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Reproductive Performance of Rabbits Fed Maize-Based Diets Containing FB_1 Strain of Fusarium Verticillioides (Sacc.)

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Abstract
Thirty sexually matured mixed breeds of bucks (1.35 to 1.37kg in weight) were fed maize-based diets containing mycotoxin (FB_1) produce by Fusarium verticillioides (Sacc.) at levels of 1.70mg/kg diet (Treatment 1), 1. 80 mg/kg diet (Treatment 2) and 1.90 mg/kg diet (Treatment 3) for a period of eight weeks. At the end of the feeding trial, five bucks were randomly selected from each treatment and were mated at a ratio of 1:2 to thirty sexually matured mixed breeds of dry-does (weighing between 1.55 and 1.64 kg) fed diets containing 0.48 mg/kg diet (Treatment 4). The bucks were sacrificed after five days of mating and the testes carefully collected and processed for quantitative testicular histology. By the end of the first trimester, the does were also sacrificed and their uteri carefully dissected. The results showed no significant ($p>0.05$) differences in the testes weight, testicular elements and daily sperm production rates between the dietary treatments. There were also no significant ($p>0.05$) differences in the fertility rate, litter size and foetal crown-rump length among the treatment groups. And there were no foetal mortality in utero. These findings suggested that fumonisin B_1 at approximately 1.90mg/kg diet does not impair reproductive performance in rabbits.

***Keywords**: reproductive performance, FB_1, spermaticidal, embryotoxic.*
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Introduction

Fusarium verticillioides (Sacc.) is one of the most common fungi associated with crops worldwide particularly maize. According to Visconti and Doko (1994), fumonisin B_1 (FB1) is the most prevalent of at least fifteen identified members of fumonisins. They are mycotoxins produced by *F. verticillioides* and a significant accumulation of them in maize occurs when the weather conditions favour fusarium kernel rot (De Leon and Pandey, 1989). They are known to be heat stable, light stable, water soluble, poorly absorbed, metabolized and rapidly excreted by animals. Thus, most of them would eventually end up being recycled into the environment in a manner that will concentrate its spatial distribution. Hence, the amount that enters the environment may be quite large (WHO, 2000). Although there are some evidences that fumonisins can be metabolized by some micro-organisms yet, little is known

about the environmental fate of them after they are either excreted or processed (Duvick *et al,* 1998).

Maize is the only commodity that contains significant amount of fumonisins and is consumed either directly or processed into products for human or animal consumption.

Dry milling of the maize results in the distribution of FB_1 into the bran, germ and flour (Bullerman and Tsai, 1994). According to WHO (2000), FB_1 levels in animal feedstuff could be exceptionally high reaching maximum value of 2mg/kg feedstuff in Asia. Whereas in foodstuffs, the highest FB_1 level was 16mg/kg foodstuff in Europe and a minimum of 0.7mg/kg foodstuff in Latin America. More interestingly FB_1 may be present in beer where maize was used as a wort additive (Scott *et al.*, 1995).

Prelusky *et al.* (1996a) reported that FB_1 was hepatotoxic in all the animal species tested but nephrotoxic in swine, rats, rabbits, mice and ruminants. However, FB_1 has not been reported in milk, meat or eggs from animals fed grains containing FB_1 at levels that would not affect the health of the animals (Scott *et al.,* 1994.). Human exposure estimates for the USA, Canada, Switzerland, Netherlands and Africa ranged from 0. 017 to 44 µg per kg body weight per day (CSMPB, 1996a, b). But there are no confirmed records of acute fumonisins toxicity in humans as well as no data on occupation inhalation exposure so far (WHO, 2000). Meanwhile available correlation studies from South Africa and China suggest a link between dietary fumonisin exposure and oesophageal cancer (IARC, 1993). Similarly, a study has reported a disease outbreak characterized by abdominal pains, borborygmi and diarrhoea in India, suspected to be associated with food-borne fungal infection (Bhat *et al.,* 1997)

Concern about the reproductive and developmental effects of FB_1 on animals including humans, gained attention when Harrison *et al.* (1990) observed abortions in pregnant sows fed fumonisin-contaminated diets and when Hendricks (1999) opined that a cluster of birth defects among residents in Brownsville (Texas) might be associated with the consumption of maize. Even at that, there are still very scanty or no data to support the conclusion that consumption of FB_1 has reproductive toxicity, embryo toxicity or crosses the placenta to the foetuses or resulted in the transfer to chicken eggs or has neonatal effects (Vudathala *et al.,* 1994).

