Research Article **IJAR (2019) 3:22**

International Journal of Animal Research (ISSN:2575-7822)

Gonadal, extra gonadal sperm reserve and daily sperm production of breeder cocks fed graded levels of dietary fumonisin B1

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ABSTRACT

Fumonisin B1 (FB1), a secondary metabolite of the fungus fusarium verticillioides is known to be consumed by farm animals and has been reported to be associated with various farm animal diseases. To account for potential reproductive effects \of fumonisin in cocks meant for breeding purpose, sixty pre-pubertal breeder cocks of about 16 weeks of age were randomly assigned to four diets containing 0.2, 5.2, 10.2 and 15.2mg FB1/ kg constituting diets 1, 2, 3 and 4 respectively. After 16 weeks of feeding trial all the pubertal cocks were sacrificed. Their testes and epididimydes were carefully dissected, removed, weighed and recorded. The left and right organs were homogenized separately. Dietary FB1 did not significantly ($p > 0.05$) influence both the gonadal and extra gonadal weights of the cock. The gonadal sperm reserves (GSR) of cocks fed the control diet (5.54x 107/ testis) was significantly superior ($p<0.05$) to those fed diet 4 (2.66 X 107/ testis). Expressing the GSR on per gram of testis basis, cocks fed with the control diet had a significantly higher value when compared with cocks fed diet 4. The GSR in the left testis both on per testis and gram of testis bases was superior to those of the right testis. The dietary FB1 levels significantly decreased the extra gonadal sperm reserves (ESR) of the cocks which ranged from 4.21X107/epididymis for cocks on diet 1 to 1.33X 107 /epididymis for cocks fed diet 4.

The daily sperm production (DSP) of the cocks both on per testis and per gram testis were significantly reduced as the inclusion levels of dietary FB1 increased. The DSP of cocks fed diet 4 were half the DSP of cocks fed the control diet. The study revealed that cocks intended for breeding purpose should not be exposed to dietary FB1 higher than 10.2 ppm FB1 for optimum reproductive performance.

Keywords: Gonadal sperm reserve, Extragonadal sperm reserve, Daily sperm production, Breeder cocks, Fumonisin.

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How to cite this article:
Ogunlade, Jacob Taiwo.Gonadal, extra gonadal sperm reserve and daily sperm production of breeder cocks fed graded levels of dietary fumonisin B1. International Journal of Animal Research, 2019; 3:22.

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Introduction

The continuous rise in the global demand for animal flesh has necessitated an improved reproductive efficiency of livestock since animal production is mostly dependent on animal reproduction which ensures the multiplication and perpetuation of animal species (Ogunlade, 2015). However, one of the greatest setbacks to achieving improved reproductive efficiency of livestock particularly in the humid tropical condition is the nutritional hazard that livestock are exposed to through an underestimation of the adverse mycotoxin influence on performance and reproductive efficiency of livestock (Egbunike, 1995). Fumonisins are novel mycotoxins produced by Fusarium molds most notably *Fusarium verticillioides.* These mycotoxins occur as contaminants of agricultural products, particularly maize worldwide (Nelson *et al.,* 1991, Gbore, 2009). Maize, which is the major cereal utilized in the formulation of livestock feeds as well as a major dietary staple in several parts of the world, is the only commodity that contains significant amounts of fumonisins (Shephard *et al.,* 1996) hence, the potential for fumonisins to be found in feeds and feed stuffs is high. Most studies on the toxicology of fumonisins have concentrated on fumonisin B1(FB_1) because it has been reported to be the most abundant and most toxic in naturally contaminated foods and feeds followed by fumonisins B2(FB_2) and FB_3 (Murphy *et al.,*1993).

