

Effect of T-2 and HT-2 Toxin during the Growing Period on Body Weight, Lipid Peroxide and Glutathione Redox Status of Broiler Chickens

Mária Weber¹, Krisztián Balogh^{2,3}, Judit Fodor³, Márta Erdélyi², Zsolt Ancsin², Miklós Mézes²

¹ Department of Pig and Small Animal Breeding, Institute of Animal Husbandry, ² Department of Nutrition, Szent István University, Gödöllő, Hungary

³ Research Group of Animal Breeding and Hygiene, Faculty of Animal Science, University of Kaposvár, Hungary

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Abstract

The effect of T-2 and HT-2 toxin using different doses in the starter (0-21 days: 1.04 mg T-2 toxin and 0.49 mg HT-2 toxin·kg⁻¹ feed), and finisher diets (22-39 days: 0.12 mg T-2 toxin and 0.02 mg HT-2 toxin·kg⁻¹ feed) was investigated in broiler chickens. Birds were divided into two groups fed with control and T-2 and HT-2 toxin contaminated diets. Pathological signs of toxicity were investigated on days 21 and 39 of the trial, individual liveweight was measured weekly. Five birds from each group were sacrificed on the 21st and 39th days of treatment, when blood plasma, red blood cell, liver and kidney samples were taken, in which malondialdehyde and reduced glutathione concentration and glutathione-peroxidase activity were determined.

Pathological signs (lesions in the oral cavity and on the tongue, inflammation in the small intestine) were found in the group fed T-2 and HT-2 toxin contaminated feed on day 21 compared to control. Body weight was significantly lower as a result of feeding T-2 and HT-2 toxin contaminated diet. However, the contamination did not cause a significant increase of malondialdehyde content in the analysed tissues. Reduced glutathione content was significantly lower in the liver homogenate on day 39 than that of the control. Glutathione peroxidase activity also did not differ significantly in blood plasma, red blood cell haemolysates and kidney homogenates, while it was significantly higher in the liver homogenates of the mycotoxin-challenged birds.

In conclusion, it can be stated that T-2 and HT-2 toxin exposure has long-term effects in broiler chickens.

T-2 toxin, HT-2 toxin, malondialdehyde, glutathione, glutathione peroxidase, chicken

Fusarium moulds are widespread in the temperate climate, producing trichothecene mycotoxins, e.g. T-2 toxin, HT-2 toxin, T-2 triol and T-2 tetraol (Scott 1990). Due to their occurrence and diverse effects, in particular impairment of the immune response (Weber et al. 2008), these mycotoxins have great importance in animal nutrition. The trichothecene mycotoxins, such as T-2 toxin and its metabolites compromise the growth rate and development of poultry (Leeson et al. 1995), and affect the antioxidant status (Atroshi et al. 2002; Rizzo et al. 1994; Weber et al. 2007), primarily due to their pro-oxidant effect (Mézes et al. 1998; Surai 2002). Among the Fusarium mycotoxins, the T-2 toxin and its natural metabolites are excreted relatively quickly (within about 72 h) from the organism after ingestion. Therefore, the rate of their accumulation in the different tissues is low (e.g. 0.2% in muscles and 0.4% in the liver). Thus the main detrimental effects of the T-2 toxin and its metabolites are not due to their accumulation, but rather the result of other, sometimes long-term effects caused immediately after absorption and metabolism of toxins in different tissues (WHO/FAO 2000).

The objective of the present study was to evaluate the long-term effect of feeding T-2 toxin contaminated diet on the growth, rate of lipid peroxidation, and also on the glutathione redox status of broiler chickens during the growing period.

Address for correspondence:

Miklós Mézes
Department of Nutrition, Szent István University
H-2103 Gödöllő, Péter Károly u. 1., Hungary

Phone: +36 28 410 735
Fax: +36 28 410 804
Email: Mezes.Miklos@mkk.szie.hu
http://www.vfu.cz/acta-vet/actavet.htm

