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FERTILITY POTENTIAL OF RABBIT BUCKS FED MAIZE-BASED DIETS CONTAINING GRADED LEVELS OF FUMONISIN B_1

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Abstract: Fumonisin B_1-contaminated maize-based diets have been reported to be mycotoxic in animal species, yet more validated data and biomarkers are needed. In this study, Fumonisin B_1 (FB_1) infected yellow-maize was used to formulate Diets 1, 2 and 3, containing 1700, 1800 and 1900 μg FB_1/kg diet, respectively. Sixty sexually matured bucks and does were used, but only the bucks were fed the FB_1-contaminated diets for 8 wk. At the end of the feeding trial, the treated bucks were mated to the dry does that were fed FB_1-free yellow maize-based diet. Effects on testis and live weight, feed utilisation, conception rate, embryo development and spermatozoa production per gram testis were monitored. Results indicated significant depression ($P<0.05$) in feed intake, from 546.77±12.09 in Diet 1 to 509.84±21.98 g/wk in Diet 3. Weight gain was drastically reduced ($P<0.05$) from 34.13±9.32 in Diet 1 to 20.38±22.13 g/wk in Diet 3. Meanwhile, some of the untreated does were pregnant in all the treatments, indicating that FB_1 concentration at 2.0 mg/kg diet may not be spermaticidal and there were no abnormalities in the embryos. It was observed that the paired testis weight value in Diet 1 (3.06±0.31 g) was not significantly different ($P>0.05$) from that of Diet 3 (2.94±0.23 g). The testicular elements were distorted by the dietary FB_1 but did not follow a definite pattern. Consequently, FB_1 concentration <2.0 mg/kg diet may not affect the fertility potentials of bucks orally dosed for a relatively short period. This observation further elucidates earlier discoveries that FB_1 is not a reproductive toxicant.

Key Words: rabbit, fumonisin B_1, feeding trial, reproductive toxicant, spermatozoa production, testicular elements.

INTRODUCTION

The choice of rabbit keeping, especially as a laboratory animal compared to other animal species, is perhaps due to its high reproductive potential. This includes early onset of spermatogenesis at 40-50 d of age and ability of the buck to mate at least once in a day when fully matured (Somade, 1985; Lebas *et al.,* 1986). According to Hafez (1970), a medium-sized buck could ejaculate $170×10^6$ sperm cells production per day and $24×10^6$ sperm cells production per gram testis. However, these special attributes and overwhelming reproductive potentials could be affected by variations in testis weight, sexual activity, hormonal imbalance and mycotoxicity (Hendricks, 1999; Egbunike, 1979).

Currently, studies have identified about 300 types of mycotoxins that have been reported to cause mycotoxicoses in livestock and primates, but scientists' attention has only been drawn to those that can produce toxins in agricultural commodities with potential deleterious effects on human

health (Guerzoni, 2008; WHO, 2000). Interestingly, maize has been discovered to be the only commodity that naturally contains significant amounts of Fumonisin B_1 (FB_1) both in the field and storage. It is believed that maize kernels could be infected with *Fusarium verticillioides* before or after harvest. Since maize is an important staple food for man and a feed ingredient for livestock, there is the possibility for FB_1 to be naturally ubiquitous in foods and feeds (Sanchis *et al.,* 1995; Bullerman and Tsai, 1994). Recently, 60% of 5 211 samples of maize-based food and feed collected worldwide were found to be contaminated with FB_1. Least concentrations of 0.7-2.0 mg/kg were reported to be detected in both visibly damaged and undamaged maize kernels, including those that appeared seemingly healthy in storage (WHO, 2000).

FB_1 has been reported to be hepatotoxic, nephrotoxic and is known to cause reproductive defects in all the animal species tested (Gelderblom *et al.,* 1994). Furthermore, Harrison *et al.* (1990) observed abortions in pregnant sows fed with fumonisin-contaminated diets and (Hendricks, 1999) speculated that a cluster of birth defects might be associated with the consumption of maize believed to be infected with fumonisins. In contrast to these reports, LaBorde *et al.* (1997) stated that there were insufficient data to support the conclusion that FB_1 is a reproductive toxicant when consumed by farm animals or humans. Against this backdrop, WHO (2000) suggested areas for further research, hence the present study was conducted to monitor effects of dietary FB_1 on live weight, feed utilisation, testis weight, conception rate, embryo development and spermatozoa production per gram testis. Essentially, this will provide basic information on reproductive toxicity potentials of dietary FB_1 in animals including humans.

