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Effect of Dietary Fumonisin B_1 on Reproductive Organs and Semen Quality Indices of Breeder Cocks

Ogunlade, Jacob Taiwo
Department of Animal Production and Health Sciences, Faculty of Agricultural Sciences,
Ekiti State University, Ado-Ekiti, Nigeria.
*E-mail of the corresponding author : taiwoogunlade2003@yahoo.com

Abstract
An experiment was conducted to investigate the implications of fumonisin B_1(FB_1), a toxic metabolite of *Fusarium verticillioides* consumed by farm animals, on reproductive organs and semen quality indices of breeder cocks in a sixteen-week feeding trial. 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 15.2 mg FB_1/kg constituting diets 1,2,3 and 4 respectively. At puberty, semen samples were collected from the cocks for three weeks and evaluated. Subsequently, the cocks were killed by decapitation, their reproductive systems carefully removed, weighed and recorded. The absolute and relative weights of the testes, epididymides and tunica albuginea of the cocks were not significantly($p>0.05$) influenced by the dietary FB_1 levels. However, the weights of the left parts of these organs were apparently heavier than those on the right side. The spermatozoa progressive motility, live spermatozoa, motile sperm per ejaculate and mass activity of the semen were significantly($p<0.05$) depressed as the dietary FB_1 levels increased. Dietary FB_1did not significantly influence the ejaculate volume, spermatozoa concentration and total spermatozoa output. The live spermatozoa of cocks fed diets 2,3 and 4 were 90.10, 88.57 and 87.25% of those fed the control diet(1) respectively. The mass activity ranged from very turbulent motion for the cocks on control diet to slow motion for those on diet 4. This study revealed that exposure of cocks to be used for breeding purpose to dietary FB_1 higher than 10.2ppm will impair the fertility capacity of their semen.
Keywords: Fumonisin B_1, Reproductive organs, Semen quality, Fertility.

1. Introduction

Animal production is mostly dependent on animal reproduction which ensures the multiplication of animals and hence, the perpetuation of the species (Ogunlade *et al.,* 2006). As the demand for animal flesh continues to increase, the necessity for increasing the reproductive efficiency of livestock becomes more evident. Unfortunately, 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 (Gbore, 2009).

Among other factors, the quality of ingredients used in ration formulation plays a vital role in the regulation of the reproduction capacity of animals. For effective animal reproduction, optimal production and quality of semen are of great importance especially when artificial insemination is used for poultry breeding programmes. Unfortunately the presence of mycotoxins in the feed and some feedstuffs with their attendant adverse influence on performance and reproductive efficiency of livestock (Egbunike, 1995) is a limiting factor. Petzingher and Weidenbach, (2002) reported that toxins with oestrogenic effects were observed to adversely affect breeding.

Fusarium verticilloides (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 (the major cereal used as an energy source in poultry) is the only commodity that contains significant amounts of fumonisin.

Although, several naturally occurring fumonisins are known, fumonisin B_1 (FB_1) has been reported to be the most abundant and most toxic which represents approximately 70% of the total concentration in naturally contaminated foods and feeds (Murphy *et al.*, 1993)

Available studies have implicated fumonisins to be hepatotoxic, carcinogenic, nephrotoxic, and mutagenic (Harrison *et al.*, 1990; Kellerman *et al.*, 1990; Marasas *et al.*, 1998).

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), the report of Bradlaw *et al.*, (1994) that 1.8µg fumonisin/ml inhibited re-aggregagtion and growth of chicken embryo neura retina cells and the report of Javed *et al.*, (1993) that injection of purified FB_1 into fertile chicken eggs resulted in time-and dose-dependent embryophatic and embryocidal effects.