Materials and Methods

A total of 40 day-old Hubbard broiler cockerels were studied for 39 days. The birds were divided into two groups fed with control ('C' n = 20) and T-2 toxin contaminated ('T-2' n = 20) diets. The nutrient content of the diets met the requirements for broiler chickens (Table 1) according to the Hungarian standards (Hungarian Feed Code 2004a). The nutrient content of the diets was determined according to the Hungarian National Standard methods (Hungarian Feed Code 2004b). Complete feed in mash form was fed in the first phase (1-21 days) with < 0.01 mg·kg⁻¹ T-2 toxin in the control, while the experimentally contaminated diets contained 1.04 mg·kg⁻¹ T-2 toxin and 0.49 mg·kg⁻¹ HT-2 toxin, respectively. The same control complete feed with < 0.01 mg·kg⁻¹ T-2 toxin was fed in the second phase (22-39 days) with the same nutrient content as in the first phase, while the experimentally contaminated diets were loaded with 0.12 mg·kg⁻¹ T-2 toxin and 0.02 mg·kg⁻¹ HT-2 toxin, respectively.

Table 1. Nutrient and mycotoxin content of the diets

Nutrient	Starter control	Finisher control	Starter 'T-2'	Finisher 'T-2'
Dry matter g·kg ⁻¹	898.80	895.30	897.90	896.10
Crude protein g·kg ⁻¹ D.M.	196.37	187.25	193.45	187.40
Crude fat g·kg ⁻¹ D.M.	30.15	31.30	30.29	31.25
Crude fibre g·kg ⁻¹ D.M.	23.59	24.10	25.39	24.40
Crude ash g·kg ⁻¹ D.M.	62.97	63.15	63.70	63.40
Nitrogen free extract g·kg ⁻¹ D.M.	686.92	694.20	687.16	693.55
T-2 toxin mg·kg ⁻¹ D.M.	< 0.01	< 0.01	1.04	0.12
HT-2 toxin mg·kg ⁻¹ D.M.	< 0.01	< 0.01	0.49	0.02

D.M. = dry matter

However, there is no official regulation in the European Union for the maximum allowed level of T-2 toxin in the feeds, only a guideline for the limit value of 0.5 mg T-2 toxin·kg⁻¹ is proposed (Eriksen and Pettersson 2004). T-2 toxin contamination in the first phase of the present experiment was much higher than the aforementioned maximum guideline limit value. The partially purified toxin preparation was dissolved in acetone and then sprayed onto the complete feed (100 ml/50 kg of feed). T-2 toxin was produced experimentally on maize by *Fusarium sporotrichoides* strain NRRL 3229 (Agricultural Research Service Culture Collection, National Centre for Agricultural Utilization Research, Peoria, IL) according to the method of Fodor et al. (2006). Extraction and purification of the toxin were carried out according to Burmeister (1971). The T-2 and HT-2 toxin concentrations in the feed were measured using the HPLC technique (Central Veterinary Institute, Budapest) according to the relevant European Union directive (EU 2005). The amounts of related trichothecene metabolites, T-2 triol and T-2 tetraol were under the detection limit.

Body weight was measured on the 7th, 14th, 21st, 35th and 39th days of the trial. Five animals from each group were sacrificed on 21st and 39th days, necropsy was carried out, and blood and tissue (liver and kidney) samples were collected. Blood samples were stored at cooled place (+ 4 °C) until processing, then the plasma was separated from the blood cells by centrifugation. Red blood cells were lysed with deionized water (ratio 1 : 9) and by freezing and thawing. Liver and kidney samples were homogenized in nine-fold volume of 0.65% (w/v) sodium-chloride. The samples were stored at -20 °C until analyses.

For determination of malondialdehyde (MDA) concentration in the blood samples, the method of Placer et al. (1966) and for liver and kidney samples the method of Mihara et al. (1980) were used. The glutathione peroxidase (GSHPx) activity was measured by the method of Lawrence and Burk (1976). Protein content of the blood plasma and the red blood cell haemolysate was determined by the biuret method (Weichselbaum 1948). Total protein concentrations in the 10,000 g supernatant fraction of liver and kidney homogenates were measured according to the method of Lowry et al. (1951). The GSH concentration was analysed using the method of Sedlak and Lindsay (1968).

Statistical evaluation of the results was carried out by paired Student's *t*-test after calculating the means and standard deviations (S.D.) with Statistica™ 4.0 (Statsoft Inc. 1993) software. The experiment was approved by the Animal Experimental Committee of the Faculty of Agricultural and Environmental Sciences of the Szent István University (2/2005).

