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Effect of chronic T-2 toxin exposure in rabbit bucks, determination of the No Observed Adverse Effect Level (NOAEL)

Melinda Kovács[a,b,*], Gábor Tornyos[a], Zsolt Matics[b], Miklós Mézes[c], Krisztián Balogh[c], Veronika Rajli[a], Zsófia Bloch-Bodnár[a], Miklós Rusvai[d], Míra Mándoki[d], Sándor Cseh[d]

[a] Kaposvár University Faculty of Animal Science, Guba S. 40, 7400 Kaposvár, Hungary
[b] MTA-KE Mycotoxins in the Food Chain Research Group, Guba S. 40, 7400 Kaposvár, Hungary
[c] SZIU Faculty of Agricultural and Environmental Sciences, Páter K. 1, 2001 Gödöllő, Hungary
[d] SZIU Faculty of Veterinary Science, István 2, 1078 Budapest, Hungary

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ABSTRACT

T-2 toxin (T-2) was administered to adult Pannon White ($n = 10$/group) male rabbits for 65 days, first in a suspension by gavage (0.05, 0.1 or 0.2 mg/animal/day), and secondly mixed into the feed (0.33 and 0.66 mg/kg feed). In the first experiment 0.1 mg T-2 exposure resulted in temporary decrease in feed intake, slower increase in the gonadotropin-releasing hormone (GnRH) induced testosterone synthesis, slight centrolobular infiltration in the liver and a slight hyperplasia of the Leydig cells. In addition to the temporary feed refusal effect, 0.2 mg T-2 caused a temporary decrease in plasma albumin and urea concentrations, lesser glutathione peroxidase (GPx) activity in the seminal plasma, a greater (by 320%) ratio of spermatozoa with cytoplasmic droplets, slower increase in the GnRH-induced testosterone synthesis, centrolobular infiltration in the liver, slightly hyperaemic testes and increased proliferative activity of the Leydig cells. The two smaller doses applied in feed (0.33 and 0.66 mg/kg) did not cause any significant adverse effect, and no feed refusal was observed. According to these results the No Observed Adverse Effect Level (NOAEL) of T-2 for adult rabbit males was found to be <0.1 mg/animal/day (<0.02 mg/kg b.w./day).

1. Introduction

Secondary toxic metabolites of *Fusarium* molds (fusariotoxins) are frequent contaminants in cereal and other plant products (Scott, 1990). *Fusarium* molds predominantly produce two types of mycotoxins: the non-estrogenic trichothecenes and the mycoestrogens including zearalenone and its zearalenol metabolites. T-2 toxin (T-2) is the most acute toxic compound among trichothecenes: it inhibits protein, DNA and RNA synthesis (therefore injuring organs with rapidly dividing cell populations), alters cellular membrane functions, stimulates lipid peroxidation, is cytotoxic and immunotoxic and induces apoptosis (SCF, 2001).

The effect of T-2 on reproduction has been widely studied in female animals, while knowledge about its effect on spermatozoa, testosterone production, i.e. reproduction processes in males is limited. T-2 was found to inhibit testosterone secretion in gerbil testicular interstitial cells in vitro (WHO, 2001). Yang et al. (2010) investigated the effects of T-2 on semen quality, fertility and serum testosterone concentration in mice after intra-peritoneal injection of 0, 5, 10 and 15 mg/kg b.w. T-2 on 7 consecutive days. A dose-dependent reduction of sperm count in the testes and epididymidis and increase in the number of abnormal spermatozoa occurred. Semen quality of bulls was also impaired after a treatment with a small

* Corresponding author at: Kaposvár University, Guba S. Street 40, 7400 Kaposvár, Hungary. Tel.: +36 82505800; fax: +36 82505970.
E-mail address: kovacs.melinda@ke.hu (M. Kovács).

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dose of T-2 and HT-2 toxin (117.5 μg T-2/day and 1425 μg HT-2/day) for about 14 weeks, with poor progressive motility and poor morphology of spermatozoa being observed. The effect persisted until 5 month after withdrawal of the moldy hay (Alm et al., 2002). The subsequent effect of T-2 applied to rabbits in larger doses (4 mg/animal/day for 3 days) was manifested by a decreased sperm motility, increased number of spermatozoa with morphological abnormalities, decrease in concentration of citric acid in seminal plasma, and decrease in testosterone concentration after 48 days following a 3-day long sub-chronic toxicosis (Kovács et al., 2011). Interaction of T-2 with reproduction in rabbits was reported by WHO (2001), but the studies were criticized due to their poor description. However, in addition to taking reproductive disorders into consideration from an animal production perspective, studying effects in rabbits can be of special interest, as this animal species is known to be a good model of human reproductive toxicology. Using the rabbit in reproductive toxicological studies has several advantages, e.g. the male rabbit is the smallest, least expensive animal that can be ejaculated with an artificial vagina, permitting longitudinal evaluation of semen characteristics (Morton, 1988). The recognition of the importance of sperm morphology measurements in men exposed to various agents, and the repeatability of morphometric measurements of rabbit sperm add to the value of the male rabbit as an especially useful model (Foote and Carney, 2000).

