Spermatogenesis, gonadal sperm reserves and fertility of rabbits fed micro doses of fumonisin

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Abstract

The effects of micro doses of dietary fumonisin, a metabolite of *Fusarium verticillioides*, on spermatogenesis, gonadal sperm reserves and fertility of rabbits were studied. Relative paired testis weight; sperm production rate and sperm storage potential were not adversely affected in rabbits which were exposed to varied minute levels of dietary fumonisin of about 153-161 µg/kg body weight/day over a period of 8 weeks. The toxin also failed to exert any influence ($P > 0.05$) on seminiferous tubular diameter and volume percent of testicular elements. Male fertility as evidenced by conception rate, litter size and embryo survival and normalcy was also not affected ($P > 0.05$). However, the frequency of occurrence of stages II and VIII of the cycle of the seminiferous epithelium were significantly ($P < 0.05$) influenced with increased dietary fumonisin levels. The results suggest that the ingestion of *Fusarium*-infected feed that would result in the liberation of about 153 µg of fumonisin/kg of body weight per day for a short time may not influence spermatogenesis, fertility in the male or the normalcy of the resulting foetuses.

Key words: Rabbits, Spermatogenesis, Gonadal Sperm Reserves, Fertility and Fumonisin.

Introduction

Available data on livestock production in Nigeria clearly indicate that we are far from the biological potential for rate and efficiency of gain, fertility and breeding efficiency. As the demand for animal flesh continues to soar, the necessity for increasing the reproductive efficiency of livestock becomes more evident since this may be the area where man can expect the most significant economic achievements in livestock production. Among other factors; nutrition plays a very important role in the regulation of the reproductive efficiency of animals. Most of the ingredients for ration formulation however contain some obnoxious materials, such as

mycotoxins, which have been reported (Egbunike, 1995) not to be conducive to reproduction. Toxins with oestrogenic effects for example, have been reported to impede breeding (Petzinger and Weindenbach, 2002).

Fusarium verticillioides (Sacc.) Nirenberg is one of the most prevalent fungi associated with human and animal dietary staples such as maize (Marasas *et al.*, 1984; Nelson *et al.*, 1991). This species of fungus produces the novel mycotoxin, fumonisin, and Shephard *et al.* (1996) reported that maize is the only commodity that contains significant amounts of fumonisin, and the importance of this ingredient in the food of man

and his livestock cannot be overemphasized. Hence, the potential for fumonisins to be found in feeds and foodstuffs is high.

The carcinogenicity, hepatotoxicity and mutagenicity as well as the effects on feed intake and body weight gain by dietary fumonisins in animals have been well documented (Marasas *et al.*, 1988; Harrison *et al.*, 1990; Kellerman *et al.*, 1990; Gelderblom *et al.*, 1991; Colvin and Harrison, 1992). However, interest in these areas had far outweighed those in the effect of fumonisins on the reproductive efficiency of animals.

Concern about the reproductive and developmental effects of fumonisins originated with the observation of abortions in pregnant sows fed fumonisin-contaminated diets (Harrison *et al.*, 1990) and the report of Bradlaw *et al.* (1994) that 1.8 µg fumonisin/ml inhibited reaggregation and growth of chicken embryo neural retina cells, a commonly used *in vitro* assay for screening potential developmental toxins.

With the above in mind, and the fact that reproductive inefficiency is recognised as the most costly and limiting constraint to efficient animal production, the present investigation was designed with the aims of evaluating the effects of micro doses of fumonisin on spermatogenesis, gonadal sperm reserves and male fertility in the rabbit.

Materials and Methods

Thirty crosses of adult bucks aged 20-24 weeks weighing between 1000 and 1650g were randomly and equally, by live weight, in a completely randomized design assigned to three treatments of low, medium and high *F. verticillioides*-infected maize-based diets containing 1960, 2080 and 2190 µg fumonisin/kg respectively, with five replicates each.

After the 8-week feeding trial, 5 males per treatment were used to mate untreated female rabbits at the rate of one male to two females. Thereafter, all the male rabbits were sacrificed by stunning and decapitation, right testis dissected and processed as described by Egbunike and Steinbach (1972). Seminiferous tubular diameters were measured following the procedure of Egbunike and Steinbach (1972) and the mean tubular diameter for each animal was calculated from duplicate measurements of 20 round tubules per testis. Sperm production rates were determined by quantitative testicular histology as outlined by Swierstra (1966) and slightly modified by Egbunike and Steinbach (1972). The lifespan of the spermatids with round nuclei was estimated to be 7.32 days from the lengths and cellular associations of the different stages of the cycle of seminiferous epithelium (Ortavant, 1959) and the duration of the whole cycle was 12.0 days (Clermont *et al.*, 1959). The evaluation of the sperm storage potential of the experimental male rabbits involved direct haemocytometer counts of homogenates of testes after 1:40 (v/v) dilution in 0.154M-NaCl (Egbunike *et al.*, 1976).