Results

Pathological signs

The effects of T-2 and HT-2 toxin were found at necropsy on day 21 and also on day 39 but with different extents and rates of occurrence in the group fed mycotoxin contaminated feed. The pathological signs appeared as lesions in the oral cavity and on the tongue, also

Table 2. Clinical signs of intoxication after long term feeding of T-2 and HT-2 toxin contaminated feed

Group	Lesions in oral cavity and on tongue		Inflammation in the intestine	
	Day 39	Day 21	Day 39	Day 21
Control	0/10	0/10	0/10	0/10
T-2+HT-2 toxin treated	6/10	3/10	4/10	1/10

haemorrhage and inflammatory symptoms especially in the proximal part of the small intestine were found (Table 2).

Body weight

Body weight of the birds was significantly lower after feeding T-2 and HT-2 toxin

contaminated feed at the end of the starter (high toxin concentration) period on day 21 (Table 3). During the second phase of the trial with a lower dose of mycotoxin contamination, compensatory growth occurred in the toxin-fed group.

Table 3. Body weight of broiler chickens after long term feeding of T-2 and HT-2 toxin-contaminated feed (mean \pm SD)

Age days	Body weight (g)	
	Control	T-2+HT-2 toxin
0	41.6 \pm 1.0	41.4 \pm 2.7
7	125.0 \pm 19.5	121.1 \pm 13.0
14	319.8 \pm 48.2	280.7 \pm 34.5
21	665.2 ^a \pm 103.9	574.1 ^b \pm 80.1
28	1028.3 \pm 200.7	936.7 \pm 199.5
35	1508.8 \pm 292.4	1387.5 \pm 278.2
39	1795.4 \pm 341.3	1630.4 \pm 310.6

^{a,b} Different superscripts in the same row mean significant difference ($P < 0.05$)

Table 4. Malondialdehyde content in blood plasma, red blood cell haemolysate, liver and kidney homogenates after long term feeding of T-2 + HT-2 toxin-contaminated feed (mean \pm SD)

Sample	Age days	Control	T-2 + HT-2 toxin treated
Blood plasma ($\mu\text{mol}\cdot\text{l}^{-1}$)	21	6.3 \pm 2.88	2.62 \pm 0.34
	39	7.13 \pm 1.94	6.90 \pm 1.65
RBC haemolysate ($\mu\text{mol}\cdot\text{l}^{-1}$)	21	7.92 \pm 0.78 ^a	6.44 \pm 0.59 ^b
	39	8.52 \pm 2.80	7.74 \pm 3.08
Liver homogenate ($\mu\text{mol}\cdot\text{g}^{-1}$ wet weight)	21	3.46 \pm 0.85	3.02 \pm 0.55
	39	5.26 \pm 1.80	5.02 \pm 1.15
Kidney homogenate ($\mu\text{mol}\cdot\text{g}^{-1}$ wet weight)	21	5.87 \pm 0.74	7.27 \pm 2.19
	39	6.85 \pm 1.24 ^a	4.14 \pm 1.22 ^b

^{a,b} Different superscripts in the same row mean significant difference ($P < 0.05$)

Malondialdehyde content

Lipid peroxidation was followed by measuring the concentration of the malondialdehyde, a meta-stable end-product of peroxidation of non-saturated fatty acids. There were no significant changes in the malondialdehyde content of blood plasma and liver homogenate following the feeding of T-2 and HT-2 toxin-contaminated diet. However, significantly lower malondialdehyde content was found in the red blood cell haemolysates on day 21 and in kidney homogenates on day 39 of the birds from toxin group compared to control (Table 4).

Reduced glutathione content

Neither high nor low toxin contamination of the diet caused significant changes in the reduced glutathione content of blood plasma, red blood cell haemolysate and kidney homogenate. However, significantly lower content of reduced glutathione was found in the 10,000 g supernatant fraction of liver homogenate on day 39 of the birds from toxin group compared to control (Table 5).

Glutathione peroxidase activity

Glutathione peroxidase activity did not differ significantly in the blood plasma, red blood cell haemolysate and 10,000 g supernatant fraction of kidney homogenates as an effect of feeding T-2 and HT-2 toxin-contaminated diet (Table 6). On the contrary, the enzyme activity was significantly higher in the 10,000 g supernatant fraction of liver homogenates of the birds from the toxin group at both times of samplings, namely on days 21 and 39, compared to control (Table 6).