In several studies, it has been concluded that the major factors with regard to male subfertility or infertility are being sought among environmental and industrial chemicals, antibiotics and cytotoxic drugs, heavy metals, and dietary toxins, e.g. mycotoxins. People all over the world can be exposed to prolonged intake of mycotoxins in small doses. According to the most recent overview (Van der Fels-Klerx and Stratakou, 2010) on the occurrence of T-2 and HT-2 toxin (HT-2) in grain and grain-based commodities in Europe, the incidence and concentrations of these toxins were the greatest in oats through 2007 with reduced amounts being detected in recent years. The contamination of barley and maize except in some incidental cases was little, wheat, durum wheat, triticale and rye were not susceptible to T-2/HT-2 contamination. Of the cereal derived food products, oat products were contaminated to the greatest extent. Because the consumption of wheat products in Europe exceeds that oat derived products, even the lesser contamination of wheat may also be significant in case of longer exposure. There are currently no EC limits for T-2 and HT-2 and no recent risk assessments on these grains in the European community, mainly because of a lack of a complete up-to-date overview of the occurrence of these two toxins.

To determine the No Observed Adverse Effect Level (NOAEL) and by this providing data for the calculation of the tolerable limit levels of T-2 in bucks, two experiments were conducted, in which the toxin was administered during 65 days in small doses, first in a suspension by gavage (Experiment 1), and secondly mixed into the feed (Experiment 2).

2. Materials and methods

2.1. Animals, housing and treatments

Pannon White ($n = 10$/group) male rabbits (weight: 4050–4500 g, age: 9 month) trained to ejaculate into an artificial vagina were used in the experiments. Rabbits were individually housed in wire mesh cages (42 cm × 50 cm) in a closed building, with 16 light hours per day. Average temperature ranged from 16 to 18 °C and the farm had overpressure ventilation. The animals received a commercial diet containing 10.3 MJ digestible energy (DE)/kg, 15.5% crude protein, 4.0% crude fat and 14.7% crude fiber, ad libitum, and had free access to drinking water from weight-valve self-drinkers.

T-2 was produced experimentally on corn grits by *Fusarium sporotrichioides* strain NRRL 3299, as described by Fodor et al. (2006). In the first experiment T-2 was extracted and purified according to the methodology described previously (Kovács et al., 2011). Treated animals received 0.05, 0.1 or 0.2 mg/animal/day T-2 toxin, while control animals received toxin free suspension by gavage for 65 days. The toxin containing suspension was prepared by dissolving the crystallized T-2 in ethanol, and thereafter diluted with distilled water to get ethanol concentration ≤2%.

Taking the results of the first experiment into consideration, in the second experiment the fungal culture was mixed to the diet of the rabbits so, that the T-2 concentrations corresponded to the two smaller doses of the first experiment, i.e. 0.33 and 0.66 mg/kg complete feed. Control animals received a diet with no detectable T-2 toxin content.

Individual feed consumption was recorded daily. The body weight was measured weekly. Animal health status was checked every day.

In both experiments, semen was collected with an artificial vagina after 65 days of toxin exposure. Subsequently, the GnRH-challenge test wasconducted. The animals were treated with 0.2 ml GnRH-analog i.m. (Receptal, Intervet, The Netherlands). The concentrations of testosterone were determined from blood samples collected from the marginal ear vein just prior to GnRH-analog injection (0 min) and thereafter in the 25th, 50th, 75th, 90th and 115th minute.

Blood samples were taken at the beginning (Day 0), on the 30th day and at the end (Day 65) of the experiment to determine certain parameters of clinical chemistry and the antioxidant status. At the end of the experiment animals were euthanized by intravenous barbiturate overdose, exsanguinated, necropsied, the weight of liver, spleen, kidneys and testes was measured, the relative weight (expressed as % of body weight) calculated, and the testes were subjected to histo-pathological examination.

The experimental protocol was authorized by the Food Chain Safety and Animal Health Directorate of the Somogy County Agricultural Office, under permission number 23.1/02322/007/2008.