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Ameliorative effects of vitamin E on sperm morphological defects of cocks fed fumonisin B_1 contaminated diets

Fumonisin B1 ile kontamine rasyonlarla beslenen horozların sperm morfolojik kusurları üzerine E vitamininin iyileştirici etkileri

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ABSTRACT

The study investigated the ameliorative effect of vitamin E on testicular parameters, semen characteristics and sperm morphology of mature cocks fed Fumonisin B_1 (FB_1) contaminated diets. Twenty four weeks old 160 cocks were used for the 16 weeks experiment. The cocks were assigned to eight experimental diets, six diets contained varying inclusion levels of FB_1 with and without the inclusion of vitamin E. Diets 2, 3, and 4 contained 10, 20, and 30 mg kg^{-1} FB_1 respectively without vitamin E while Diets 6, 7, and 8 contained 10, 20, and 30 mg kg^{-1} FB_1 respectively with 200 mg kg^{-1} vitamin E each. Diet 1 was the control without vitamin E while Diet 5 was the control with vitamin E. Each group was replicated four times with five cocks per replicate in a completely randomized design. There were significant (P<0.05) reductions in the left, right, and paired testicular weights and volumes as well as the paired epididymal weight of the cocks fed diets contaminated with varying levels of FB_1 while the testicular densities were not significantly (P>0.05) influenced. The ejaculate volume, sperm motility, total sperm cells, total motile cells, mass activity, and total live cells of the cocks fed varying levels of FB_1 were equally significantly (P<0.05) reduced. However, significant (P<0.05) increases were observed in the secondary sperm morphological abnormalities such as curved tails, headless tails, and rudimentary tails. Inclusion of vitamin E in the diets significantly (P<0.05) improved the affected parameters, especially, among the cocks fed 10 mg FB_1/kg diet. Therefore, vitamin E is recommended as a feed additive to improve the reproductive potentials of cocks fed FB_1-contaminated diets.

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ÖZ

Bu çalışmada, E vitamini takviyesinin, Fumonisin B1 (FB1) kontamine diyetlerle beslenen erişkin horozların testis parametreleri, semen özellikleri ve sperm morfolojisi üzerindeki iyileştirici etkisi araştırılmıştır. Bu amaçla, 16 hafta süren deneme için 24 haftalık yaştaki 160 horoz kullanılmıştır. Materyal, farklı FB1 düzeyleri içeren altı rasyonun yanında, E vitamini içeren ve içermeyen rasyon olmak üzere sekiz gruba ayrılmışlardır. 2, 3 ve 4 nolu grup, E vitamini içermeyip sırasıyla 10, 20 ve 30 mg kg^{-1} FB1 içerirken, 6, 7 ve 8 nolu grup, 200 mg kg^{-1} E vitamininin yanında sırasıyla 10, 20 ve 30 mg kg^{-1} FB1 içermektedir. 1 nolu grup, E vitamini içermeyen kontrol grubu ve 5 nolu grup E vitamini içerikli kontrol grubudur. Hayvanlar her bir gruba, beş horozdan oluşan dörder tekerrür olacak şekilde şansa bağlı olarak dağıtılmışlardır. İnceleme sonucunda, farklı düzeylerde FB1 kontamine rasyonlarla beslenen horozların sol, sağ ve eşli testis ağırlıkları ve hacimlerinin yanı sıra eşli epididimal ağırlıklarında önemli (P<0.05) azalmalar olduğu, testis yoğunluklarındaki değişimin ise anlamlı olmadığı (P>0.05).) görülmüştür. Ayrıca, farklı seviyelerde FB1 ile beslenen horozların ejakülat hacmi, sperm motilitesi, toplam sperm hücresi, toplam hareketli hücreler, kütle aktivitesi ve toplam canlı hücre sayısı önemli ölçüde azalmıştır (P<0.05). Bununla birlikte, kavisli kuyruk, başsız kuyruk ve ilkel kuyruk gibi ikincil sperm morfolojik anormalliklerinde önemli artışlar gözlenmiştir (P<0.05). Rasyonlara E vitamininin dahil edilmesi, özellikle 10 mg FB1/kg içeren rasyonla beslenen horozlarda, etkilenen parametreleri önemli ölçüde iyileştirmiştir (P<0.05). Bu nedenle, FB1 ile kontamine rasyonlarla beslenen horozların üreme potansiyellerini geliştirmek için yem katkı maddesi olarak E vitamini önerilmektedir.

