Preliminary Results on the Effect of Chronic T-2 Toxin Exposure in Rabbit Bucks

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Summary

The aim of the present study was to examine the chronic effect of T-2 toxin on feed consumption and sperm quality. Pannon White (n=10/group) male rabbits (weight: 4050-4500 g, age: 9 month) trained to ejaculate into artificial vagina were exposed to 0 (control), 0.05, 0.1 or 0.2 mg/animal/day of T-2 toxin by gavage for 63 days. On the 63rd day of the experiment semen was collected with an artificial vagina, and the following traits were evaluated: pH, concentration, morphology, motility with CASA, concentration of seminal plasma components such as citric acid, zinc and fructose. At the end of the experiment animals were necropsied and the testes were subjected to histopathological examination. T-2 toxin in 0.1 mg and 0.2 daily dose significantly decreased feed intake in the first two weeks but no significant difference between groups were observed from the 4th week. Among the sperm quality traits examined only the ratio of spermatozoa with cytoplasmic droplets increased by 320% in animals treated with the highest dose of T-2. The 0.1 mg/animal/day toxin exposure resulted in a slight hyperplasia of the Leydig cells, while the highest dose (0.2 mg/animal/day) caused hyperaemia, increased proliferative activity and hyperplasia of the Leydig cells. According to the preliminary results it seems, that adult male rabbits may tolerate the concentration of 0.05 mg/animal/day T-2 toxin.

Key words

T-2 toxin, rabbit, sperm quality

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Aim

Mycotoxins are frequently existing contaminants in cereal and other plant products (Scott, 1990). Like many other environmental factors, mycotoxins may cause subfertility or infertility in males (Sprando et al., 2005; Ewuola and Egbunike, 2010). T-2 toxin is the most acute toxic compound among trichothecens: it inhibits protein, DNA and RNA synthesis (therefore injuring organs with rapidly dividing cell populations), alters cellular membrane functions, stimulates lipidperoxidation, it is cytotoxic and immunotoxic and induces apoptosis (SCF, 2001). Our knowledge about mechanisms of action and the effects of certain mycotoxins on male reproduction processes is incomplete since it has not been widely studied. In a pilot study we examined the subsequent effect of T-2 toxin applied in high dose (0.78-0.99 mg/kg bw) for 3 days on the male reproductive processes in rabbits (Kovács et al., 2011). Already one day of T-2 toxin treatment dramatically decreased voluntary feed intake and remained lower during the first 2 weeks after the withdrawal of the toxin. The ratio of spermatozoa showing progressive forward motility decreased in the semen samples of toxin treated animals, while the ratio of spermatozoa with abnormal morphology increased, even after 48 days following the withdrawal of the toxin.

The aim of the present study was to examine the chronic effect of T-2 toxin in low doses on feed consumption and sperm quality.

Materials and methods

Pannon White (n=10/group) male rabbits (weight: 4050-4500 g, age: 9 month) with adequate sexual functioning and trained to ejaculate into artificial vagina were used in the experiment. The animals received a commercial diet containing 10.3 MJ DE/kg, 15.5% crude protein, 4.0% crude fat and 14.7% crude fibre, *ad libitum*. T-2 toxin was produced experimentally on corn grits by *Fusarium sporotrichioides* strain NRRL 3299, as described by Fodor et al. (2006). T-2 was extracted and purified according to the methodology described by Kovács et al. (2011). Exposed animals received 0.05, 0.1 or 0.2 mg/animal/day T-2 by gavage for 63 days. Control animals received toxin fee suspension by gavage for 63 days. Every day the individual feed consumption was recorded. The body weight was measured weekly. Animal health status was checked daily. On the 63rd day of the experiment semen was collected with an artificial vagina. The following traits were evaluated: pH, concentration (improved Neubauer cell counting chamber), morphology (Spermac™ staining, Beernem, Belgium), as well as the motility (Medealab™ CASA System, Erlangen, Germany) of spermatozoa. The concentration of seminal plasma components such as citric acid (Citric Acid Test, FertiPro, Belgium), zinc (Zinc, Wako Chemicals GmbH, Germany), and fructose (Fructose Test, FertiPro, Belgium) were also measured (WHO, 1999). At the end of the experiment animals were necropsied and the testes were subjected to histopathological examination.