Besides, there is little or no awareness of the prevalence of FB_1 in maize grown in the tropics by most Nigerians. This warranted the present investigatory study to examine the effects of maize-based diets containing FB_1 on the reproductive performance of rabbits.

Materials and Methods

Yellow maize acquired from the Grains Unit, International Institute of Tropical Agriculture (IITA), Ibadan, was inoculated with *F. verticillioides* and cultured according to the procedures of Gelderblom *et al.* (1988), Bezuidenhout *et al*. (1988)

and Laurent *et al,* (1989a) in the Mycotoxin Laboratory, Pathology Unit ,IITA, Ibadan. After the incubation period, the maize was ground to a size of less than 2mm (Visconti and Boenke, 1995) and the FB_1 yield was determined according to the procedures of Sydenham and Shepherd, (1996). Uninfected yellow maize was also ground and mixed proportionally with the infected yellow maize to obtain the required FB_1 concentrations per treatment diet. Four experimental dietary levels containing 1.70mg FB_1/kg diet (Treatment 1), 1.80 mg FB_1/kg diet (Treatment 2), 1.90mg FB_1/kg diet (Treatment 3) and 0.48mg FB_1/kg diet (Treatment 4) were formulated and compounded on weekly basis in accordance with Lang, (1981) dietary recommendation for resting adult rabbits.

In the feeding trial conducted in the rabbitory unit of the Teaching and Research Farm, University of Ibadan, Ibadan (7^0 3'N, 3^0 54'E; relative humidity of 80-85%; 200m above sea level and day time temperature of 25-28^0 C between September and November). Thirty sexually matured mixed breeds of bucks weighing between 1.35 and 1.37kg were randomly allotted to treatment 1, 2 and 3 in a completely randomized design such that each treatment has ten replicates. Thirty sexually matured mixed breeds of dry-does weighing between 1.55 and 1.64kg were all in treatment 4. The animals procured from the rabbitory unit of the Institute of Agricultural Research and Training (IAR&T), Ibadan, were treated against ecto- and endo-parasites, followed by administration of antistress; allowed two weeks of adaptation and then housed in pairs in standard hutches. During the eight-week feeding trial, the animals were fed the compounded diets *ad libitum* and clean fresh water was supplied regularly.

At the end of the experimental period, five bucks were randomly selected from treatments 1, 2 and 3 and mated to the dry-does in a ratio of one buck to two does, morning and evening for a day. The bucks were sacrificed after five days of mating and the testes carefully collected and weighed. The right testes were immediately sampled, fixed in aqueous Boun's fixative and processed as reported by Carlton, (1980) for quantitative testicular histology. The testicular elements were determined microscopically as prescribed by Ortavant, (1959), while the daily sperm production rates were determined as outlined by Swierstra, (1966). By the end of the first trimester, the does were equally sacrificed and their uteri carefully dissected longitudinally to check for conception (fertility rate), count the foetuses therein (litter size), measure the crown-rump length of the foetuses (foetal developmental abnormality) and to check for mummification (foetal mortality rate).

The data so collected were subjected to analysis of variance using Statistical Analysis Software (SAS, 1999) and the treatment means were separated using Duncan's Multiple Range Test of the same software.

Results and Discussion

Spermatogenesis in bucks

Table 1 shows the testes weight and testicular elements of bucks fed maize-based diets containing FB_1. The mean values of the paired testes weight, relative paired testes weight and the testicular elements (i.e, spermatogonia, primary & secondary spermatocytes, round and elongated spermatids and spermatozoa) obtained for bucks in the three treatment diets were not significantly different ($p>0.05$) from one another. Also sperm production rates estimated in the present study were not significantly different ($p>0.05$) among the treatment means. However, they were greater than the standards (24×10^6/gram testes and 350×10^6/testis respectively) estimated for medium-size breed of rabbits (Hafez, 1970). The apparently higher values obtained could have resulted from the estimation procedures adopted, climatic variability, mating frequency and age of the bucks.

Table 1: Spermatogenic cycle of Bucks fed maize-based diets containing FB_1.