1. Introduction

The nutritional quality of a feed depends on a variety of factors, including feed presentation, microbial contamination, and content of anti-nutritional factors, digestibility, palatability, and intestinal healthfulness. Common microbial contamination of raw materials, such as maize, used in feed production in the tropics is due to that of fumonisin. These are mycotoxins produced mostly by *Fusarium verticillioides* (Tardieu et al. 2019). The most abundant fumonisin found in high levels and responsible for contamination of human food and animal feed is fumonisin B1 (FB_1). Among many negative effects of FB_1 earlier documented by other authors are haematotoxicity, carcinogenicity, hepatotoxicity, and mutagenicity in farm animals (Gbore 2009; Ogunlade and Egbunike 2013; Ogunlade 2019). The adverse effects of FB_1 on feed intake and body weight gain were equally profound (Ewuola et al. 2008). The reproductive toxicity effect of FB_1 was also emphasized by Gbore (2009) to have resulted in reduced sperm per ejaculate and morphological abnormalities in the semen of boars fed fumonisin contaminated diets. Other semen characteristics used as yardstick reproductive potentials were also reportedly affected by FB_1 contamination in feed. Ogunlade (2015) previously reported a significant reduction in spermatozoa progressive motility, motile sperm per ejaculate, and mass activity of breeder cocks fed fumonisin contaminated diets. Apart from the adverse effect of FB_1 on sperm production and morphology, sexual function and fertility in adult male and female animals are seriously hampered as well as causing developmental toxicity in the offspring. In rabbits, the serum reproductive hormones such as luteinizing hormone, follicle-stimulating hormone, prolactin, prostaglandin F 2α (PGF2α), and estradiol levels were significantly lowered among those fed diets containing 7.5 mg kg^{-1} FB_1 (Gbore and Adu 2017). This was explained to be responsible for the long gestation lengths and lower kit weights recorded among this group when compared to the other treatment groups.

Recent studies have outlined the importance of synthetic antioxidants such as vitamins in protecting the living organism against the toxic effects of mycotoxins (Alpsoy et al. 2009; Adu and Gbore 2015). Vitamin E is one such important dietary antioxidant capable of ameliorating the adverse effect of reactive oxygen species implicated in causing oxidative stress. Vitamin E treatment was also reported to have significantly ameliorated aflatoxin-induced biochemical alterations and lipid peroxidation in the testis of mice (Verma et al. 2001). Increased vitamin E supplementation of poultry males was reported to have significantly increased the α-tocopherol level in semen resulting in increased resistance to oxidative stress imposed by mycotoxins (Surai et al. 2019). In another development, aflatoxin B_1-associated immunotoxic effects in chicks were reportedly mitigated by dietary intake of Vitamin E by the hens (Khan et al. 2014). Another study also showed that vitamin E supplementation in female rabbits' diets counteracted the adverse effects of FB_1 on reproductive hormones, gestation length, kit weight, and milk production (Gbore and Adu 2017). Considering the importance of the quality and wholesomeness of feed in the reproductive potentials of cocks, more studies are required and the outcomes to be documented as baseline and guideline in accepting or rejecting the hypothesis that dietary vitamin E can mitigate the adverse effects of FB_1 on reproductive potentials of domestic cocks.

