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Response of Yaffa Breeder Cocks to Dietary Fumonisin B_1: Volumetric Proportions of Spermatogenic Elements, Tubular Diameter and Pubertal Age

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Abstract: Sixty pre-pubertal Yaffa breeder cocks of about 16 weeks old were used to evaluate the reproductive effect of fumonisin B_1(FB_1), a toxic secondary metabolite of *Fusarium verticillioides* on pubertal age, tubular diameter and volumetric proportions of spermatogenic elements. The cocks were randomly allotted to diets containing 0.2(control), 5.2, 10.2 and 15.2ppmFB_1 in a 16-weeks feeding trial. At week 22, semen collection was conducted at 48hours interval until the ages at puberty of the cocks were established. At the end of the experiment, half of the right testis of each cock was removed and processed histologically for evaluation of diameter of seminiferous tubules and volumetric proportions of testicular elements. Results showed that cocks that were fed diets containing 0.2, 5.2 and 10.2ppmFB_1 attained puberty two and half ($2^{1/2}$) weeks earlier than cocks that were fed 15.2ppmFB_1. Tubular diameter (130.78µm) of cocks that were fed 0.2ppmFB_1 was significantly ($P < 0.05$) superior to the diameter of 107.38, 104.05 and 103.10µm obtained from cocks that were fed 5.2, 10.2 and 15.2ppmFB_1 respectively. Spermatogonia A and B of cocks that were fed 0.2 and 5.2ppm FB_1 were significantly higher than those of cocks fed 10.2 and 15.2ppmFB_1. Round spermatids, sertoli cells and lumen of cocks fed 15.2ppmFB_1 were significantly impaired. The proportions of basement membrane and cytoplasm in the testes of cocks fed 15.2ppmFB_1 were significantly higher suggesting an impairment of spermatogenic element in the cocks. This study revealed that exposure of cocks to be used for breeding purpose to dietary FB_1 higher than 10.2ppm will impede the reproductive efficiency of the cocks.

Keywords: Fumonisin B_1, Spermatogenic Elements, Tubular Diameter, Pubertal Age, Breeder Cocks

1. Introduction

Fumonisins are mycotoxins produced by fusarium molds, most notably *Fusarium verticilliodes.* These mycotoxins occur as contaminants of agricultural products, particularly maize, worldwide [21, 18]. Shepard *et. al.* [28] reported that maize, which is the major cereal utilized in the formulation of livestock feeds particularly, poultry, is the only commodity that contains significant amounts of fumonisins; therefore, the potential for fumonisins to be found in feeds and feedstuffs is high. Although several naturally occurring fumonisins are known, fumonisin B_1 has been reported to be the most abundant and most toxic [8, 16].

The haematotoxicity, carcinogenicity, hepatotoxicity, mutagenicity, as well as the effects on feed intake and body weight gain of dietary fumonisin in animals have been well documented [23, 14, 11, 12, 9, 5].

Speculations about the adverse effect of fumonisin on reproductive efficiency of animals as earlier reported by Harrison *et. al.*, [11] and Bradlaw *et. al.*, [2] were corroborated by recent observation of reduced sperm per ejaculate and morphological abnormalities in semen of boars fed fumonisin contaminated diets [8], significant reduction in spermatozoa progressive motility, motile sperm per ejaculate and mass activity of breeder cocks fed fumonisin contaminated diets [22] and the report of Ogunlade [21] that gonadal, extra gonadal sperm reserve and daily sperm production were adversely affected in breeder cocks fed dietary fumonisin B_1. More research data are needed to validate or negate the conclusion that consumption of FB_1 has reproductive toxicity. This necessitated the present investigation to assess the effect of dietary fumonisin B_1 on

volumetric proportions of spermatogenic elements, tubular diameter and pubertal age of breeder cocks.

