

## EFFECT OF T-2 TOXIN ON FEED INTAKE AND DIGESTION OF RABBITS

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### Introduction

The trichotecen structured mycotoxins are produced by some *Fusarium* and approximately 5 other fungi genera. From the point of view of the animal husbandry, among the identified more than 40 mycotoxins, it is the T-2 toxin, the diacetoxyscirpenol (DAS) and the vomitoxin (deoxynivalenol, DOS) that are the most important. The farm animals get these toxins mostly by the ingestion of contaminated corn or wheat. Owing to certain milling procedures, the concentration of toxin in meals and brans can be elevated (Cheeke and Shull, 1985).

Data and observations concerning the effect of trichotecens upon the rabbit's organism, are limited. Gentry and Cooper (1982) described the decrease in hematocrit, white blood cell count and serum alkaline phosphatase activity, after a single intravenous administration of 0.5 mg T-2 toxin per kg body weight. The plasma clotting time was prolonged. The perorally applied 2.0 mg/kg T-2 produced oral lesions, diarrhea and anorexia but the hematological and biochemical parameters did not change significantly. One of the eight rabbits, injected intravenously and one of the five animal, given the toxin by intubation, were found dead 24 and 36 hours after the treatment.

Hanika (1984) observed diarrhea and nephrosis as signs of the citrinin intake of the rabbits. Khera et al. (1986) examined the effect of vomitoxin on the reproduction of the female rabbit. The consumption of feed, containing 0.024 % vomitoxin, caused 100 % of embryonic mortality. In our experiment we wanted to supply data about the effect of moderate quantities of T-2 toxin on rabbit's voluntary feed intake and nutrients' digestibility.

### Material and Method

On the basis of previous experiences (Glávits et al., 1988) two subtoxic experimental feeds were used: 12.5 and 25 mg T-2 toxin per kg (in the following: "12.5 ppm T-2" and "25 ppm T-2" mark the feed and the group of rabbit in question). The concentration were set up so that, in case of the entire consumption of the daily ration (110 gram per animal), the toxin intake does not reach the 1.0 mg per kg body weight, which proved unable to produce diarrhea or to kill the animal after 10 days' application (Glávits and Ványi, 1988) but is high enough for an expressed biological effect. It is worth mentioning that the naturally contaminated feeds generally contain less trichotecene mycotoxins: 0.17-2.40 ppm T-2, 0.05-0.28 ppm HT-2, 0.15-0.85 ppm neosolaniol and 0.50-0.85 ppm DAS (Bata et al., 1984). The daily ration corresponds to the maintenance requirement of the animals (De Blas et al., 1985).

The digestibility trials were carried out with the so-called self-control method, using 2 x 6, 4-month-old NZW female rabbits (body weight :  $2.75 \pm 0.14$  kg), according to the following experimental design.

- Preliminary period. Feeding of a typical commercial rabbit feed mixture ("basal diet") during 10 days. The pellets contained the solvent of the toxin, i.e. 20 ml/kg dimethyl-sulfoxid (DMSO). The natural and chemical composition and the calculated nutritive value are given in Table 1.

- The main period of the basal diet: controlled feeding and quantitative, individual collection of feces, cecotroph and urine during 8 days.

- Transition or adjustment period of toxin-containing feed: 7 days.

- Main (collection) period of the toxin-containing feeds (as described previous): eight days.

The chemical composition of feed and feces samples was established according to A.O.A.C. (1975). The DE and ME content were calculated by the equation of Hoffmann et al. (1972) and Fekete and Papp (1981). The digestibility coefficients were calculated according to the classical formula. Statistical analyses were performed as described by Pearce (1965).

At the end of the experiment the animals were killed and a complete postmortem examination was performed. Tissue samples were taken from the liver, spleen, kidney, adrenal gland, stomach, small intestine, ampulla ilei, mesenteric lymph nodes and bone marrow (sternum) of each animal and fixed in neutral-buffered (5 %) formalin solution for histological examination. The embedding was made in paraffin and the sections were stained with hemalaun and eosin.

The concentration of trichotecene toxins in the feces, cecotroph and urine (mixed samples per treatment) was determined after Bata et al. (1984), using HPTLC (high-performance thin-layer chromatography) and gas chromatography.

