

RESPONSE OF MALE RABBITS TO T-2 EXPOSURE

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ABSTRACT

In several studies it has been concluded that the major factors with regard to male subfertility or infertility can be attributable to environmental factors, like mycotoxins, as frequently occurring dietary toxins. In a pilot study we examined the subsequent effect of T-2 toxin applied in high dose (4 mg/animal) for 3 days on the male reproductive processes in rabbits. One day of T-2 toxin treatment dramatically decreased voluntary feed intake which also remained lower during the first 2 weeks after the withdrawal of the toxin. Body weight gain of the contaminated rabbits decreased by 88% on days 17 and 29 compared to controls. The subsequent effect of T-2 toxin applied to rabbits in high dose manifested a decrease in sperm motility, an increase in the number of spermatozoa with morphological abnormalities, and decrease in the testosterone level even after 48 days following a 3-day long acute toxicosis. In another study male rabbits were exposed to 0 (control), 0.05, 0.1 or 0.2 mg/animal/day T-2 toxin by gavage for 63 days. T-2 toxin in 0.1 mg and 0.2 daily doses significantly decreased feed intake, which returned to normal values after the 2nd and 3rd week, respectively for each group. None of the sperm quality parameters examined showed significant difference between groups, except the ratio of spermatozoa with cytoplasmic droplets, which increased in animals treated with the highest dose of T-2. The two lower doses applied via feed (i.e. mixed into the feed to get 0.33 and 0.66 ppm T-2 containing diets) had no significant effect on feed intake, body weight, and any spermatological parameters. According to the results of the two chronic experiments the two lower doses may be tolerated by adult male rabbits without any detrimental changes in reproductive parameters.

Key words: T-2 toxin, reproduction, male, rabbit

INTRODUCTION

Mycotoxins are of great economic, scientific and public health significance all over the world. They are biologically active secondary fungal metabolites found as contaminants of food- and feedstuff. *Fusaria* are moulds predominantly producing two types of mycotoxins: the non-estrogenic trichothecene and the mycoestrogens including zearalenone and its zearalenol metabolites. These fusariotoxins are frequently existing contaminants in cereal and other plant products (Scott, 1990). T-2 toxin is the most acute toxic compound among trichothecens: it inhibits protein, DNA and RNA synthesis, alters cellular membrane functions, stimulates lipidperoxidation, it is cytotoxic and immunotoxic and induces apoptosis (Scientific Committee on Food, SCF, 2001). While the toxic effect on reproduction in females are quite well known, our knowledge about mechanisms of action and the effects of certain mycotoxins on spermatozoa and male reproduction is incomplete. The aim of our studies was to examine the subsequent effect of T-2 toxin applied in high dose, and in case of chronic exposure to low doses, on the male reproductive processes in rabbits.

MATERIALS AND METHODS

Three experiments were carried out. Pannon White male rabbits (weight: 4050-4500 g, age: 9 month) trained to ejaculate into an artificial vagina were used. 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. The

feedstuffs provided were available *ad libitum*, and the rabbits also had free access to drinking water from weight-valve self-drinkers.

T-2 toxin was produced experimentally on corn grits by *Fusarium sporotrichioides* strain NRRL 3299 as described by Fodor *et al.* (2006). Exposed animals received 4 mg/animal/day T-2 toxin by gavage for 3 days (exp. 1), 0.05, 0.1 and 0.2 mg/animal/day T-2 toxin for 63 days by gavage (exp. 2), while 0.33 and 0.66 ppm for 63 days in feed (exp. 3.) (Table 1)

Table 1: Toxin exposure and sampling

Experiment	Dose of T-2	Time of exposure	Toxin application	Sampling time
1	4 mg/animal/day	3 days	gavage	48 days after the withdrawn of T-2
2	0.05, 0.1 and 0.2 mg/animal/day	63 days	gavage	after 63 days of exposure
3	0.33 and 0.66 ppm	63 days	in feed	after 63 days of exposure

Every day the individual feed consumption was recorded. The body weight was measured weekly, while animal health status was checked 3 times a day.

On the 51st (exp. 1) or 63rd day (exp. 2 and 3) of the experiment semen was collected and the GnRH-challenge test was carried out. The levels of testosterone hormone were determined from blood samples collected from the marginal ear vein just prior to GnRH-analogue injection (0 minutes) and thereafter in the 25th, 50th, 75th, 90th and 115th minute collections.

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.

The following spermatological parameters were evaluated: pH, concentration, morphology and acrosomal integrity, as well as the total motility and fast/slow forward motility of spermatozoa. Testosterone concentration was determined with a direct ³H-radioimmunoassay method.

All data were analysed by using the Multiway ANOVA procedure of SPSS (2002), version 10.0.

RESULTS AND DISCUSSION

Experiment .1.

One day of T-2 toxin treatment dramatically decreased voluntary feed intake (by 27 % compared to control, $P < 0.05$) which remained low ($P < 0.05$) during the first 2 weeks after the withdrawal of the toxin. Body weight gain of the contaminated rabbits decreased by 88 % on days 17 and 29 compared to controls ($P < 0.05$). No effect of toxin treatment was detected on pH and quantity of the semen or concentration of spermatozoa. The ratio of spermatozoa showing progressive forward motility decreased from 65 to 53 % in the semen samples of toxin treated animals compared to controls ($P > 0.05$). The ratio of spermatozoa with abnormal morphology increased ($P < 0.05$) in the ejaculates collected from the toxin treated animals. T-2 toxin decreased the basic testosterone level by 45 % compared to control ($P < 0.01$) and resulted in lower ($P < 0.05$) GnRH induced testosterone concentration (Figure 1).

