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Short Term Effects of Herbal Mixture and T-2 Toxin Exposure on Some Glutathione Redox and Lipid Peroxidation Parameters of Blood in Broiler Chickens

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Abstract

Short-term effects of T-2 toxin (1.13 mg/kg feed) was investigated in broiler chicken, in connection with dietary addition of a medicinal herbal mixture. Three-week old Cobb 540 broiler chickens (n=120) were randomly assigned into five experimental groups. In three experimental groups Herbamix® Basic Premix were used (300, 600 and 1500 mg/kg) as two weeks long pre-treatment. Samplings were done at every twelve hour. Parameters of lipid peroxidation and glutathione redox system were measured in blood plasma and red blood cell haemolysates. T-2 toxin treatment increased the GSH concentration and GPx activity of plasma at 12h and 24h, while most marked elevations were found in T-2 toxin+medium Herbamix dose group. The results revealed that T-2 toxin had pro-oxidant effect, and consequently activated the glutathione redox system of broiler chickens. The applied herbal mixture had only moderate effect against the mild oxidative stress caused by T-2 toxin at the dose applied.

Keywords: T-2 toxin, lipid peroxidation, glutathione redox system, medicinal herb.

1. Introduction

T-2 toxin is the most toxic trichothecene mycotoxin produced by *Fusarium* moulds in temperate climate. It belongs to the 'type A' trichothecenes, and has a risk to the poultry industry because of its toxicity and occurrence in feedstuffs [4].

Fusarium moulds may produce a number of mycotoxins with different chemical structures, depending on temperature and humidity in the environment [22].The presence and quantity of substrates in the grains (e.g. starch), and the presence of oxygen are needed for the growth of the mould T-2 toxin has an 12,13-epoxy group in its chemical structure, which is a reactive compound and might be responsible for its pro-oxidant effects. Poultry species are sensitive for this mycotoxin, thus T-2 toxin contamination of the feed above 1mg/kg can result in economic loss, because of lower meat producion, related to reduced feed intake and inhibited protein synthesis (Schuhmacher-15].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). Most of the previous studies also revealed that T-2 toxin has marked effect on the antioxidant status of animals [1,17] because of its pro-oxidant properties [14]. However, in other studies neither the rate of lipid peroxidation processes nor the antioxidant parameters showed significant alterations even at high T-2 doses [8,13]. Oxygen free radical formation and consequently lipid peroxidation increased in farm animals because of long-term exposure of trichothecenes in context of biochemical changes in cells, which affect the amount and/or activity of the biological antioxidant system, as well [10,17].Several herbal products contain antioxidant substances capable of scavenging free radicals and enhancing antioxidant enzymes. 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

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capable of scavenging free radicals and enhancing antioxidant enzymes [11].The purpose of present study was to investigate the short-term effect of T-2 toxin on lipid peroxidation processes and on the glutathione redox system in broiler chicken, in connection with the dietary addition of a medicinal herb mixture.

2. 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.In three experimental groups Herbamix® Basic Premix were used in three different concentrations (1 kg feed) 300mg, 600 mg and 1500mg as two weeks long pre-treatment, to evaluate its effect against short-term T-2 toxin exposure. The main components of the applied herbal mixture are rosemary (*Rosmarinus officinalis*), oregano (*Origanum vulgare*), thyme oil (*Thymus vulgaris*) and Mary thistle (*Silybum marianum*).T-2 toxin was produced by *Fusarium sporotrichioides* (NRRL 3299) strains on corn substrate according to Fodor [7].T-2 toxin and HT-2 toxin concentration were measured based on the method of Trebstein [18].with HPLC after immunoaffinity cleanup. The experimentally mycotoxin-contaminated diets (1kg) contained: 1.13 mg T-2 toxin and <0,1 mg HT-2 toxin.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). The short-term trial lasted for 48 hours, after 12 hours of feed deprivation. Six birds of each group were slaughtered at 12th, 24th, 36th and 48th hours of the experiment. After cervical dislocation, blood samples were collected into EDTA-Na2 containing tubes. The whole blood was separated by centrifugation (2,500×g, 20 min) and blood plasma was collected. Red blood cell haemolysates (RBC) were made using 9-fold distilled water. The samples were stored at -70^0C until analysis. From the samples collected parameters of lipid peroxidation processes (malondialdehyde, MDA) and glutathione redox system (reduced glutathione /GSH/ concentration and glutathione-peroxidase /GPx/ activity) were measured.MDA content was determined using the 2-thiobarbituric acid method according to Placer [12].The concentration of MDA was calculated using standard curves of increasing 1,1,3,3 tetraethoxypropane (Fluka, Buchs). Reduced glutathione (GSH) content was measured as described by Sedlak and Lindsay [16].Glutathione peroxidase (GPx) activity was determined according to Lawrence and Burk [9].GSH content and GPx activity were expressed in protein content, which was determined by the biuret method [21].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).