With the above in mind and the fact that little is known about the potential for fumonisin to influence reproductive performance in cocks, this study was designed to evaluate the effects of graded levels of dietary fumonisin B_1 on the reproductive organs weight and seminal quality in 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 (Nelson *et al*., 1994). The ground cultured maize was substituted for ground autoclaved, non-cultured maize in various proportions to formulate four 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 & Robert, 2000). 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 randomised design such that each experimental diet had 15 breeder cocks replicated thrice with 5 cocks per replicate. The birds were individually housed in previously sanitised 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. *Semen Collection and Evaluation*

At 22 weeks of age, all the cocks in each treatment were subjected to the double hand lumbar massage system of Burrows and Quinn (1937) for the purpose of training cocks for semen collection and to establish the age at puberty for the cocks. Semen collection for analyses started when cocks were 25 weeks old (one week after attaining puberty) and semen samples were collected from each cock at 72 hours interval for a period of three weeks. Ejaculate from each cock was collected with a calibrated micro syringe and the ejaculate volume was read off directly in the calibrated micro syringe to the nearest 0.01ml and recorded. Semen colour was evaluated by visual appraisal directly from the collection syringe under natural light. The colour was rated from creamy white to translucent. The mass activity of the spermatozoa of each cock was determined by placing a drop of undiluted semen of each cock on a clean, pre-warmed (37^0c) glass slide and examined with a microscope, under low power. This was scored according to the intensity of the wave motion, from absence of wave motion (0) to slow motion (+), rapid motion (+ +) and turbulent motion (+ + +) characterised by the appearance of dark prominent wave in a rapid motion. The spermatozoa progressive motility was evaluated as described by Kastelic *et al.*, (2001). Evaluation of semen for spermatozoaconcentration was done according to the method of Zaneveld and Polakoski (1977) and percent live spermatozoa was determined as described by (Gbore, 2009).

2.3. *Testicular and Epididymal Characteristics*

At the end of semen collection and evaluation, all the breeder cocks were sacrificed and their reproductive system carefully dissected. The testes, tunica albuginea and the epididymides were then carefully trimmed free of adhering fat and connective tissues, weighed and recorded. The relative weight of these organs (expressed as percentage of live weight) were determined.

2.4. *Statistical Analysis*

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

3. Results

Table 2 shows the testicular and epididymal characteristics of breeder cocks fed graded level of fumonisin B_1. The results revealed that the testes, epididymides and tunica albuginea weights of the cocks were not significantly ($P>0.05$) influenced by the dietary FB_1 levels. However, the weights of the left testis, left epididymis and left tunica albuginea of the cocks appeared to be superior to the weight of those on the right side across the treatments. The semen characteristics of breeder cocks fed graded levels of dietary FB_1 are shown in Table 3. The spermatozoa progressive motility, percent live spermatozoa, motile sperm per ejaculate and mass activity were significantly ($P<0.05$) altered by the dietary FB_1 levels. The progressive motility decreased significantly as the levels of dietary fumonisin B1 increased. Cocks fed the control diet had the highest spermatozoa motility (67.20%) while those fed diet 4 had the least (61.60%). The percent live spermatozoa of cocks fed diets 2, 3 and 4 were 90.10%, 88.57% and 87.25% of those on the control diet (1) respectively. The mass activity ranged from very turbulent motion for the cocks on the control diet to slow motion for those on diet 4. Number of motile sperm per ejaculate of the cocks decreased significantly as the dietary FB_1 increased. The pattern of decrease followed the same trend with the spermatozoa progressive motility. Semen colour was

not significantly influenced by the dietary FB_1 levels across the treatments. Although the ejaculate volume, spermatozoa concentration and the total spermatozoa output tended to decline as the dietary FB_1 increased, the parameters were not significantly influenced among the treatments.

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

Ingredients	Treatment			
	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[a]	-	1.74	3.48	5.22
Wheat Offals	29.20	29.20	29.20	29.20
Soybean 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
Oyter 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. Energ (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).