MATERIAL AND METHODS

Experimental site

The feeding trial was conducted between September and November on the University of Ibadan Teaching and Research Farm, located at latitude 7°3'N, longitude 3°54'E and 200 m above sea level, with relative humidity of 80-85% and daytime temperature of 25-28°C. The laboratory analyses were carried out at the University of Ibadan's Department of Animal Science Animal Physiology Laboratory, as well as the International Livestock Research Institute (ILRI) Analytical Laboratory at the International Institute of Tropical Agriculture (IITA), Ibadan.

Experimental diets

One hundred kilograms of yellow maize (*Zea mays*) was acquired from IITA, Ibadan prior to starting the feeding trial. Although the natural FB_1 concentration was not determined, some of the yellow maize grain was inoculated with *F. verticillioides* and cultured in the Plant Pathology Laboratory, IITA, Ibadan, in accordance with Gelderblom *et al.* (1992) and Nelson and Ross (1992) methods and the remainder was autoclaved. The infected maize was ground and substituted for autoclaved non-cultured yellow maize in various proportions to formulate the diets (Table 1). Samples of the diets were analysed using Fumonisin Quantitative Test Kits (Neorgen Corp., USA®) adopting enzyme-linked immunosorbent assay procedure described by Pestka *et al.* (1994). This process was repeatedly carried out until approximately 1 700, 1 800 and 1 900 µg FB_1/kg diet were obtained to represent Diets 1, 2 and 3 respectively. The graded levels used in the present study followed the trend of less than 2 mg total fumonisins/kg diet, reported by the WHO (2000) to be the least natural occurrence detected in some maize-based food and feed samples collected worldwide.

Management of experimental animals

Sixty sexually matured resting bucks and dry does of non-descript breeds of around 20-24 wk of age were procured from the Rabbit Unit, Institute of Agricultural Research and Training (IAR&T), Ibadan. After 2 wk of acclimatisation, 30 bucks weighing between 1.35 and 1.36 kg were randomly allotted to the 3 diets with 10 replicates each in a completely randomised design. The bucks were paired in standard hutches under good hygiene, offered the experimental diets and clean drinking water *ad libitum* for 8 wk. The dry does were kept in multipurpose hutches and fed with autoclaved non-cultured yellow maize-based diet, formulated to satisfy their nutrient requirements prior to mating with the treated bucks. The diet was later adjusted to suit their physiological needs during pregnancy as recommended by NRC (1998) and Lang (1981a).

Data collection and analysis

Feed intake (dry matter intake) was derived from the differences in feed offered and the left over which was recorded weekly. During the 7th wk of feeding trial, faeces (dry matter output) from the paired bucks were collected, mixed thoroughly and weighed on a weekly basis. Aliquots (10%) of the diets and faecal samples were taken for apparent nutrient digestibility for dry matter, crude protein, crude fiber, neutral detergent fiber, acid detergent fibre, acid detergent lignin and organic matter. Feeds and faeces were analysed according to AOAC methods (2006). The differences in initial and final weights represented weight gain and was recorded every week.

Experimental diets composition

Gross composition and proximate analysis of the experimental diets fed to resting rabbit bucks are presented in Table 1. The FB_1 screening revealed approximately 1 700, 1 800 and 1 900 µg/kg diet for diets 1, 2 and 3 respectively. The same ingredients at the same inclusion levels were used to formulate the diets. The proximate analysis indicated 13% crude protein, 14% crude fibre and gross energy of 2 225 kcal/kg diet. This concurred with the recommendations of NRC (1998) and Lang (1981b) for adult resting rabbits.