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

Data were analysed by using the Multiway ANOVA procedure of SPSS, version 10.0. The significance of differences was tested by LSD post hoc test. A value of $P<0.05$ was considered as significant.

Results and discussion

No significant difference in body weight attributable to toxic effect was detected. In the first week T-2 toxin in 0.1 mg and 0.2 daily doses significantly decreased voluntary feed intake by 63 and 47%, respectively, compared to controls (Table 1). On the second week even the lowest dose (0.05 mg/animal/day) resulted in a temporary decreased feed consumption ($P<0.002$), in the 3rd week the feed refusal effect was detectable only in case of the highest dose (0.20 mg/animal/day, $P<0.005$). After the 2nd week feed consumption increased in the toxin treated animals, and no significant difference between groups was observed from the 4th week, indicating the adaptation to the toxin.

Table 1. Effect of T-2 on voluntary feed intake (g/day, mean±SD)

Weeks	Daily T-2 exposure (mg/animal)[1]				P
	0	0.05	0.10	0.20	
1.	155±39^{c}	118±40^{bc}	98±32^{ab}	73±29^{a}	0.000
2.	141±38^{b}	98±32^{a}	93±45^{a}	77±25^{a}	0.002
3.	139±39^{b}	122±24^{ab}	111±35^{ab}	83±33^{a}	0.005
4.	130±31	121±11	129±22	101±30	NS
5.	125±21	118±16	123±33	114±16	NS
6.	154±29	140±16	143±29	140±28	NS
7.	151±34	144±21	152±22	140±29	NS
8.	134±25	117±14	133±29	130±30	NS
9.	128±18	128±19	133±21	130±28	NS

[1] n=10 in each group, means with different superscripts are significantly different ($P<0.05$)

None of the sperm quality traits examined showed significant difference between groups, except the ratio of spermatozoa with cytoplasmic droplets, which increased by 320% in animals treated with the highest dose of T-2 (Table 2). In average 2.8% (SE=0.76), 0.7% (SE=0.2), 4.5% (SE=0.8), 13.3% (SE=0.6) and 1.4% (SE=0.96) of the sperm cells was microcephal, macrocephal, showed broken tail, rolled tail, or lack of acrosoma, respectively. Despite the lack of statistical significance between groups in sperm motility, the trend of the data shows a negative toxin effect in case of this parameter (Table 2).

According to the histological examinations control animals and animals treated with 0.05 mg/animal/day T-2 showed active testes, physiological spermatogenesis and intact parenchyma. Daily 0.1 mg/animal exposure resulted in a slight hyperplasia of the Leydig cells, while the highest dose (0.2 mg/animal/day) caused increased proliferative activity of the Leydig cells beside hyperplasia. In the latter case the testes were slightly hyperaemic.

Rabbit can be considered rather sensitive to T-2 toxin, as they have a relatively low (1.1 mg/kg bw) LD_{50} value (Wannemacher and Wiener, 1997). The SCF (2002) has established a combined

Table 2. Effect of T-2 on certain sperm quality parameters (mean±SD)