Table 2: **Testicular and epididymis characteristics of breeder cocks fed graded levels of dietary FB_1**

Parameters	Treatment				
	Diet 1	Diet 2	Diet 3	Diet 4	SEM
	0.2ppm FB_1	5.2ppm FB_1	10.2ppm FB_1	15.2ppmFB_1	
Live Body Weight (kg)	2.40	2.38	2.44	2.42	90.96
Right Testis Weight (g)	12.35	12.06	13.32	12.28	1.79
Left Testis Weight (g)	12.66	12.55	12.47	12.35	1.76
Paired Testes Weight (g)	25.01	24.61	24.79	24.63	3.50
Rel*. Paired Testes Weight (%)	2.00	2.00	2.00	2.00	0.07
Right Epididymis Weight (g)	1.78	1.68	1.70	1.59	0.33
Left Epididymis Weight (g)	1.86	1.71	1.65	1.60	0.32
Paired Epididymis Weight (g)	3.64	3.39	3.35	3.19	0.62
Rel*. Paired Epididymis Wt. (%)	0.15	0.14	0.14	0.13	0.02
Right Tunica Albuginea Weight (g)	0.70	0.62	0.69	0.53	0.11
Left Tunica Albuginea Weight (g)	0.71	0.64	0.70	0.60	0.30
Paired Tunica Albuginea Weight (g)	1.41	1.26	1.39	1.13	0.09
Rel*. Paired Tunica Albuginea Weight (g)	0.06	0.05	0.06	0.05	0.01

Values shown on the table are means. SEM : Standard Error of Means,
* Relative to Live Weight, ppm: Part per million (equivalent of mg/kg)

4. Discussion

4.1. Reproductive organs' weight

In this study, the live body weight of the cocks, the absolute and relative weight of the testes, epididymides and tunica albuginea of the breeder cocks fed graded levels of dietary FB_1 were not statistically different among the treatments. Since all the cocks used in this investigation were of similar age and breed, the non significant

influence of dietary FB_1 on the weight of the testes, tunica albuginea and epididymides is consistent with the report of Colenbrander and Kemp (1990) that testes size is directly related to age of the animal. Ogunlade *et al.*, (2006) reported that testes weights of rabbits fed fumonisin contaminated diet were not significantly affected regardless of the treatment.

Similarly, Gbore (2009) observed and reported a non-significant difference in the gross and relative weights of the testes and epididymides of the boars exposed to varied levels of FB_1. The paired testes weight of the cocks across the treatments were within the range of normal values (14-60grammes) reported by Hafez (1993) and comparable with those reported for White Rock, Rhode Island Red and White Leghorn by Egbunike and Oluyemi (1979) and Nkanga (1989).

In this study, the absolute weights of the right and left testes, epididymides and tunica albuginea were statistically similar (Clulow and Jones, 1982), however, the left organs (testes, epididymides and tunica albuginea) were observed to be heavier than the right organs across the treatments. Similar observation was reported by Nkanga (1989) for cocks, Egbunike (1998) for boar and Ogunlade *et al.*, (2006) for rabbit fed fumonisin contaminated diet. These results are however contradictory to the report of king and Mc Lelland (1975).

Table 3: **Semen characteristics of breeder cocks fed graded levels of dietary FB_1**

Parameters	**Treatment**				
	Diet 1	**Diet 2**	**Diet 3**	**Diet 4**	**SEM**
	0.2ppm FB_1	**5.2ppm FB_1**	**10.2ppm FB_1**	**1 5.2ppm FB_1**	
Ejaculate Volume (ml)	0.12	0.11	0.11	0.11	0.002
Spermatozoa Progressive Motility (%)	67.20^{a}	65.00^{ab}	62.60^{ab}	61.60^{b}	1.70
Spermatozoa Concentration ($x10^7$/ml)	33.01	30.00	31.00	30.00	1.01
Total Sperm/ejaculate ($x10^6$)	39.50	33.00	33.09	33.04	1.27
Motile Sperm/ejaculate ($x10^6$)	22.24^{a}	22.31^{ab}	21.22^{ab}	20.35^{b}	1.08
Live Spermatozoa (%)	91.00^{a}	82.00^{b}	80.60^{bc}	79.40^{c}	0.48
Semen Colour	Creamy white	Creamy white	Creamy white	Creamy white	
Mass Activity	+ + +	+ +	+ +	+	

a, b, c : Means differently superscripted across the row are significantly($p<0.05$) different. SEM : Standard Error of Means, ppm : Part per million (equivalent of mg/kg)
+ + + : Very turbulent motion; + + : Rapid wave motion; + : Slow wave motion.