Parameters	Treatments (mean ± SEM)		
Mycotixin Conc. (ppm FB_1/kg diet)	Diet 1 (1.7)	Diet 2 (1.8)	Diet 3 (1.9)
Paired tastes weight (g)	3.06 ± 0.31	3.26 ± 0.25	2. 94 ± 0.23
Relative paired testes weight (g)	0.19 ± 0.02	0.20 ± 0.01	0.19 ± 0.01
Spermatogonia (%)	12.99 ± 1. 76	18.05 ± 2.18	14.07 ± 1.92
1^0 Spermatocytes (%)	17.41 ± 1.06	18.36 ± 2.04	20.68 ± 2.47
2^0 Spermatocytes (%)	24.50 ± 2.21	23.79 ± 1.43	22.49 ± 1.00
Round spermatids (%)	10.41 ± 0.90	9.46 ± 1.05	11.10 ± 1.62
Elongated spermatids (%)	9.23 ± 0.80	8.92 ± 0.01	9.85 ± 1.44
Spermatozoa (%)	9.09 ± 1.60	9.79 ± 1.05	10.76 ± 1.78
DSP/gram testis ($x10^6$)	373.00 ± 29.00	328.00 ± 10.00	311.00 ± 29.00
DSP/testis ($x10^6$)	698.00 ± 42.00	577.00 ± 37.00	517.00 ± 63.00

SEM: Standard error of the means; DSP: Daily sperm production

This suggest that there were no spermaticidal effects of FB_1 in the treated bucks, probably due to the ability of most animals to rapidly eliminate FB_1 from their systems especially when orally dosed at micro-doses for a relatively short period (Hopmans *et al*., 1997). The results conformed to the findings of Floss *et al.* (1994a), Voss *et al.* (1996a) and the report of WHO, (2000) that FB_1 has no reproductive toxicity or developmental effects on animals, particularly at micro-doses that would not ordinarily affect the health of the animals. However, daily sperm production per gram testis and per testis decreased with increase in dietary fumonisin, which could be an indication of mild developmental effects on the bucks' spermatogenesis.

Breeding of rabbits

The reproductive performance of bucks mated to dry-does fed maize-based diet containing micro-dose of FB_1 is shown in Table 2. There were no significant

differences ($p>0.05$) in the mean fertility rate, litter size, foetal crown-rump length among the three treatment groups and there were no foetal mortality *in utero*.

Table 2: Reproductive performance of Bucks mated to Dry-does fed maize-based diets containing micro-dose of FB_1.

Parameters	Treatments (mean ± SEM)		
Mycotoxin Conc. (ppm FB_1/kg diet)	Diet 1(1.7)	Diet 2 (1.8)	Diet 3 (1.9)
Fertility rate (%)	20.00	10. 000	40.00
Litter size	2.00 ± 1.26	0.80 ± 0.80	5.20 ± 2.18
Foetal crown-rump length (cm)	2.70 ± 0.00	4.70 ± 0.00	3.34 ± 0.14
Foetal mortality rate (%)	0.00	0.00	0.00

SEM: Standard error of the means.

The occurrence of conception and the absence of mummification recorded in this study are clear indications that there was no reproductive toxicity of FB_1 on the rabbits. This could be attributed largely to the unaffected high sperm production potentials of the treated bucks resulting in pregnancy and possibly may be due to the placenta barrier characteristics between the foetuses and the dams that prevented *in utero* foetal mortality (mummification). It could also be partly due to the dams' ability to have partially hydrolyzed FB_1 (Prelusky *et al.*, 1996b). These findings corroborate the reports of Gross *et al.* (1994), LaBorde *et al.*, (1997) and Collins *et al.* (1998a) that FB_1 has no reproductive toxicity, embryotoxicity and teratogenic effects on animals, mostly when dosed orally at micro-doses.

Conclusion

The findings in this present study indicated that fumonisin B_1 at approximately 1.90mg/kg diet seems apparently not to have spermaticidal and embryotoxic effects on rabbits. There was no evidence of FB_1 crossing the placenta to cause developmental abnormalities in the foetuses examined. However, there might have been some damages on the physiological status as reflected in the daily sperm production and possibly, gradual accumulation of FB_1 in the carcasses which might in turns, pose residual health hazard to humans when consumed.

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