At the end of the 8-wk feeding trial, 5 bucks were picked randomly from each treatment and hand-mated to 10 untreated dry does per treatment group in the morning and evening for a day in 1 buck/2 does ratio. After 10 d of mating, all the 30 mated does were sacrificed and

Table 1: Ingredients and chemical composition of the experimental diets of rabbit bucks.

	Diet 1	Diet 2	Diet 3
Determined composition (g/kg)			
Dry matter	878	880	913
Ash	96	111	108
Crude protein	132	132	132
Crude fibre	140	140	140
Hemicelluloses[1]	394	360	338
Cellulose[2]	128	118	136
Gross energy (kcal/kg)	2225	2225	2225
Total FB_1 (µg/kg diet)	1690	1820	1910

Gross composition of 1000 g for diets 1 to 3 (ingredients g/kg); Yellow maize: 250, Wheat offer: 250, Brewer's dried grain: 100, Rice husk: 300, Soybean meal: 50, Fish meal: 25, Bone ash: 20, Premix: 5.
[1] Hemicellulose calculated as neutral detergent fibre – acid detergent fibre.
[2] Cellulose calculated as acid detergent fibre – acid detergent lignin.

their uteri carefully dissected longitudinally to check for conception as well as to count life and dead embryo(s) therein. To determine embryonal resorption, the *corpora lutea* in the ovaries were carefully counted using a hand lens. Crown-rump-length of the embryos was estimated by measuring between the forehead and the base of the tail using a measuring tape (Butterfly®).

Immediately after mating, all the bucks were sacrificed and their testes were carefully collected and weighed; testes volume was determined by Archimedes' principle of water displacement. The right testes were sampled, fixed in aqueous Bouin's fixative for 24 h, dehydrated for 1 h each in graded levels of ethyl alcohol, cleared in chloroform, embedded in paraffin wax and sectioned with microtome at 7 µm thick. The slides were stained with haematoxylin-eosin for histopathological examinations as described by Culling (1974). The seminiferous tubular diameter, volume percent of testicular elements and seminiferous epithelial cycle were determined microscopically using a 25-point ocular graticule at 800 times magnification using oil immersion as reported by Ortavant (1959). The spermatozoa production per gram testis was determined as outlined by Swiestra (1966), assuming a shrinkage value of 47.6%, a volume percent of round spermatid nuclei of 6.25 and a life span of round spermatids of 1.25 d in this study. The data sets collected were subjected to statistical analysis of variance according to SAS (1999) and the mean values were separated using Duncan's multiple range testing procedure from the same software.

RESULTS AND DISCUSSION

Feed utilisation by the experimental animals

Feed utilisation by the rabbit bucks fed graded levels of FB_1 is shown in Table 2. Mean feed intake values ranged from 509.84 to 546.77 g/wk, while mean weight gain varied from 20.38 to 34.13 g/wk. It was found that an increase in dietary FB_1 level significantly ($P<0.05$) depressed feed intake and weight gain. Apparent digestibility coefficients for the neutral detergent fibre values varied from 50.93 to 53.84% and 61.28 to 63.73% nitrogen retention values, but they were not statistically influenced ($P>0.05$) by FB_1. These results indicated that when dietary FB_1 was increased from 1.7 to 1.9 mg/kg, appetite and weight gain were significantly reduced, in a similar way to the reports on rabbits exposed to high doses of FB_1 intravenously (Gumprecht *et al.,* 1995) and via enteral feeding (Bucci *et al.*, 1996). These works observed how FB_1 could inhibit

Table 2: Feed utilisation by rabbit bucks fed graded levels of FB_1.