The toxigenic nature of FB_1 on reproduction in pigs(Gbore, 2009), Danicke *et.al.,* 2004, Harrison *et.al.,* 1990), rabbits (Ogunlade *et. al.*, 2006), rats (Flynn *et.al.,* 1996), Syrian hamsters(Floss *et.al.,* 1994), chicken embryos(Javed *et al.,* 1993, Bacon *et al.,* 1995) and human beings (Hendricks,1999) have been well documented. However, little is known about the potential for fumonisins to influence the gonadal and extra gonadal sperm reserves in breeder cocks . Therefore, the present investigation was designed with the aims of evaluating the effects of graded levels of dietary fumonisin B1 on gonadal, extra gonadal and daily sperm production of breeder cocks since reproductive inefficiency is recognized as the most costly and limiting constraint to efficient animal production

Materials and Methods

Experimental site

The experiment was carried out in the Poultry Unit of the Teaching and Research Farm of the University of Ibadan, Ibadan Nigeria. (7^0 20^1N; 3^0 50^1 E; 200m above sea level)

Experimental Feed Ingredient and Diets

Apparently healthy maize grains intended for inoculation with *F. verticillioides* were autoclaved and cultured with a toxigenic strain of *Fusarium verticillioides* (MRC 286) at the Mycotoxin Laboratory of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria to produce FB_1 as described earlier (Nelson *et al.,* 1994).

Four experimental diets were formulated by substituting ground *F. verticillioides* cultured maize for ground autoclaved, non-cultured maize in various proportions to formulate diets containing 0.2, 5.2, 10.2 and 15.2 mg FB_1/kg as determined using the fumonisin quantitative 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 (Oluyemi and Robert, 2000)

Experimental Birds and Design

Sixty 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 (after a two-week physiological adjustment period in a completely randomized design such that each experimental diet had 15 breeders cocks replicated thrice with 5 cocks per replicate. The birds were individually housed in previously sanitized cages and were fed their respective diets *adlibitum* for a period of 16 weeks.

Evaluation of gonadal and extra gonadal sperm reserves.

At the end of the feeding trial, all the breeder cocks were sacrificed and their reproductive system carefully dissected.

The testes and the epididimydes were then carefully trimmed free of adhering fat and connective tissues and weighed. Left and right organs were homogenized separately according to the methods described earlier (Igboeli and Rakha, 1971), while sperm concentrations were determined by direct haemocytometer count after proper dilution in 0.154M Nacl (Egbunike *et al.,* 1975)

Evaluation of Daily Sperm Production (DSP)

The number of late spermatids and spermatozoa in homogenized testes was determined by the haemocytometrical counts. Nkanga (1989) indicated that the actual counts of the maturing spermatids represents 48.25% or 0.4825 of the cycle of the seminiferous epithelium with a duration of 1.93 days. The daily sperm production was therefore calculated with the formula proposed by Aman (1970).

$$\text{DSP} = \frac{\text{Testis sperm count}}{\text{Time divisor}} = \frac{\text{Testis sperm count}}{1.93}$$

Statistical Analysis

The design of this experiment is complete randomized design. Data obtained were subjected to standard statistical analysis using ANOVA procedure of Statistical Analysis Systems Institute (SAS, 1999) while the treatment means were separated using the Duncan's Multiple Range Test of SAS (1999).

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

Ingredients	Treatment			
	Diet 1 0.2ppmFB1	Diet 2 5.2ppmFB1	Diet 3 10.2ppmFB1	Diet 4 15.2ppmFB1
Non-Cultured Maize	40.000	38.26	36.52	34.78
Cultured Maize [a]	-	1.74	3.48	5.22
Wheat Offals	29.20	29.20	29.20	29.20
Soybeans Meal	8.00	8.00	8.00	8.00
Fish Meal	2.00	2.00	2.00	2.00
Palm Kernel Cake	17.00	17.00	17.00	17.00
Bone 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
Premix [b]	0.25	0.25	0.25	0.25
Methionine	0.10	0.10	0.10	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 *Fasarium verticillioides* [b] To provide per kg of diet; Vit A (8,000 i.u);

Results

Table 2 shows the gonadal and extra gonadal sperm reserves of breeder cocks fed graded levels of dietary fumonisin B_1. The gonadal sperm reserve (GSR) of breeders cocks fed the control diet (5.54 X 10^7/t) was significantly higher than the GSR of cocks fed diet 4 (2.66 X 10^7/t). Expressing the GSR on per gram of testis basis, cocks fed the control diet also had a significantly higher value (4.35 X10^6/g) when compared with cocks fed diet 4 (2.18 X10^6/g).