2. Materials and Methods

2.1. Experimental Materials and Operations

Autoclaved maize grains were cultured with a toxigenic strain of *F. verticillioides* (MRC 286) at the Mycotoxin Laboratory of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria to produce FB_1 as described earlier [17]. The ground cultured maize was substituted for ground autoclaved, non-cultured maize in various proportions to formulate four diets containing 0.2, 5.2 and 15.2 mg FB_1/Kg as determined using the fumonisin CD-ELISA test kit (Neorgen Corp., USA) constituting diets 1 (control), 2, 3 and 4 respectively. The diets were isocaloric and isonitrogenous and satisfied the nutritional specifications of breeder cocks [26]. After a 2-week physiological adjustment period, 60 pre-pubertal breeder cocks of about 16 weeks of age sourced from a reputable commercial farm in Abeokuta, Ogun State, Nigeria were randomly allotted to the experimental diets in a completely randomized design such that each experimental diet had 15 breeder cocks replicated thrice with 5 cocks per replicate. The birds were individually housed in previously sanitized cages. The feeding trial was conducted at the Poultry Unit of the Teaching and Research Farm, University of Ibadan, Ibadan, Nigeria ($7^0 20^1$N, $3^0 50^1$E, 200m above sea level with an average day time temperature of 24-25^{0}C and relative humidity of 80-85%) and lasted for 16 weeks. The cocks were fed their respective diets *ad libitum* daily. Table 1 shows the gross composition of the experimental diets fed to the cocks.

2.2. Evaluation of Pubertal Age

At 22 weeks of age, all the breeder cocks in each treatment were subjected to semen collection training as described by Burrows and Quinn [3] and modified by Ogunlade [22]. Semen collection was conducted at 48hours interval for a period of two weeks to establish the exact period that puberty was attained by the cocks. A calibrated micro syringe was used to collect exudates from the papillae of the cocks. The exudate was smeared on a pre-warmed, clean glass slide and examined with a microscope for the presence of spermatozoa. The age at which spermatozoa were observed in the exudate of 50% of cocks in each treatment was considered as pubertal age.

2.3. Evaluation of Tubular Diameter

After the ages at puberty of the cocks were determined, all the breeder cocks were sacrificed. Their testes were carefully dissected and trimmed free of adhering fat and connective tissues. Half of the right testis of cocks in each treatment was taken and fixed in aqueous Bouin's fixative (75ml of picric-acid, 200ml of formalin and 5ml of glacia acetic acid) for 24hours. Following fixation, the testes samples were subjected to further histological processes as described by Rowett [27]. The diameter of seminiferous tubules was determined by observing the vertical and horizontal diameters of randomly selected seminiferous tubules using a light microscope with calibrated eye piece. Twenty seminiferous tubules were observed in each testis sample and the average of the vertical and horizontal diameter of each seminiferous tubule was recorded as the diameter of such tubule. Precaution was taken to ensure that no seminiferous tubule was examined twice.

2.4. Identification of Spermatogenic Elements

The stages of transformations of the round spermatids to free spermatozoa were identified by a combined method of assessments of acrosome development as revealed by PAS technique [13] and by nuclear morphology shown by haematoxyline-eosin stained paraffin embedded tissues [6].

Sperm cells were identified at their different stages of development following their reactions to the staining procedures used. Spermatogonia were identified by their position next to the basement membrane and by their nuclear diameter. The spermatocytes were identified by their nuclear diameters and their positions in the seminiferous epithelium. The spermatids were recognized by the alteration of the shape and position of their nuclei following elongation. Sertoli cells were found closely adjacent of each other sandwiched by developing germ cells.

2.5. Volumetric Proportions of Testicular Elements

The volumetric proportions of testicular elements in the seminiferous epithelium were determined using the methods of Chakley [4]. A twenty-five point ocular graticule (Carl Zeirs, Oberkochen) was used in the estimation. Twenty microscopic fields were observed per slide under light microscope using X10-eye piece and X100 objective lenses (oil immersion).