3. Results and discussions

In the T-2 toxin treated group MDA content in RBC increased ($p<0.05$) at 12h as compared to control (Table 2), while Herbamix treatment had beneficial effect resulting lower MDA concentration at 36h in both plasma and RBC as compared to T-2 toxin alone (Table 1 and 2). As effect of T-2 toxin treatment the GSH concentration of plasma increased moderately at 12h and 24h as compared to control (Table 1), while significant increase was measured in plasma and also in RBC haemolysate at 24h in T-2 toxin+highest Herbamix dose group (Table 1 and 2). GPx activity of plasma was also increased ($p<0.05$) by the T-2 toxin treatment at 12h (Table 1), while most marked ($p<0.05$) elevations were found in T-2 toxin+medium Herbamix dose group in plasma at 12h and 36h (Table 1), and in RBC haemolysate at 24h (Table 2).

In the present experiment the contamination level of T-2 toxin in feed was 2.25-fold than tolerable level for broilers as proposed by Eriksen and Pettersson [5].and 4.5-fold as the proposed maximum level of the European Commission [6].Although in a long-term experiment with broiler chickens [20] investigated pathophysiological changes associated with the consumption of mycotoxin contaminated (2.35 mg T-2 toxin /kg feed) diet, T-2 toxin did not enhance the lipid peroxidation processes but had effect on the glutathione redox system in blood plasma. Using lower mycotoxin contaminated diet (1.04 mg T-2 toxin /kg feed and 0.49 mg HT-2 toxin /kg feed) Weber [20] did not find alterations in MDA concentration, GSH concentration and GPx activity of blood plasma during the starter phase.

Table 1. Individual and combined effect of T-2 toxin and different doses of medicinal herb mixture (Herbamix™) on some parameters of glutathione redox system and lipid peroxidation processes of blood plasma (mean±SD; n=6).

	CONTROL	T-2 TOXIN	H1+ T-2 TOXIN	H2+ T-2 TOXIN	H5+ T-2 TOXIN
MALONDIALDEHYDE (MDA) (MMOL/L)					
12TH HOUR	4,22±	3,72±	4,43±	3,92±	**4,35±**
	0,68	0,41	0,77	1,10	**0,46**
24TH HOUR	2,99a±	3,26ab±	4,90b±	4,20ab±	**3,84AB±**
	1,48	1,09	0,60	0,93	**0,98**
36TH HOUR	4,75ab±	4,92b±	3,47ab±	4,05ab±	**2,44A±**
	2,32	1,36	1,33	1,10	**0,62**
48TH HOUR	3,43a±	3,08a±	4,32ab±	3,80a±	**6,09B±**
	1,03	1,56	0,46	0,86	**1,29**
REDUCED GLUTATHIONE (GSH) (MMOL/G PROTEIN CONTENT)					
12TH HOUR	10,28ab±	11,86b±	9,64ab±	10,79ab±	**8,95A±**
	0,81	2,19	1,29	1,85	**0,69**
24TH HOUR	7,95a±	9,27ab±	8,29ab±	10,18ab±	**10,41B±**
	0,99	2,00	1,40	1,35	**1,69**
36TH HOUR	10,21ab±	9,07ab±	9,22ab±	11,16b±	**7,90A±**
	0,79	1,60	1,80	1,37	**1,17**
48TH HOUR	6,91±	5,69±	5,98±	5,42±	**5,87±**
	0,56	1,17	1,06	0,41	**1,33**
GLUTATHIONE PEROXIDASE (GPX) (U/G PROTEIN)					
12TH HOUR	5,16a±	8,50b±	6,51a±	9,29bc±	**6,88AB±**
	0,37	1,47	1,24	1,32	**0,88**
24TH HOUR	8,24b±	8,07b±	6,02a±	6,88ab±	**7,78AB±**
	0,66	1,25	1,11	1,58	**1,32**
36TH HOUR	7,71a±	8,70ab±	8,00a±	10,90b±	**9,15AB±**
	1,99	0,82	1,73	1,12	**1,99**
48TH HOUR	**7,10±**	**6,26±**	**5,76±**	**6,51±**	**6,25±**
	1,15	**1,36**	**0,96**	**0,64**	**1,23**