The result also revealed that GSR in the left bases was superior to those of the right testis. testis both on per testis and gram of testis

Table 2: Gonadal and extra gonadal sperm reserves of breeder cocks fed graded levels of dietary fumonisin B1

Parameters	Treatments				
	Diet 1 0.2ppmFB1	Diet 2 5.2ppmFB1	Diet 3 10.2ppmFB1	Diet 4 15.2ppmFB1	SEM
Right epididymis wt (g)	1.78	1.68	1.70	1.59	0.33
Left epididymis wt (g)	1.86	1.71	1.65	1.60	0.32
Right testes wt (g)	12.35	12.06	13.32	12.28	1.79
Left testes wt (g)	12.66	12.55	12.47	12.35	1.76
Gonadal sperm reserves (×107)					
Right (R)/t	2.96^{a}	2.58^{ab}	2.64^{ab}	1.83^{b}	0.32
Left (L)/t	8.12^{a}	5.61^{ab}	5.57^{ab}	3.50^{b}	1.13
Paired (R+L)	11.07^{a}	8.19^{ab}	8.21^{ab}	5.32^{b}	1.37
GSR/t (R+L)/2	5.54^{a}	4.10^{ab}	4.10^{ab}	2.66^{b}	0.69
GSR/gram (×10^{6})					
Right (R)/g	2.54	2.59	2.11	1.50	0.42
Left (L)/g	6.15^{a}	5.30^{ab}	4.45^{ab}	2.86^{b}	0.82
Paired (R+L)	8.69^{a}	7.89^{ab}	6.56^{ab}	4.36^{b}	1.12
GSR/g (R+L)/2	4.35^{a}	3.95^{ab}	3.29^{ab}	2.18^{b}	0.56
Extra Gonadal sperm Reserve (×10^{7})					
Right (R)	3.11^{a}	2.00^{ab}	2.16^{ab}	1.14^{b}	0.36
Left (L)	5.30^{a}	2.38^{b}	2.29^{b}	1.52^{b}	0.64
Paired (R+L)	8.41^{a}	4.38^{b}	4.45^{b}	2.66^{b}	0.68
ESR/Epididymis (R+L)/2	4.21^{a}	2.19^{b}	2.23^{b}	1.33^{b}	0.34
ESR/gram (×10^{6})					
Right (R)/g	2.48	1.80	1.26	0.73	0.57
Left (L)/g	4.58	1.55	1.30	0.94	1.29
Paired (R+L)	7.06	3.35	2.56	1.67	1.78
ESR/epididymis (R+L)/2	3.53	1.68	1.28	0.84	0.89

Values shown on the table are means
SEM: Standard Error of Means
a, b : Means differently superscripted across the rows are significantly different ($p<0.05$)
ESR: Extra gonadal sperm reserve
GSR: Gonadal sperm reserve
g: gram
t: testis
ppm: Part per million (equivalent of mg/kg)

ESR significantly decreased as the inclusion level of FB_1 in the diet increased. Cocks that were fed the control diet had a significantly superior ESR ($8.41X10^7$/epididymis) compared to the cocks fed 15.2 ppm FB_1 ($2.66 X 10^7$/epididymis). Similarly, the ESR values obtained for the cocks on per gram epididymis followed the same trend with ESR per epididymis.

Table 3 shows the daily sperm production (DSP) of breeder cocks fed graded levels of fumonisin B_1. The results revealed that the daily sperm production (per testis and per gram) testis of breeder cocks fed the control diet was significantly higher ($p< 0.05$) than the DSP of breeder cocks fed diet 4 (15.2ppm FB_1). The DSP per gram of right testis apparently decreased with increased levels of dietary fumonisin, however, the values for DSP were not significantly different ($p>0.05$)

Table 3: Daily sperm production of breeder cocks fed graded levels of fumonisin B1