Table 1. *Gross composition (%) of the experimental diets fed to the breeder cocks.*

Treatments				
Ingredients	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**
	0.2ppm FB_1	**5.2ppm FB_1**	**10.2ppm FB_1**	**15.2ppm FB_1**
Non-Cultured Maize	40.00	38.26	36.52	34.78
Cultured Maize		1.74	3.48	5.22
Soyabean meal	29.20	29.20	29.20	29.20
Soyabean meal	8.00	8.00	8.00	8.00
Fish meal	2.00	2.00	2.00	2.00
Oyster shell	1.00	1.00	1.00	1.00
Salt (NaCl)	0.25	0.25	0.25	0.25

Treatments				
Ingredients	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**
	0.2ppm FB_1	**5.2ppm FB_1**	**10.2ppm FB_1**	**15.2ppm FB_1**
Premix[b]	0.25	0.25	0.25	0.25
Methionine	0.10	0.10	0.100	0.10
Lysine	0.20	0.20	0.20	0.20
Total	100.00	100.00	100.00	100.00
Analysed Nutrients				
Crude Protein (%)	16.68	16.66	16.64	16.51
Crude Fibre (%)	6.52	6.46	6.40	6.38
Met. Energy (Kcal/kg)	2,561.84	2,515.32	2,472.61	2,441.28

[a] Inoculated with Fusarium verticillioides.
[b] To provide per kg of diet: Vit. A (8,000i.u); Vit. D3 (2,000i.u); Vit. E (5 i.u); Vit K (3.2mg); Choline chloride (3,000mg); Folic acid (0.5mg), Mn(56mg); I(1mg); Fe(20mg); Cu(10mg); Zn(50mg); Co(1.25mg); Riboflavin (4.2mg); Vit. B12(0.01mg); Pantothenic acid (5mg); Nicotinic acid(20mg); ppm: Part per million (equivalent of mg/kg).

The following testicular elements were identified and classified accordingly; Spermatogonia A and B, Primary and Secondary spermatocytes, Round and Elongated spermatids, Spermatozoa, Sertolic cells, Basement membrane, Lumen, Interstitial cells and Cytoplasm.

The volume of each element in the testis was estimated using the following formula:

$$\text{Volume of element} = \frac{\text{Total "hit"on the element}}{\text{Total possible "hits"} - \text{number of artifacts "hit"}}$$

3. Results

Figure 1 shows the age at puberty (in days) of breeder cocks fed graded levels of dietary fumonisin B_1. The results revealed that cocks that were fed the control diet attained puberty on the 175th day of age while cocks that were fed 5.2ppm, 10.2ppm and 15.2ppm dietary fumonisin B_1 became pubertal at 179th, 179th and 195th days of age respectively. Cocks fed 0.2 (control), 5.2 and 10.2ppm FB_1 attained puberty two and half weeks earlier than cocks fed 15.2ppm FB_1.

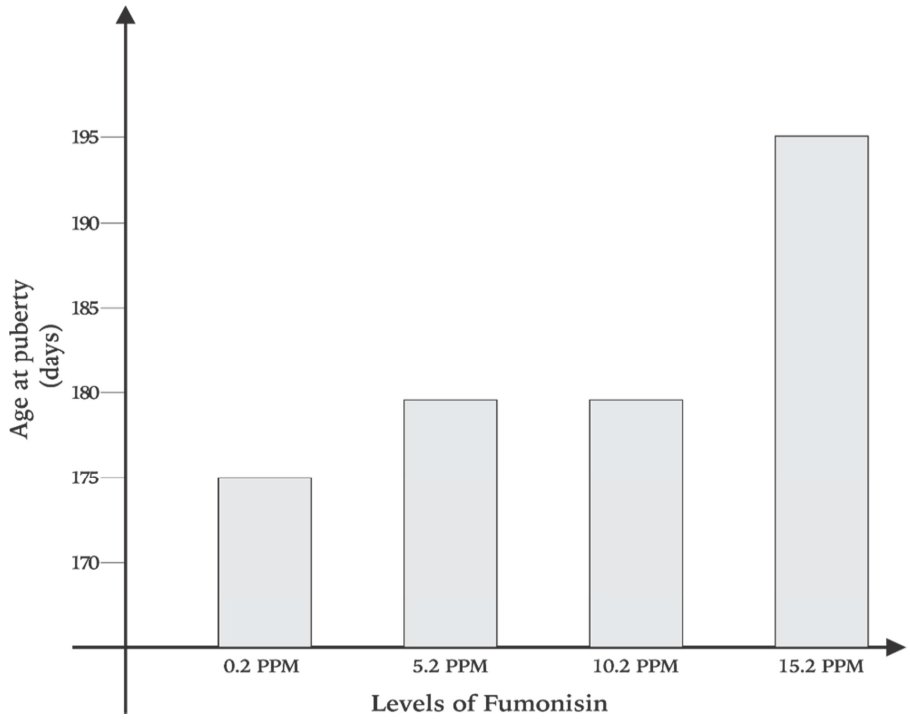

Figure 1. *Age at puberty (days) of breeder cocks fed graded levels of dietary fumonisin B_1.*