To determine the fertility rates, the mated female rabbits were sacrificed at the end of the first trimester (10 days *post coitum*) and their uteri cut open longitudinally to check for conception, count the embryos therein and measure the crown-rump lengths of the embryos.

The data were subjected to statistical analysis using analysis of variance procedure of SAS (1999). The treatment means were compared using the Duncan's procedure of the same software.

Results

The relative paired testes weight (expressed as percentage of live weight), seminiferous tubule diameter, volume percent of testicular elements,

the frequency of occurrence of the stages of the cycle of seminiferous epithelium, except stages II and VIII, daily sperm production rate and the efficiency of spermatogenesis (daily sperm production per g testis) and sperm storage potentials did not differ significantly ($P>0.05$) among the treatments (Table 1). The frequency of occurrence of stages II and VIII of the seminiferous epithelial cycle significantly ($P<0.05$) decreased and increased, respectively with increased dietary fumonisin levels.

Similarly, the fertility in the three treatments was not significantly different ($P>0.05$). Table 2 summarises the effect which breeding female rabbits with males that had consumed varied

micro doses of dietary fumonisin had on their fertility. There were no significant differences ($P>0.05$) in litter size 10 days *post coitum*. Crown-rump length and the survival rate of the embryo were similar ($P>0.05$) even though the ovulation rates of the females were likewise similar across the treatments (12.52 ± 1.22 ova shed).

Discussion

Earlier studies have established that testicular weight and sperm production are highly related (Swierstra, 1966; Egbunike, 1973). In the present study, the relative paired testes weight, gonadal sperm storage potentials, seminiferous tubular diameter and volume percent of testicular elements of rabbits fed fumonisin-contaminated

Table 1: Effect of dietary fumonisin on testis function of rabbits (Mean $\pm$ SEM*)

Parameter	Dietary fumonisin level		
	Diet 1 (Low)	Diet 2 (Medium)	Diet 3 (High)
Weight			
Body weight (g)	1638.00 $\pm$ 70.41	1583.33 $\pm$ 56.22	1365.00 $\pm$ 48.90
Paired Testis wt (%**)	0.19 $\pm$ 0.02	0.20 $\pm$ 0.01	0.19 $\pm$ 0.01
Histometry			
Seminiferous tubular diameter (μ m)	115.92 $\pm$ 11.56	125.32 $\pm$ 10.48	13.94 $\pm$ 8.48
Volume of testicular elements (%)			
Spermatogonia	12.99 $\pm$ 1.76	18.05 $\pm$ 2.18	14.07 $\pm$ 1.92
Primary spermatocytes	17.41 $\pm$ 1.06	18.36 $\pm$ 2.04	20.68 $\pm$ 2.47
Secondary spermatocytes	24.45 $\pm$ 2.21	23.79 $\pm$ 1.43	22.49 $\pm$ 1.00
Spermatids (round)	10.41 $\pm$ 0.90	9.46 $\pm$ 1.05	11.10 $\pm$ 1.62
Spermatids (elongated)	9.23 $\pm$ 0.80	8.92 $\pm$ 1.01	9.85 $\pm$ 1.44
Spermatozoa	9.09 $\pm$ 1.61	9.79 $\pm$ 1.05	10.76 $\pm$ 1.78
Sertoli cells	6.41 $\pm$ 1.34	2.46 $\pm$ 0.59	1.83 $\pm$ 0.48
Interstitial tissue	3.62 $\pm$ 0.91	3.57 $\pm$ 0.76	2.90 $\pm$ 0.80
Lumen	9.59 $\pm$ 0.68	7.37 $\pm$ 1.77	6.32 $\pm$ 1.01
Seminiferous epithelial cycle (%)			
(a) Stage I	18.88 $\pm$ 4.32	16.89 $\pm$ 3.47	17.25 $\pm$ 4.73
(b) Stage II	27.38 $\pm$ 2.25 ^a	18.56 $\pm$ 1.96 ^b	21.38 $\pm$ 2.16 ^{ab}
(c) Stage III	11.75 $\pm$ 3.32	6.55 $\pm$ 1.92	11.88 $\pm$ 2.37
(d) Stage IV	9.63 $\pm$ 0.86	12.44 $\pm$ 1.89	12.00 $\pm$ 1.50
(e) Stage V	8.50 $\pm$ 0.71	10.11 $\pm$ 1.71	8.88 $\pm$ 1.66
(f) Stage VI	8.38 $\pm$ 2.19	10.11 $\pm$ 0.93	7.75 $\pm$ 0.96
(g) Stage VII	7.88 $\pm$ 1.97	10.56 $\pm$ 1.69	9.13 $\pm$ 2.13
(h) Stage VIII	7.63 $\pm$ 2.49 ^b	15.89 $\pm$ 1.94 ^a	11.75 $\pm$ 2.49 ^{ab}
Daily sperm production ($\times 10^6$)			
(a) per testis	6.98 $\pm$ 2.42	5.77 $\pm$ 0.37	5.17 $\pm$ 0.63
(b) per g testis	3.73 $\pm$ 0.29	3.28 $\pm$ 0.10	3.11 $\pm$ 0.29
Testicular sperm reserves ($\times 10^9$)			
(a) per testis	1.42 $\pm$ 0.41	1.46 $\pm$ 0.17	1.16 $\pm$ 0.12
(b) per g testis	0.84 $\pm$ 0.19	0.94 $\pm$ 0.13	0.85 $\pm$ 0.09