Table 5. Reduced glutathione content in blood plasma, red blood cell haemolysate, and 10,000 g supernatant fraction of liver and kidney homogenates after long term feeding of T-2 + HT-2 toxin-contaminated feed (mean \pm SD)

Sample	Age days	Control	T-2 + HT-2 toxin treated
Blood plasma ($\mu\text{mol}\cdot\text{l}^{-1}$)	21	6.29 \pm 1.28	5.18 \pm 2.07
	39	4.80 \pm 0.69	5.25 \pm 0.83
RBC haemolysate ($\mu\text{mol}\cdot\text{l}^{-1}$)	21	9.36 \pm 4.18	13.59 \pm 2.91
	39	10.33 \pm 1.82	10.73 \pm 2.43
Liver homogenate ($\mu\text{mol}\cdot\text{g}^{-1}$ protein)	21	2.56 \pm 0.60	3.00 \pm 0.66
	39	1.82 \pm 2.59 ^a	0.61 \pm 0.77 ^b
Kidney homogenate ($\mu\text{mol}\cdot\text{g}^{-1}$ protein)	21	1.08 \pm 0.26	1.43 \pm 0.26
	39	0.96 \pm 0.46	1.57 \pm 0.07

^{a,b} Different superscripts in the same row mean significant difference ($P < 0.05$)

Table 6. Glutathione peroxidase activity in blood plasma, red blood cell haemolysate, and 10,000 g supernatant fraction of liver and kidney homogenates after long-term feeding of T-2 + HT-2 toxin-contaminated feed (mean \pm SD)

Sample	Age days	Glutathione peroxidase activity	
		Control	T-2 + HT-2 toxin
Blood plasma ($\mu\text{kat}\cdot\text{l}^{-1}$)	21	8.67 \pm 1.51	7.31 \pm 2.72
	39	6.99 \pm 1.64	7.59 \pm 0.96
RBC haemolysates ($\mu\text{kat}\cdot\text{l}^{-1}$)	21	4.84 \pm 1.62	6.50 \pm 2.16
	39	5.51 \pm 1.26	5.45 \pm 1.20
Liver homogenate (nkat·g ⁻¹ 10 000 g supernatant protein)	21	47.26 \pm 8.68 ^a	58.28 \pm 5.85 ^b
	39	31.90 \pm 6.51 ^a	41.58 \pm 10.52 ^b
Kidney homogenate (nkat·g ⁻¹ 10 000 g supernatant protein)	21	19.21 \pm 8.35	25.22 \pm 7.68
	39d	24.88 \pm 8.02	27.05 \pm 5.01

^{a,b} Different superscripts in the same row mean significant difference ($P < 0.05$)

Changes of biochemical markers of lipid peroxidation and glutathione redox system are in line with the findings of Leal et al. (1999) and Weber et al. (2006). Significant difference was found at the end of the high dose T-2 and HT-2 toxin-challenge on day 21 but also at the end of the trial when the mycotoxin contamination of the diet was much lower. The higher glutathione peroxidase activity in the liver homogenate at both samplings suggests that T-2 and HT-2 toxin load, even at low level, activates the antioxidant defence system in a tissue-specific manner (Brigelius-Flohé 1999). Activation of glutathione peroxidase might be the result of post-translational activation of the enzyme due to the well known inhibitory effect of T-2 toxin on protein synthesis (Ueno et al. 1973).

These findings support our hypothesis that the effects of a high level T-2 and HT-2 toxin-contaminated feed in broiler chickens persist for a longer period of time even when it is followed by a lower challenge.

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Discussion

The clinical signs of T-2 and HT-2 toxicity were the same as previously described (Joffe et al. 1971; Gentry 1982). Severity of these alterations decreased but did not completely cease during the second phase of the trial when the mycotoxin content of the diet was much lower. It means that a high dose of T-2 and HT-2 toxin-load has a long-term effect even if followed by a much lower level of contamination. The same effect was found for live weight, which was significantly lower in the T-2 group at the end of the starter period with a high level toxin contamination (1.04 mg·kg⁻¹ T-2 toxin and 0.49 mg·kg⁻¹ HT-2 toxin), but the difference between the two groups was negligible at the end of the trial. That was caused partly by the lower dose of mycotoxin contamination (0.12 mg·kg⁻¹ T-2 toxin and 0.02 mg·kg⁻¹ HT-2 toxin) during the second phase of the trial and also the well known compensatory growth ability of poultry species.

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