Parameters	Treatments				
	Diet 1	Diet 2	Diet 3	Diet4	SEM
	0.2ppm FB1	5.2ppm FB1	10.2ppm FB1	15.2ppm FB1	
DSP ($\times10^7$)					
Right Testis (R)	1.53^a	1.34^{ab}	1.37^{ab}	0.95^b	0.17
Left Testis (L)	4.21^a	2.92^{ab}	2.89^{ab}	1.81^b	0.58
Paired Testes (R+L)	5.74^a	4.26^{ab}	4.25^{ab}	2.76^b	0.71
DSP/testis (R+L)/2	2.87^a	2.14^{ab}	2.13^{ab}	1.38^b	0.36
DSP per gram ($\times10^6$)					
Right (R)	1.32	1.34	1.10	0.77	0.22
Left (L)	3.19^a	2.75^{ab}	2.31^{ab}	1.46^b	0.43
Paired (R+L)	4.51^a	4.09^{ab}	3.41^{ab}	2.25^b	0.58
DSP/g	2.26^a	2.05^{ab}	1.71^{ab}	1.13^b	0.29

Values shown on the table are means
a,b: Means differently superscripted across the rows are significantly different ($p<0.05$)
ppm: part per million (equivalent to mg/kg)
SEM: Standard Error of Means

Discussion

In this study, significant treatment effects were observed in gonadal and extra gonadal sperm reserve of the experimental cocks. Gonadal sperm reserves (GSR) when expressed on per testis and gram of testis bases was significantly higher in breeder cocks fed diet 1 followed by those fed diets 2,3 and 4 respectively.

These results were an indication that the testes of breeders cocks fed dietary fumonisin B_1 may have progressively suffered testicular degeneration and impaired spermatocytogenesis with a concomitant drop in the rate and efficiency of spermatozoa production. Similar observation was reported by Ogunlade (2015) for cocks, Gbore (2009) for boars, Ogunlade *et al.,* (2006), for rabbits fed graded levels of dietary fumonisin B_1 and Egbunike (1979) for rats injected with micro doses of aflatoxin B_1

Furthermore, the higher GSR values recorded for the left testes when compared with the right testes across the treatments in this study were directly related to the weight of the testes (Ogunlade 2015). These result confirmed the close relation between organ weight and sperm reserves as reported by Ogunlade *et al.,*

(2006), Egbunike and Elemo(1978)and Swierstra(1970).

The distribution of spermatozoa in the three epididymal compartments of the testes across the treatments was significantly influenced. The pattern of influence of the dietary treatments on epididymal sperm reserves followed similar trend with gonadal sperm reserve. This further indicates the negatives impact the dietary FB_1at the levels used in this study had on the ability of the testes to synthesize spermatozoa.

However ESR on per gram of testis basis was not significantly affected by the dietary treatments.

The daily sperm production (DSP) on per testis and gram bases were significantly higher in cocks fed the control diet. This result was a manifestation of the heavier weight of the testes of cocks on the control diet. Testicular weights have been reported to have a high correlation with sperm reserves in the testis or epididymis and therefore a reflection of sperm production. These results are consistent with those of Egbunike and Nkanga (1999) and Ogunlade *et al.,* (2006). The numerical values obtained in this study are superior to those of Cerolini *et al.,* (2003) but differ slightly from those of Surrai *et al.,* (2000) and Maldjan *et al.,* (2002). The variations in values are probably due to differences in species, which agrees with the report of Ganner and Hafez (1993) that every species has its capacity for sperm production which is genetically determined. DSP of the cocks on per gram of testicular parenchyma revealed that sperm production in cocks on diet 1 was almost as twice of cocks on diet 4 suggesting that efficiency of sperm production is not just a function or reflection of testicular weight alone, but also the degree of spermatogenesis. It was observed in this study that cocks fed diets 1 and 2 had more spermatogenetic elements, interstitial cells, sertoli cells and more spermatozoa. Thus, the efficiency of daily sperm production is partly determined by the level of stimulation of the seminiferous tubules by testosterone from interstitial cells.

Conclusion

This study has demonstrated that feeding diets containing fumonisin B_1 (FB_1) as high as 15.2 ppm to cocks intended for breeding puporse will result in significant depression in gonadal and extra gonadal sperm reserve as well as daily sperm production which will impair the fertility capacity of the cocks.

Acknowledgements

I wish to express my gratitude to the members of staff of Mycotoxin Laboratory, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria for the supply of *Fusarium verticillioides* inoculum and the quantification of the toxin in the experimental materials.

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