Parameter	Daily T-2 exposure (mg/animal)[1]				P
	0	0.05	0.10	0.20	
Sperm cell count (10^6/ml)	300 ± 136	345 ± 359	265 ± 139	277 ± 111	0.390
pH	7.14 ± 0.14	7.23 ± 0.27	7.06 ± 0.22	7.09 ± 0.16	0.825
Citric acid (mg/ml)	3.23 ± 1.6	4.06 ± 1.86	3.56 ± 1.2	3.51 ± 1.79	0.817
Fructose (mg/ml)	1.43 ± 0.7	1.4 ± 0.7	1.2 ± 0.7	0.9 ± 0.3	0.559
Zn (µg/ml)	596 ± 145	686 ± 115	537 ± 223	675 ± 207	0.562
Normal morphology (%)	70 ± 14	69 ± 19	77 ± 8	70 ± 7	0.692
Retention of cytoplasmic droplets	3^a ± 0.6	5.4^{ab} ± 2.0	2.9^a ± 1.0	9.6^b ± 2.4	0.01
Motility (%)	85.4 ± 5.5	74.6 ± 9.0	72.9 ± 18.1	79.0 ± 9.0	0.133
Immotile cells (%)	11.4 ± 5.0	22.6 ± 6.8	25.1 ± 17.8	16.9 ± 9.5	0.072

[1] n=10 in each group, means with different superscripts are significantly different (P<0.05)

t-TDI (temporary Tolerable Daily Intake) of 0.06 µg/kg bw/day for the sum of T-2 and HT-2. In mouse and rats 0.23 and 0.5 mg/kg bw/day have been identified as NOAELs (no observed adverse effect level) for T-2 in chronic toxicosis by the Scientific Committee on Food (SCF, 2001). According to the committee's report this t-TDI would protect against the chronic, subchronic and reproductive effects. The T-2 toxin dose we applied was 0.01-0.02-0.05 mg/kg bw/day, it represents about 0.3-0.6-1.3 mg/kg feed contamination. The 0.3 ppm can be considered as a risk under practical conditions, as approximately about 5% of examined feed samples in Hungary contained 0.1-0.5 ppm T-2 in the average of the last 5 years (unpublished data).

Trichothecenes are shown to alter the serotonin activity in the central nervous system. The serotoninerg receptors are important mediators in appetite regulation, so an increased serotonin concentration can be the reason of the decreased appetite (Smith, 1992). In previous experiments the lowest toxin concentration was 0.01 mg/kg bw in rabbit, which did not cause the feed refusal phenomenon of T-2 (Fekete and Huszenyicza, 1993). This may be supported by our results, as 0.01 mg/kg bw (0.05 mg/animal) T-2 resulted in a temporary decrease in feed intake, experienced only in the 2nd week compared to the 1st week's data.

From the results according to which there was no significant difference in the number of spermatozoa and the ratio of the cells with abnormal morphology, it may be concluded that T-2 toxin in the doses applied did not expound direct cytotoxic effect on the sperm cells. Observed effects are probably attributable to the altered Leydig cell function, e.g. testosterone production, which could be proved by the detection of testosterone production under T-2 exposure. Nikodemusz and Mezes (1992) observed that GnRH injection induced significantly lower testosterone production in gander treated with T-2 toxin. Our previous result obtained in male rabbit is very similar since we found that T-2 toxin applied in high dose induced acute functional disturbances in the Leydig cells, indicated by the decreased basic testosterone level, which resulted in a hypo-androgenic status (Kovács et al., 2011). The higher ratio of spermatozoa with cytoplasmic droplets and the decreased motility (not significant) in the toxin treated group can be the result of the impaired epididymal function, i.e. maturation of spermatozoa, which is known to be influenced by testosterone (Yeung, 1995; Parkinson, 2009).

Conclusions

According to the preliminary results it can be concluded, that adult male rabbits may tolerate the concentration of 0.05 and 0.1 mg/animal/day T-2 toxin, without any significant decay in sperm quality, though 0.1 mg/animal/day toxin caused slight alteration in the Leydig cells. 0.2 mg T-2 had a more pronounced feed refusal effect, dramatically increased the ratio of spermatozoa with cytoplasmic droplets, caused hyperaemy in the testes, proliferation and hyperplasia in the Leydig cells.

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