## Results

The fundamental data (effective, average daily feed consumption, changes in body weight, dry matter content of the feces, daily T-2 toxin and digestible energy intake) of the digestibility trial are summarized in Table 2. The digestibility coefficients of nutrients of basal and toxin-containing feed are visualized in Table 3. The concentration of T-2 and HT-2 toxins, neosolaniol, T-2 triol and T-2 tetraol in the feces, cecotroph and urine, collected during the trial, is shown in Table 4.

The rabbits, exsanguinated at the end of the experiment, showed emaciation, subacute catarrhal gastritis, the necrosis of the lymphoid cells of the intestinal mucosa, depletion and necrosis in the lymphoid follicles of the ampulla ilei (sacculus rotundus), in the spleen and lymph nodes. Necrosis of cells, belonging to the MPS (mononuclear phagocyte system) in the liver was found. In cell colonies of the myeloid hemocytogenesis the necrosis and depletion of blast-cells were characteristic. The described necrotic alteration affected the 5 to 30 % of the cell colonies and there was no difference between the animals of groups "12.5 ppm T-2" and "25 ppm T-2".

### Discussion

The continuous consumption of a diet of subtoxic T-2 concentration (12.5 and 25 ppm, i.e. 0.19 and 0.28 mg per kg of body weight per day) decreased the voluntary feed intake by 60 to 70 %. Our results are close to the observations, made on swine, where refusal of feed containing greater than 16 ppm T-2 toxin have been described (Cheeke and Shull, 1985). The dry matter content of the feed significantly down (from 52-55 to 42-43 %) but there was no difference between the two toxin-treatment (Table 2).

The nutrients' digestibility of the 12.5 ppm T-2 toxin containing feed mixture proved to be better (by 2-6 %), that of the 25 ppm concentration by 4-11 % worse than the corresponding parameters of the basal diet (Table 3). We explain this phenomenon with the following: in the first case the digestibility improving effect (Fekete and Gippert, 1981) of the diminution of the feed intake might be greater than the damaging influence of the toxin. The knowledge of the above described fact could help in interpreting of natural, slight, subclinical mycotoxicoses: namely the data of feed conversion and feed intake mustn't be treated separately.

The subacute catarrhal gastritis may be the consequence of the direct cytopathic effect of T-2 toxin. The damage of lymphoid cells in the intestinal mucosa suggests the suppression of the local immune response, as for the lesions of the B- and T-cell-dependent zones of the lymphoid organs, they indicate the suppression of the humoral and cell-mediated immune responses. Necrosis of MPS cells shows the disturbance of the phagocyte function and the impaired granulocytogenesis in the bone marrow can reflect in an impaired natural resistance.

The toxin concentration of the feces, cecotroph and urine is proportional to the intake (Table 4), consequently one can estimate the toxin-loading of the animal on the basis of feces analysis. The relatively high T-2 content of the cecotroph - by means of the reingestion - can aggravate the damage of the organism.

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Table 1 : Composition and nutritive value of the basal diets

Ingredients	%
Alfalfa meal (21 % CP)	59.3
Corn	10.0
Wheat	10.0
Oats	10.0
Soybean meal (extr. solv. 48 % CP)	3.0
Animal fat (47 % EE)	4.0
Wheat straw	2.0
Mineral-vitamin-amino acid suppl.	2.0
<del>XX</del>	100.0
Digestible energy, MJ/kg	10.56
Crude protein, %	16.5
Digestible crude protein, %	10.6
Crude fiber, %	11.8
Lysine, g/kg	7.4
Methionine + cystine, g/kg	6.2
<del>XX</del>	
<del>XX</del> ) Calculated values	

Table 2 : Data of the digestibility trial (mean  $\pm$  SEM)

Parameters	Diets and corresponding groups			
	Basal I.	"12.5 ppm T-2"	Basal II.	"25 ppm T-2"
Number of animals	6	6	6	6
Feed intake, g/day	110	41 $\pm$ 7	110	34 $\pm$ 3
Change of BW, g/day	12 $\pm$ 6	- 13 $\pm$ 7	14 $\pm$ 6	- 18 $\pm$ 26
DM content of feces, %	52 $\pm$ 5	42 $\pm$ 5 <sup>**</sup>	55 $\pm$ 5	43 $\pm$ 2 <sup>**</sup>
Toxin intake, mg/kg BW/d	0	0.19	0	0.28
Energy intake, MJ DE/day <sup>*</sup>	1.33	0.52	1.30	0.40
Maintenance requirement, MJ DE	1.18	1.18	1.18	1.18