4.2. Semen quality indices

The spermatozoa progressive motility decreased significantly with increased levels of dietary FB_1. This result suggests that biochemical and physiological changes might have occured in cocks consuming 15.2ppm dietary FB_1 resulting in the depression of nutritive substances for sperm motility and viability in the seminal fluid. Ogunlade *et al.,* (2004) observed poor digestion, poor absorption and poor utilization of protein in rabbits fed high level of dietary fumonisin. Similar observation was reported for pig by Gbore and Egbunike (2007). However the results obtained for spermatozoa motility in this study were superior to 37.1% reported by Nwakalor *et al.,* (1986) but comparable with 64% motility reported by Ledec *et al.*, (1981) and those reported for local and exotic cocks (Nkanga, 1989). The results were however inferior to 81.67% and 70% reported by (Gbore 2009) for boars fed 0.2 and 5.0 mg FB_1 respectively and those of Egbunike and Nkanga (1999). This may be due to differing susceptibility of both animal species to the mycotoxin as observed by Voss *et al*., (1996.)

The apparent lack of significant difference in the spermatozoa concentration of the experimental cocks across the treatments suggests that they were potentially fertile as their spermatozoa concentration were more than the minimum number of sperm, 100 million (Munro, 1938), 40 – 70 million (Taneja and Gowe, 1962), 25 million (De Reviers and Williams, 1981) required for optimum fertility in natural mating. For artificial insemination programmes and by the recommendation of 25 million spermatozoa (De-Reviers and Williams, 1981) for high level of fertility in chickens, it thus implies that spermatozoa concentration obtained from the cocks in this study will successfully inseminate 13 hens for breeder cocks on diet 1 as against 12 hens for breeder cocks on diet 4. This result further suggested that spermiogenesis was not impaired in the cocks across the treatments. However, Beasley (1999) obtained a significant reduction in spermatozoa concentration of bob white quails fed T-2 toxin treated diet.

Huang and Johnson (1996) established that there is a positive relationship between testicular size and sperm production. It then implies that males with large testes would produce superior ejaculate volume, and total spermatozoa per ejaculate.

The apparent lack of statistical significance in the ejaculate volume and total sperm per ejaculate of

cocks fed 0.2ppm dietary FB_1 which also have the heaviest testes as compared to cocks fed other diets may be occasioned by the failure of fumonisin B_1 to induce any significant depression in the weights and contents of the testes of cocks fed higher levels of dietary FB_1 compared to those fed 0.2ppm FB_1. Although the ejaculate volume obtained for the cocks in this study were considerably lower than the 0.34ml and 0.25ml obtained by Nkanga (1989) for exotic and local breeds of cocks respectively and 0.58ml and 0.80ml reported by Nwagu *et al.,* (1996) for White and Red Rhode Island Red cocks respectively, the ejaculate volume however fell within the range of 0.1 to 1.0ml reported for cocks by Sturkie (1970). Factors such as age, method of semen collection and amount of seminal fluid in the semen as reported by (Ledec *et al.,* 1981) may be responsible for the existing variation in the ejaculate volume in this study and those of others.

The pattern and mode of influence of dietary FB_1 on motile sperm per ejaculate followed the same trend with spermatozoa progressive motility. The significantly lower motile sperm per ejaculate of the cocks fed diet containing 15.2ppm dietary FB_1 may be attributed to the adverse effect of dietary FB_1 on spermatozoa motility. Semen colour was consistent irrespective of dietary FB_1 levels and fell within the normal colour range reported by Sturkie (1970). The slow swirling characteristics in the mass activity of breeder cocks fed 15.2pp dietary FB_1 is directly related to the motility of the spermatozoa and may be fumonisin concentration dependent.

5. Conclusion

This study has shown that dietary FB_1 adversely affected spermatozoa progressive motility, motile sperm per ejaculate and live spermatozoa in cocks. The exposure of cocks to be used for breeding purpose to dietary FB_1 higher than 10.2ppm may result in significant depression in spermatozoa motility and live spermatozoa, which might impair fertility capacity of cocks.

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