	Treatments (Mean±standard error)		
FB_1 concentration (µg/kg diet)	Diet 1 (1700)	Diet 2 (1800)	Diet 3 (1900)
No. of bucks	10	10	10
Final live weight (kg)	1.64±0.07	1.58±0.06	1.55±0.06
Weekly feed intake[1] (g)	546.8±12.09[a]	541.0±23.0[ab]	509.8±22.0[b]
Weekly faecal output[2] (g)	249.1±12.9	257.4±11.0	232.8±9.5
Apparent digestibility coefficients (%)			
Nitrogen retention	63.28±0.42	61.28±0.82	63.73±0.36
Ash	19.39±0.07[b]	29.44±0.99[a]	27.63±1.50[a]
Neutral detergent fibre	53.84±0.48	50.93±0.53	51.94±0.97
Acid detergent fibre	17.15±1.02[b]	4.18±2.19[c]	24.91±0.10[a]
Weekly weight gain (g)	34.13±9.32[a]	26.43±10.55[ab]	20.38±22.13[b]

[1] Dry matter intake. [2] Dry matter output.
[a,b,c] :Means in the same row bearing different superscripts differ significantly ($P<0.05$).

some enzymes like sphingomyelinase, ceramide synthase as well as sphingosine kinase and lyase known to cause a variety of alterations in cellular regulations which may led to reduce appetite and growth. The percentage values of nutrient were expressed as their apparent digestibility and both values were within the normal ranges reported for healthy rabbits (Lebas *et al.*, 1986). However, the acid detergent fibre did not follow a particular trend. The marked differences in hemicelluloses values determined for the diets could be largely due to the dry matter content of the experimental diets, which was perhaps influenced by the hygroscopic nature of FB_1(WHO, 2000). In similar studies, Bondy *et al.* (1998) and Gelderblom *et al.* (1994) reported significant depression in body weight and feed consumption in rats.

Mating of the experimental animals

Conception rate and embryonal development in the untreated rabbit does mated with bucks fed graded levels of FB_1 is given in Table 3. No statistical differences (*P*>0.05) were observed in any of the parameters measured among the treatments. No dead embryos were recorded in any of the treatments. The irregularity of these results recorded could possibly be due to the manual mating technique adopted in the study, which did not always guarantee successful mating. More importantly, it could be a reflection that dietary FB_1 doses from 1.7 to 1.9 mg/kg may not distort epididymal functions in rabbit bucks. This was evidenced by the conception rate, litter size, normal embryonal development without dead ones and testis weight as well as high spermatozoa production rate per gram testis among the treatments. The occurrence of pregnancy in some of the untreated does across the treatments, without any dead embryos in any of the untreated does, is proof enough that FB_1 may not remove epididymal functions in rabbit bucks at dietary concentrations of less than 2.0 mg/kg diet. This observation was consistent with earlier findings that fumonisin consumption did not cross the placenta in rabbit does (LaBorde *et al.,* 1997), rats (Collins *et al.,* 1998) and mice (Reddy *et al.,* 1996). Similarly, in Syrian hamsters, Floss *et al.* (1994) observed no maternal toxicity at 0.25 mg FB_1/kg body weight.

Histopathological examination of the experimental animals

Table 4 shows the histopathological examination of testes from rabbit bucks fed graded levels of FB_1. There were no statistical differences (*P*>0.05) in the paired testis weight, which ranged from 2.94 to 3.26 g, relative testes weight (0.19 to 0.20 g), elongated spermatids (8.92 to 9.85%), as well as spermatozoa values of 9.09 to 10.76%. Similarly, values of spermatozoa production per gram testis, seminiferous epithelial stages I and V were not also statistically different (*P*>0.05). Meanwhile, there was no significant decrease in the final live weight from Diet 1 to Diet 3, with

Table 3: Conception rate and embryonal development in untreated rabbit does mated with bucks fed graded levels of FB_1.

	Treatments (Mean±standard error)[1]		
FB_1 concentration (µg/kg diet)	Diet 1 (1700)	Diet 2 (1800)	Diet 3 (1900)
No. of does	10	10	10
Conception rate (%)	20.0±0.82	10.0±0.22	40.0±1.33
Number of embryo per doe	2.0±1.26	0.80±0.80	5.20±2.18
Crown-rump-length (cm)	2.70±0.00	4.70±0.00	3.34±0.14
Embryonal resorption (%)	50.0±1.66	25.0±0.56	30.4±0.92

No statistical differences (*P*>0.05) were observed for any of traits.

Table 4: Histopathological examination of testes from rabbit bucks fed graded levels of FB_1.

