44	3.23± 0.62
48th hour	1.99± 0.37	2.54± 0.48	2.84± 0.49	2.64± 0.58	2.72± 0.61	2.31± 0.37

^{a,b} Means designated with different letters within the same rows mean significant difference ($p<0.05$)

Addition of herbal mixture has moderate effect against the mild oxidative stress as caused by DON or T-2 toxin at the dose applied. No clinical signs of toxicity and mortality was observed at the dose level applied, which supported by the relatively high tolerance of broiler chicken to DON (Dänicke et al., 2001) or T-2 toxin (Eriksen and Pettersson, 2004). The results revealed that addition of herbal mixture did not modify the TBARS values, probably because of the lack of marked oxidative stress in liver as effect of the mycotoxin doses used in this trial. Glutathione peroxidase activity in liver homogenate changed significantly at 24 hour sampling, when higher values were found as effect of T-2 toxin, also in combination with herbal mixture and DON in combination with herbal mixture, but also when DON was used alone. In liver homogenate significant changes were found at 48 hour sampling, where T-2 toxin load resulted higher activity when it was used together with herbal mixture, as compared to herbal mixture supplemented group. T-2 toxin induced glutathione peroxidase activity, without additional effect of herbal mixture. Lack of effect of DON probably explained the high tolerance of broiler chicken which is probably caused by the higher rate of metabolism in the liver (Awad et al., 2014). It means that both trichothecene mycotoxins activate the enzymatic antioxidant defence, in this case glutathione peroxidase activity, but herbal mixture has no additional effect, and when it used alone did not has effect. the results revealed that trichothecene mycotoxins, DON or T-2 toxin, have effect on oxygen free radical formation, and it is activate the glutathione redox system in liver of broiler chicken. Addition of herbal mixture has moderate effect against the mild oxidative stress as caused by DON or T-2 toxin at the dose applied, in antioxidant but also in xenobiotic transformation.

CONCLUSIONS

The results of this study showed that the applied trichothecene mycotoxins, DON or T-2 toxin, activated the glutathione redox system in liver of broiler chicken, while addition of herbal mixture had moderate effect against the mild oxidative stress as caused by DON or T-2 toxin at the dose applied. In conclusion the results revealed that trichothecene mycotoxins, DON or T-2 toxin, have effect on oxygen free radical formation, and it is activate the glutathione redox system in liver of broiler chicken. Addition of herbal mixture has moderate effect against the mild oxidative stress as caused by DON or T-2 toxin at the dose applied, which would important not only in antioxidant but also in xenobiotic transformation.

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