a, b: means with different superscripts on the same row differ significantly ($P<0.05$)

* SEM: Standard error of mean

** %: Percent of body weight

diets were unaffected by the toxin. It is known that the kinetics of spermatogenesis is species specific (Swierstra and Foote, 1963). Therefore, contrary to the gross degenerative changes observed in rats exposed to aflatoxin (Jayaraj *et al.*, 1968), all rabbits in the present study exhibited active spermatogenesis as indicated by the active divisions of the spermatocytes as well as the normal cellular associations in the seminiferous tubules. Also that the first four stages of the seminiferous epithelial cycle (Table 1) constituted over 50% of the cycle length, which agreed with the report of Egbunike *et al.* (1985), is an indication that the kinetics of spermatogenesis and hence sperm production were not impaired by the dietary fumonisin levels in the trial since sperm production rates are determined by the kinetics of spermatogenesis as reflected by the seminiferous epithelial cycle. This arose from the significant decrease and increase in the durations of stages II and VIII, respectively with increased dietary fumonisin levels indicating that spermatid and spermatocytes maturation are enhanced and delayed, respectively. However, daily sperm

production tended to decrease with increased dietary fumonisin.

Finn (1963) whose results indicated that the male influence on litter size was a characteristic of the males and was not due to the male-female interaction had studied the influence of the male on litter size. In the present study, the micro doses of the toxin which the male rabbits were exposed to failed to influence conception rate, litter size, embryo normalcy and mortality. The results corroborate the report of Voss *et al.* (1996), that no reproductive effects were observed in males and females exposed to about 3-4mg fumonisin B₁/kg body weight /day prior to and during mating, gestational and lactational phases of the study. Also, no developmental effects were found in the foetus during any phase of the study.

That these rabbits did not suffer adversely from the dietary toxin exposure may have been due to their nutritional status since malnutrition has been claimed to enhance the susceptibility of animals to lower concentrations of food-borne mycotoxins such as fumonisin (Nelson *et al.*, 1993) and so affect their reproductive efficiency

Table 2: *Effects of dietary fumonisin on the fertility of rabbits (Mean $\pm$ SEM*)*

<i>Parameters</i>	<i>Dietary fumonisin level</i>		
	<i>Diet 1 (Low)</i>	<i>Diet 2 (Medium)</i>	<i>Diet 3 (High)</i>
Ovulation rate	14.33 $\pm$ 1.30	11.06 $\pm$ 1.28	12.17 $\pm$ 1.10
Conception rate (%)	20.00 $\pm$ 12.25	10.00 $\pm$ 10.00	40.00 $\pm$ 18.00
Litter size	2.00 $\pm$ 1.26	0.80 $\pm$ 0.08	5.20 $\pm$ 2.18
Embryo survival rate (%)	50.00 $\pm$ 5.00	25.00 $\pm$ 0.01	69.63 $\pm$ 2.50
Foetal CR** length (cm)	2.70 $\pm$ 0.003	4.70 $\pm$ 0.004	3.34 $\pm$ 0.14

* SEM: Standard error of mean

**CR: Crown-rump.

(Panda *et al.*, 1970). Similarly, the dose and period of exposure may have been accountable for the failure of the toxin to influence all the parameters evaluated, as time-and dose -dependent embryopathic and embryocidal effects of fumonisin B₁ injected into fertile chicken eggs have been reported (Javed *et al.*, 1993).

Conclusion

Rabbits mistakenly fed for a short time with rich diet lightly contaminated by *Fusarium verticillioides* (which would produce about 153 µg of fumonisin per kg body weight) may not suffer any loss in reproductive efficiency. An investigation of the effects of the dietary fumonisin over a longer period will be necessary in order to evaluate the amount of damage, if any, and the rate of recovery thereafter.

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