FB$_1$ concentration (μg/kg diet)	Treatments (Mean±standard error)		
	Diet 1 (1700)	Diet 2 (1800)	Diet 3 (1900)
No. of bucks	10	10	10
Paired testes weight (g)	3.06±0.31	3.26±0.25	2.94±0.23
Relative testes weight(g)	0.19±0.02	0.20±0.01	0.19±0.01
Seminiferous tubular diameter (μm)	115.9±11.6[b]	125.3±10.5[a]	113.9±8.5[b]
Spermatogonia (%)	12.99±1.76[b]	18.05±2.18[a]	14.07±1.92[b]
Elongated spermatids (%)	9.23±0.80	8.92±1.01	9.85±1.44
Spermatozoa (%)	9.09±1.60	9.79±1.05	10.76±1.78
Seminiferous epithelial I (%)	18.88±4.32	16.89±3.47	17.25±4.73
Seminiferous epithelial III (%)	11.75±3.32[a]	6.55±1.92[b]	11.88±2.37[a]
Seminiferous epithelial V (%)	8.50±0.71	10.11±1.71	8.88±1.66
Seminiferous epithelial VII (%)	7.88±1.97[b]	10.56±1.69[a]	9.13±2.13[a]
Spermatozoa production/g testis ($\times 10^6$)	37.3±29.0	32.8±10.0	31.1±29.0

[a,b]: Means in the same row bearing different superscripts differ significantly ($P<0.05$). SEM: Standard error of mean.

increasing dietary FB_1. This observation might be due to the nondescript breeds suspected to be characterised by low live weight used in the study. The same trend was found in spermatozoa production rate per gram testis values, which dwindled from 37.3×10^6 in Diet 1 to 31.1×10^6 in Diet 3, even superior to the report of 24×10^6 for medium-sized bucks (Hafez, 1970). Although the testis weight and relative testis weight values were higher in Diet 2, with 3.26±0.25 g and 0.20±0.01 g respectively, there was a decrease in spermatozoa production rate per gram testis. This buttressed the observation recorded by Orgebin-Christ (1968) that in rabbits, a decrease in sperm production per unit weight of testes could occur without changes in testes weight.

This observation could be an indication that dietary FB_1 at 2.0 mg/kg diet administered for a longer period could be a developmental toxicant. However, the live weight and testis weight recorded conformed to the estimated normal ranges for healthy rabbits given by Lebas *et al.* (1986) and Hafez (1970). The values of seminiferous tubular diameter, testicular elements and stages of seminiferous epithelial cycle obtained concurred with the values reported for healthy mammals (Clermont, 1972; Courot *et al.,* 1970). This showed that there was no extensive sloughing of the epithelial cells expected to be associated with mycotoxicity (Egbunike, 1979).

It was discovered that the spermatozoa production rate per gram testis in all the treatments were even greater than the value reported to be the least for a healthy rabbit buck (Hafez, 1970). These findings supported the reports by LaBorde *et al.* (1997) in rabbits, Voss *et al.* (1996) in rats and Floss *et al.* (1994) in Syrian hamster that FB_1 is not a developmental or reproductive toxicant. However, the observations contradicted the report of Gross *et al.* (1994) that mice presented maternal toxicity and foetal developmental abnormalities when gavaged with FB_1.

CONCLUSION

The study revealed that increasing dietary FB_1 from 1.7 to 1.9 mg/kg significantly depressed feed intake and body weight gain in rabbit bucks but did not in any way affect the fertility potential. Although there were distortions in the conception rate, number of embryos per doe, crown-rump-length, paired testes weight, seminiferous tubular diameter, testicular elements and seminiferous epithelial stages, there was no definite trend. Also, there was normal spermatozoa

production rate per gram testis in all the treatments as well as normal embryonal growth and development without any dead embryos *in utero*. These results further elucidate the reports from several scientists that FB_1 consumption at micro-doses was not toxic in farm animals. Therefore, FB_1 concentration that is less than 2.0 mg/kg diet may not disrupt the epididymal functions of rabbit bucks, especially when dosed orally for a relatively short exposure.

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