Table 2 shows the diameters of seminiferous tubules of breeder cocks fed graded levels of dietary fumonisin. The mean tubular diameter (130.78µm) of the breeder cocks fed the control diet was significantly higher ($P<0.05$) than the diameter of 107.38, 104.05, 103.10µm obtained from the seminiferous tubules of cocks fed 5.2ppm, 10.2ppm and 15.2ppm respectively.

Table 2. *Effect of graded levels of dietary fumonisin B_1 on the diameter of seminiferous tubules of breeder cocks.*

Treatments					
Parameter	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**SEM**
	0.2PPMFB$_1$	**5.2PPM FB$_1$**	**10.2PPM FB$_1$**	**15.2PPM FB$_1$**	
Diameter of Seminiferous Tubules (µm)	130.78^a	107.38^b	104.05^b	103.10^b	25.31

Values shown on the table are means.
$^{a, b}$: Means differently superscripted across the row are significantly different (P<0.005).
ppm: Part per million (equivalent of mg/kg).
SEM: Standard error of means.

The results of the volumetric proportions of testicular elements of breeder cocks fed graded levels of dietary FB_1 are presented in table 3. Results showed that the proportion of spermatogonia A and B in the testes of cocks fed diets 1 and 2 were statistically similar but significantly (p<.05) superior to those of cocks fed diets 3 and 4. Cocks that were fed diet 4 had the least proportion (1.97 and 1.95% respectively) of both testicular elements. The pattern of influence of dietary FB_1, levels on the round spermatids of the cocks were the same with spermatogonia A and B. The sertoli cells of cocks fed diet 4 were significantly depressed compared with those of cocks fed diets 1, 2 and 3.

Significantly (P<0.05) higher basement membranes were obtained in the testes of cocks fed diets 3 (2.38%) and 4 (2.42%) when compared with the basement membrane of cocks fed diets 1 (1.14%) and 2 (1.52%). The proportion occupied by lumen (5.17%) in the testes of cocks fed diet 1 was significantly higher than those of cocks fed diet 4 (2.53%). The proportion of cytoplasm in the testes of the experimental breeder cocks was significantly higher in the testes of cocks fed diet 4 (23.73%), followed by the cytoplasm of cocks on diet 3 (16.74%) while statistically similar values of 8.43% and 6.84% were obtained for cocks fed diets 2 and 1 respectively.

Table 3. *Volumetric Proportions (%) of testicular elements in breeder cocks fed graded levels of dietary fumonisin B_1.*

Treatment					
Elements	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**SEM**
	0.2ppmFB$_1$	**5.22ppmFB$_1$**	**10.22ppmFB$_1$**	**5.22ppmFB$_1$**	
Spermatogonia A	4.18^a	4.01^a	2.92^{ab}	1.97^b	0.80
Spermatogonia B	6.66^a	5.81^a	3.90^b	1.95^c	1.44
Primary Spermatocytes	19.22	20.38	20.19	20.56	13.77
Secondary Spermatocytes	15.21	15.51	15.55	15.80	5.30
Round Spermatids	15.28a	14.94^a	11.53^{ab}	9.39^b	8.05
Elongated Spermatids	14.02	15.50	15.59	15.97	2.95
Spermatozoa	4.33	2.61	2.39	2.33	1.47
Sertoli Cells	5.49^a	4.90^a	4.30^a	1.76^b	1.04
Basement Membrane	1.14^b	1.52	2.38^a	2.42^a	0.19
Lumen	5.17^a	4.09^{ab}	3.04^{ab}	2.53^b	0.98
Interstitial Cells	2.46	2.30	1.58	1.39	1.39
Cytoplasm	6.84^c	8.43^c	16.74^b	23.73^a	3.53

Values shown on the table are means.
$^{a, b, c}$: Means differently superscripted across the rows are significantly different (P<0.05).
SEM: Standard error of means.
ppm: Parts per million (equivalent of mg/kg).