2. Materials and Methods

2.1. Experimental site and diets

The experimental site was the Poultry Unit, Teaching and Research Farm, the Federal University of Technology Akure, Nigeria. The study was conducted in compliance with the research ethics and guidelines of the Animal Production and Health Department of the institution (FUTA/APH/15/4750). A total of eight (8) experimental diets (Table 1) were constituted with varying inclusion levels of FB_1 and Vit E in the basal diet. Diets 1 to 4 contained 0.00, 10.00, 20.00, and 30.00 mg FB_1 per kg basal diet respectively without vitamin E while other portions of Diets 1 to 4 were further fortified with 200 mg vitamin E each to constitute Diets 5 to 8 respectively. A toxigenic strain of *F. verticillioides* (MRC 826) was sourced from the Plant Pathology Laboratory of the International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria. The fungus isolates were characterized and grown on acidified potato dextrose agar under a controlled environment in an incubator for a period of 7–14 days. After the incubation period, the mycelia were washed into a suspension with distilled water and were used to inoculate the autoclaved maize grains used for this experiment. Samples of homogeneously mixed experimental diets were quantified in replicates for FB_1 using AgraQuant® FUM tests kit (Romer Labs, Inc. USA). AgraQuant® FUM test kits are accurate and reliable enzyme-linked immunosorbent assays (ELISA) in a quantitative format.

2.2. Experimental animals and management

A total of 160 sexually matured Isa Brown cocks of twenty-four (24) weeks old were used for the experiment. The cocks were caged for two weeks before the commencement of the study for stabilization with commercial grower rations fed with fresh and cool water given *ad libitum.* At the expiration of the stabilization period, the cocks were weighed and randomly allotted to the experimental treatments. Each treatment was replicated 4 times with 5 cocks/ replicate in a completely randomized design. The experimental diets and drinking water were provided *ad libitum* throughout the sixteen weeks (16) period of the experiment. All required managerial practices such as strict bio-security measures were ensured as at and when due, appropriate vaccines, and prophylactic treatments were administered. The birds were housed in an open-sided building in a thoroughly cleaned, washed, and disinfected three tier cage system of 32 x 38 x 42 cm dimension. At the end of the feeding trial, four (4) cocks per replicate were selected and fasted overnight for semen evaluation.

2.3. Semen collection and evaluation

Semen was collected between 6 to 8 am by the manual massage technique. Semen samples were assessed within 5 minutes of the collection as described by Olarotimi and Adu (2020) for volume, gross motility, live-dead count, mass activity grade, and concentration. The mass activity was scored subjectively according to the intensity of the wave motion, from the absence of wave motion (0) to slow motion (+), rapid motion (+ +), and turbulent motion (+ + +) characterized by the appearance of dark prominent wave in a rapid motion. After semen collection, the cocks were humanely sacrificed and eviscerated for a gross examination of organs *in situ*. The reproductive tracts of the slaughtered cocks were carefully

Table 1. Ingredient composition of the experimental cock diets

Ingredients	INCLUSION LEVEL OF FB1 and Vit E							
	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8
Maize	330	330	330	330	330	330	330	330
Groundnut cake	80	80	80	80	80	80	80	80
Bone Meal	14	14	14	14	14	14	14	14
Limestone	20	20	20	20	20	20	20	20
Salt	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Corn Bran	100	100	100	100	100	100	100	100
BDG	250	250	250	250	250	250	250	250
Palm Kernel Cake	200	200	200	200	200	200	200	200
Methionine	1	1	1	1	1	1	1	1
Broiler Premix	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Fuminosin B1	0	0.01	0.02	0.03	0.00	0.01	0.02	0.03
Vitamin E	0	0	0	0	0.2	0.2	0.2	0.2
Total	1000	1000.01	1000.02	1000.03	1000.20	1000.21	1000.22	1000.23
Calculated Nutrients								
ME (Kcal Kg^{-1})	2520.66	2520.66	2520.66	2520.66	2520.66	2520.66	2520.66	2520.66
Crude Protein (%)	16.07	16.07	16.07	16.07	16.07	16.07	16.07	16.07
Calcium (%)	1	1	1	1	1	1	1	1
Phosphorus (%)	0.59	0.59	0.59	0.59	0.59	0.59	0.59	0.59
Lysine	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65
Methionine	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27
Crude Fibre (%)	6.28	6.28	6.28	6.28	6.28	6.28	6.28	6.28