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

In another long-term trial Balogh [2] also did not find significant alterations in lipid peroxidation processes and in the glutathione redox system after feeding broiler chickens with experimentally contaminated (1.5 mg T-2 toxin /kg feed) diet for 28 days. Rezar [13].in a 18 days long experiment done with 3 weeks old broilers did not find significant alterations in blood plasma MDA concentrations and GPx activity of erythrocytes in case of the applied doses (0.5, 1.5, 4.5 and 13.5 mg/kg feed) of T-2 toxin.In a short-term experiment done by Bócsai [3]. because of feeding almost 3-times higher T-2 toxin contaminated diet (3.09 mg T-2 toxin/kg) than in our study.

4. Conclusions

The glutathione redox system activated shortly after starting the mycotoxin exposure, which is supported by the significantly higher concentration of reduced glutathione and glutathione peroxidase activity in blood plasma at 24h and 48h. Our results revealed that the applied trichothecene mycotoxin, T-2 toxin, had effect on oxygen free radical formation, and consequently activated the glutathione redox system redox system of broiler chickens, namely synthesis of reduced glutathione and glutathione peroxidase. Addition of herbal mixture had only moderate effect against the mild oxidative stress caused by T-2 toxin at the dose applied.

Table 2. Individual and combined effect of T-2 toxin and different doses of medicinal herb mixture (Herbamix™) on some parameters of glutathione redox system and lipid peroxidation processes of red blood cell hemolysate (mean±SD; n=6)

	CONTROL	T-2 TOXIN	H1+ T-2 TOXIN	H2+ T-2 TOXIN	H5+ T-2 TOXIN
MALONDIALDEHYDE (MDA) (MMOL/L)					
12TH HOUR	6,46a± 1,30	8,67b± 1,10	7,91ab± 0,61	8,37b± 0,95	**7,44AB± 0,48**
24TH HOUR	9,25± 0,67	7,82± 0,71	8,71± 1,02	8,65± 0,53	**7,83± 2,46**
36TH HOUR	7,89ab± 0,68	8,44b± 1,50	6,46a± 1,37	7,03ab± 1,36	**6,27A± 0,71**
48 HOUR	11,93± 1,23	10,55± 2,59	10,14± 2,12	10,82± 2,96	**8,57± 1,93**
REDUCED GLUTATHIONE (GSH) (MMOL/G PROTEIN CONTENT)					
12TH HOUR	13,49± 2,60	12,10± 1,12	13,61± 2,79	12,89± 2,56	**12,26± 1,26**
24TH HOUR	11,84a± 1,60	11,70a± 3,29	11,94a± 1,72	15,35ab± 1,96	**17,68B± 2,09**
36TH HOUR	15,23± 3,14	12,66± 3,98	13,33± 2,98	12,87± 1,55	**13,04± 2,41**
48 HOUR	10,64± 3,01	9,86± 2,69	9,61± 2,59	11,00± 4,01	**8,62± 3,09**
GLUTATHIONE PEROXIDASE (GPX) (U/G PROTEIN)					
12TH HOUR	6,95± 1,08	5,79± 0,49	6,59± 1,43	5,88± 1,33	**5,70± 0,52**
24TH HOUR	5,30a± 0,94	5,42a± 1,34	5,63ab± 0,99	7,46bc± 1,12	**8,29C± 1,27**
36TH HOUR	6,85± 1,15	6,67± 0,90	7,03± 1,77	5,34± 1,59	**6,45± 1,28**
48 HOUR	**5,72± 0,75**	**5,41± 1,00**	**5,68± 1,06**	**5,48± 0,82**	**5,89± 1,19**

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

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