4. Discussion

Pubertal age of the cocks was determined with respect to the presence of mature spermatozoa in the exudate obtained from the distal end of the papillae within the cloaca. Breeder cocks that were fed diet 1 (0.2ppm) attained puberty four days ahead of those fed diets 2 (5.2ppm) and 3 (10.2ppm) and twenty days ahead of cocks fed diet 4. Interestingly, breeder cocks fed diets 2 and 3 became sexually matured on the same day. The delay in attainment of puberty, particularly by cocks fed diet 4(15.2ppm) could be an indication of the possible detrimental effects of fumonisin on reproductive performance of breeder cocks. The results also suggests that dietary fumonisin B_1 beyond the inclusion levels used in this study may have a more pronounced delay in attainment of sexual maturity in breeder cocks. Similar result of the detrimental effects of T-2 toxin on attainment of puberty in bob white quail was reported by Beasley [1]. The report of Ogunlade [22] that the motile sperm/ejaculate and percentage of live spermatozoa of breeder cocks fed 15.2ppm of dietary $FB1_1$ were adversely affected corroborates the results obtained in this study.

Tubular diameter of the breeder cocks ranged from 130.78µm for cocks on diet 1 to 103.10µm for cocks on diet 4. The values of tubular diameter for the respective dietary treatments are correlated to their proportion of lumen and sertoli cells. This correlation may not be unconnected with the fact that sertoli cells serve to convey nutrients and metabolites between the spermatogenic cells and the peri-tubular capillaries [15]. It can be inferred from the observed values that any diet that can influence the proportion of lumen and sertoli cells will influence the size of the

seminiferous tubules.

Since the volumetric proportion of spermatogonia A and B significantly reduced with increased levels of dietary FB_1, it is not unlikely that fumonisin B_1 might have progressively impaired the mitotic divisions and development of both the primordial germ cells and spermatogonia A and B. The significantly low value reported [21] for the daily sperm production of the same beeder cocks fed 15.2ppm dietary fumonisin may be a reflection of the impaired spermatocytogenesis associated with dietary fumonisin. Similar result was reported for rabbits fed fumonisin contaminated diets [24]. Etchu and Egbunike [7] also reported that poultry exudates are very sensitive to dietary changes.

The absence of significant treatment effects on primary and secondary spermatocytes obtained in the study are in agreement with the earlier reports of Nkanga [19], Nkanga and Egbunike [20].

The significant reduction in the proportion of sertoli cells of cocks fed diet 4 suggests that the nourishment provided during the morphological transformation of spermatids during spermiogenesis was impeded which subsequently lowered sperm production. Beasley [1] reported that impairment of spermiogenesis may result from inadequate production or transport of testosterone to the seminiferous tubule or a reduction in sertoli cell numbers necessary for the orchestration of spermiogenesis. The result obtained for basement membrane in this study are contrary to the findings of Gresson and Zlotnik [10] that exerts little or no effect on the proportion of basement membrane in the seminiferous tubules because vascular supply to the germinal epithelium is outside the basement membrane of the seminiferous tubules.

The volumetric proportion of lumen was directly related to those of spermatozoa and sertoli cells. Once the proportion of spermatozoa and sertoli cells is high, there must be a corresponding increase in the proportion of and size of the lumen to accommodate the released spermatozoa.

5. Conclusion

This study has shown that feeding diets containing fumonisin B_1 (FB_1) at a concentration 10.2ppm to cocks intended for breeding purpose for a 4 month period will delay attainment of puberty, significantly reduce the tubular diameters and volumetric proportions of some testicular elements in such cocks, thereby limiting their fertility capacity The results also suggests that dietary fumonisin B_1 beyond the inclusion levels used in this study may have a more pronounced adverse effect on the reproductive potentials of breeder cocks.

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