NFE= Nitrogen Free Extract; ME= Metabolizable Energy * Composition of premix (Nutrivitas®): 2.5 kg of premix contains: Vit. A (10000000 iu), Vit. D3 (2500000 iu), Vit. E (12000 iu), Vit. B1 (2000 mg), Niacin (25000 mg), Vit. B6 (1500 mg), Vit. B12 (10 mg), Vit. K3 (2500 mg), Biotin (75 mg), Folic Acid (2000 mg), Panthothenic Acid (7000 mg), Chlorine Chloride (50%) (200000 mg), Manganese (80000 mg), Iron (40000 mg), Copper (10000 mg), Zinc (60000 mg), Selenium (200 mg), Iodine (1500 mg), Magnesium (100 mg), Ethoxyquine (500 g), BHT (700 g), Cobalt (250 mg)

harvested. Testicular weights were recorded using a highly sensitive weighing balance in the laboratory. The testes and epididymides were separated free of adhering connective tissues and fats. The left and right testes and epididymides were measured separately and their weights recorded. The volumes of the testes were measured volumetrically using Archimede's principle of water displacement in a measuring cylinder as described by Olarotimi et al. (2015) and the result recorded. The testes' densities were calculated from the testicular weights and volumes and expressed as g ml^{-1} (Olarotimi et al. 2015).

$$Testis\ Density = Testis\ Weight / Testis Volume$$

Morphological abnormalities assay were determined as described by Olukole et al. (2014). Briefly, a total count of 400 spermatozoa in smears prepared and stained with eosin-nigrosin differential staining method and observed under a binocular microscope (Olympus CH-2 CHS Binocular Microscope, Olympus Corporation, Japan) at a magnification of x100. The morphologically defected sperm cells were classified according to Alkan et al. (2002) as the normal, abnormal, curved tail, headless tail, rudimentary tail, double tail, tailless head, double head, and bent mid-piece sperm cells.

2.4. *Statistical analysis*

Data obtained were subjected to one-way analysis of variance (ANOVA) using SAS software. The significant treatment means were compared at (P<0.05) probability level using Duncan's multiple range test (DMRT) of the software.

3. Results

3.1. *Effects of FB_1 and vitamin E on testicular parameters of cocks*

The influence of different levels of FB_1 contamination and ameliorative effects of Vitamin E on the testicular parameters of cocks is shown in Table 2. For the left and right testicular weights, significant (P<0.05) progressive reductions with increasing inclusion levels of FB_1 were observed among the cocks on Diets 2 to 4 when compared with those on the control diet and Diet 5. Their paired testicular weights equally followed this pattern with each experimental group showing a significant (P<0.05) reduction when compared with the control. The groups that received FB_1 contaminated diets fortified with Vitamin E showed significantly (P<0.05) improved testicular weights. There were non-significant (P>0.05) increases in the left and right testicular weights of cocks on Diets 6 and 7 when compared with cocks on Diets 2 and 3, respectively. However, cocks on Diet 8 did not show any significant (P>0.05) improvement in left testicular weight when compared with those on Diet 4 but the right testicular weight was significantly improved when cocks on Diets 4 and 8 were compared. Furthermore, significant (P<0.05) increases in paired testicular weights were equally observed among the cocks on Diets 6 to 8 when compared with those on Diets 2 to 4 that did not receive Vitamin E, respectively. The improvement recorded in paired testicular weights among the cocks on Diets 6 was statistically (P>0.05) comparable to the control groups while significant (P<0.05) differences were recorded among those on Diets 7 and 8 when compared with the control diet.