\* ) On the basis of chemical analysis and the present digestibility trial

\*\* )  $p < 0.05$  related to the corresponding basal value

Table 3 : The apparent digestibility coefficients (ADC) of nutrients, feeding the basal, the 12.5 and 25 ppm T-2 toxin containing diets (mean  $\pm$  SEM ; n = 6/group)

Digestibility Coefficients	Diets and the corresponding groups			
	Basal I.	"12.5 ppm T-2"	Basal II.	"25 ppm T-2"
dry matter	73.8 $\pm$ 2.2	77.0 $\pm$ 2.2	75.8 $\pm$ 0.3	69.5 $\pm$ 2.5 <sup>**</sup>
organic matter	74.5 $\pm$ 2.1	80.9 $\pm$ 2.5	76.6 $\pm$ 0.3	70.7 $\pm$ 2.5 <sup>**</sup>
crude protein	76.7 $\pm$ 2.5	80.9 $\pm$ 2.5 <sup>*</sup>	80.0 $\pm$ 0.8	74.5 $\pm$ 2.2 <sup>**</sup>
ether extract	66.2 $\pm$ 6.2	75.6 $\pm$ 3.8	74.7 $\pm$ 4.0	65.3 $\pm$ 6.6
crude fiber	14.6 $\pm$ 5.1	20.5 $\pm$ 6.7	22.2 $\pm$ 1.4	9.9 $\pm$ 4.4 <sup>**</sup>
N-free extr.	83.6 $\pm$ 1.6	85.4 $\pm$ 1.1	83.5 $\pm$ 0.3	79.5 $\pm$ 1.7 <sup>**</sup>
ash	61.1 $\pm$ 4.5	68.5 $\pm$ 3.3	62.8 $\pm$ 1.8	52.9 $\pm$ 6.0

\*)  $p < 0.1$  and \*\*)  $p < 0.05$  to the corresponding basal value

**Table 4 : Toxin content (ppm) of feces, cecotroph and urine, collected during the digestibility trial**

Samples	Toxins and metabolites				
	T-2 toxin	HT-2 toxin	Neosolantol	T-2 triol	T-2 tetraol
<b>Feces</b>					
"12.5 ppm T-2"	0.65	0.25	0.15	0.10	∅
"25 ppm T-2"	1.20	0.50	0.40	0.30	∅
<b>Cecotroph</b>					
"12.5 ppm T-2"	1.80	0.65	0.40	0.20	∅
"25 ppm T-2"	2.90	0.90	0.60	0.20	∅
<b>Urine</b>					
"12.5 ppm T-2"	0.40	0.20	0.30	0.20	∅
"25 ppm T-2"	0.85	0.40	0.55	0.30	traces

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Concentrated feed of subtoxic T-2 concentration (12.5 and 25 ppm) was fed to adult rabbits. The animals ate by 60-70 % less from the toxin-containing feeds. The dry matter content of their feces significantly (on an average: by 10 %) decreased. The nutrients' digestibility of the feed mixture, containing 12.5 ppm T-2 toxin, proved better by 2-6 %; that of the 25 ppm T-2 toxin level decreased the digestibility coefficients by 4-11 %. The toxin concentration of feces, caecotroph and urine was proportional to the intake.

L'EFFET DE T-2 TOXINE SUR LA CONSOMMATION ALIMENTAIRE  
ET DIGESTION DU LAPIN

L'aliment granulé de concentration subtoxique (12,5 et 25 ppm) a été rationné pour les lapins adultes. Les animaux ont réduit leur consommation d'aliment de 60-70 %. La teneur en matière sèche du fèces a été baissée de 10 %. La digestibilité de l'aliment contenant 12,5 ppm T-2 toxine a été améliorée de 2-6 %; celle de 25 ppm a été baissée de 4-11 %. La concentration de toxine du fèces, caecotroph et l'urine a été proportionnelle a l'ingestion.