Experiment 2

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. On the second week even the lowest dose resulted in a temporary decreased feed consumption, while in the 3rd week the feed refusal effect was detectable only in the case of rabbits administered the highest dose. After the 2nd week feed consumption increased in the toxin treated animals, and no significant difference between groups was observed from the 4th week onwards, indicating adaptation to the toxin (Table 1).

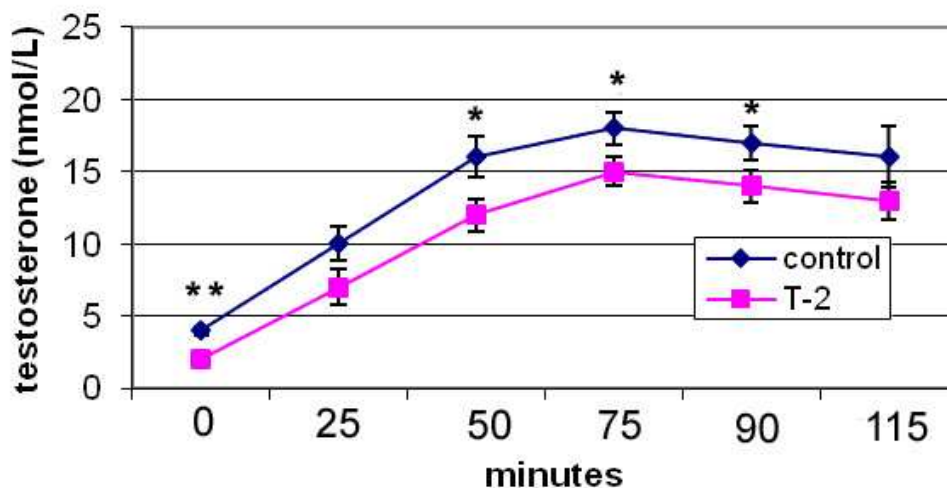


Figure 1: Basic testosterone concentration and GnRH induced testosterone production (Difference between control and T-2 groups, level of significance ** P<0.01, *P<0.05)

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 1: Voluntary feed intake (g/day, mean±SD)

Week	Daily T-2 exposure (mg/animal) n=10/group				P
	0	0.05	0.1	0.2	
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

^{a,b} P<0.05

No significant difference in basic testosterone level was observed, while the GnRH induced increase in testosterone level was lower (P<0.05) in 0.2 mg T-2 treated animals. In the case of 0.1 mg dose the increase in testosterone concentration was lower than in control animals, (P<0.05), but from min 50' the two groups did not differ significantly.

Experiment 3.

In the third experiment the two lower doses (0.05 and 0.1 mg/animal/day) were chosen and mixed into the diet to examine if the animals consumed the toxin-contaminated feed or if a feed refusal effect would be present. No difference in feed intake between groups was registered. None of the sperm quality parameters differed significantly in treated animals compared to the controls (Table 2). In case of 0.66 ppm T-2 concentration the GnRH induced increase in testosterone concentration was slightly lower compared to control but differences were not significant.

An increased serotonin concentration is thought to be the reason of the decreased or loss of appetite due to T-2 (Smith, 1992). The lowest toxin concentration in the bibliography which does not cause the feed refusal phenomenon of T-2 (Fekete and Huszenicza, 1993) is 0.01 mg/kg bw in rabbits,. In our adult male rabbits 0.05 mg/animal (i.e. 0.01 mg/kg bw) caused only a slight and temporary decrease in feed intake, which returned to normal values within a few days.

Table 2: Sperm quality parameters (mean±SD)

Parameters	Daily T-2 exposure (mg/kg feed) n=10/group	P
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	0	0.33	0.66	
Sperm cell count (10 ⁶ /ml)	213±102	244±167	211±88	0.822
Normal morphology (%)	77±12	75±9	77±10	0.883
Retention of cytoplasmic droplets (%)	3±2	4±1.7	6±3.4	0.639
Motility (%)	70±8	70±4	68±15	0.884

High concentration of T-2 resulted in decreased motility and increase in the ratio of morphologically abnormal cells, even 48 days after the exposure. A direct cytotoxic effect of T-2 toxin may be considered as one of the mechanisms of action of T-2 toxin, since trichothecenes are toxic for actively dividing cells. The subsequent effect of the toxin was probably attributable to the observations (Ettlin *et al.*, 1984), according to which, the cell types primarily affected must have been exposed to the toxin 28 days prior to the time of examination. Decreased motility in the toxin treated group can be the result of the impaired epididymal function as well, i.e. maturation of spermatozoa, leading to an impairment of sperm motility (Yeung, 1995; Parkinson, 2009). This can be supported by the increase of the ratio of cells with cytoplasmic droplets caused by 0.2 mg/animal dose in the chronic exposure. All these effects could be detrimentally influenced by the lowered testosterone production, since androgens are known to play a pivotal role in the regulation of spermatogenesis (de Kretser and Kerr, 1994).

CONCLUSIONS

The subsequent effect of T-2 toxin applied to rabbits in high dose manifested a decrease in sperm motility, an increase in the number of spermatozoa with morphological abnormalities, and a decrease in the testosterone level even after 48 days following a 3-day long acute toxicosis. Adult male rabbits may tolerate the concentration of 0.33 ppm (0.05 mg/animal/day) T-2 toxin, without any significant decay in sperm quality. The 0.2 mg/animal/day toxin had a pronounced feed refusal effect, dramatically increased the ratio of spermatozoa with cytoplasmic droplets, and reduced GnRH induced testosterone production.

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