Table 2. Testiculat characteristics of the cocks

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8	SEM	P-Value
Testicular Weight										
Left	14.87^{a}	13.80^{b}	13.40^{b}	13.33^{b}	14.92^{a}	14.23^{ab}	13.77^{b}	13.47^{b}	4.03	0.042*
Right	14.47^{a}	12.80^{b}	12.50^{b}	11.33^{c}	14.68^{a}	13.87^{ab}	13.00^{b}	11.40^{b}	2.54	0.037*
Paired	29.34^{a}	26.60^{b}	25.90^{c}	24.66^{c}	29.35^{a}	28.10^{a}	26.77^{b}	24.97^{c}	6.41	0.034*
Testicular Volume										
Left	14.01^{a}	12.67^{b}	11.89^{c}	10.47^{c}	14.11^{a}	13.97^{a}	13.05^{b}	13.00^{b}	3.57	0.025*
Right	14.02^{a}	12.33^{b}	11.00^{b}	10.97^{b}	13.99^{a}	12.50^{b}	11.05^{b}	11.33^{b}	1.84	0.041*
Paired	28.03^{a}	25.00^{b}	22.89^{bc}	21.44^{c}	28.00^{a}	26.47^{ab}	23.10^{b}	24.33^{b}	5.08	0.036*
Testicular Density										
Left	1.06	1.09	1.13	1.27	1.08	1.04	1.06	1.04	1.13	1.732^{ns}
Right	1.01	1.04	1.14	1.03	1.11	1.16	1.18	1.09	1.38	5.850^{ns}
Paired	1.04	1.06	1.13	1.15	1.13	1.10	1.13	1.07	1.26	2.063^{ns}
Epididymal Weight										
Left	0.80	0.80	0.67	0.63	0.82	0.77	0.63	0.65	0.17	0.741^{ns}
Right	0.74	0.70	0.53	0.53	0.77	0.70	0.50	0.53	0.18	0.452^{ns}
Paired	1.54^{a}	1.45^{b}	1.20^{c}	1.17^{c}	1.56^{a}	1.53^{a}	1.13^{c}	1.13^{c}	0.21	0.041*

Means in a row without common superscripts are significantly (P<0.05) different. Level of significance= ns (not significant)= P>0.05; *= P<0.05. SEM= Standard Error of Means, Diet 1= Control, Diet 2= (10 mg kg^{-1} FB1), Diet 3= (20 mg kg^{-1} FB_1), Diet 4= (30 mg kg^{-1} FB_1), Diet 5= (10 mg kg^{-1} FB_1+ 200 mg kg^{-1} Vit E), Diet 6= (20 mg kg^{-1} FB_1+200 mg kg^{-1} Vit E), Diet 7= (30 mg kg^{-1} FB_1+ 200 mg kg^{-1} Vit E).

The right, left, and paired testicular volumes of the cocks on Diets 2 to 4 were significantly (P<0.05) reduced in response to the increase in the inclusion levels of FB_1 when compared with the cocks on the control diet and Diet 5. Vitamin E supplementation, however, significantly (P>0.05) enhanced the left testicular volumes of cocks on Diets 6 to 8 when compared with those on Diets 2 to 4, respectively. While cocks on Diets 6 to 8 showed positive enhancement in the right and paired testicular volumes when compared with those on Diets 2 to 4, these increases were not significantly (P>0.05) different between the two groups. However, the paired testicular volume of the cocks on Diet 6 was statistically (P>0.05) similar to those on the control diet. For the right and left epididymal weights as well as all the testicular densities across all the experimental diets, there was no significant (P>0.05) effect of FB_1 contamination and the fortification effect of Vitamin E observed in the studied parameters when compared with one another and the control diet. However, the paired epididymal weight significantly (P<0.05) reduced when 10 mg kg^{-1} FB_1 was added to the diet when compared with the control. There were further significant (P<0.05) reductions in this parameter when 20 and 30 mg kg^{-1} FB_1 were fed to the cocks. Vitamin E inclusion in the diets only brought about a significant (P<0.05) restorative effect on paired epididymal weight among the cocks on Diet 6 when compared with the control.

3.2. Effects of FB_1 and vitamin E on semen characteristics of cocks

The semen characteristics of the cocks fed diets contaminated with varying levels of FB_1 and subsequent ameliorative effects of Vitamin E are in Table 3. The contamination of the diets with the varied levels of FB_1 had adverse effects on semen characteristics of the cocks. Ejaculate volume (EV), sperm motility (SM), total sperm cells/ejaculate (TSC/E), total live cells (TLC), and total motile cells (TMC) of the cocks on Diets 2 to 4 reduced progressively as the level of FB_1 in the diet increased with the EV of the cocks on Diets 3 and 4 showing significant (P<0.05) reduction when compared with the control. Also, the SM, TSC/E, TLC, and TMC of the cocks on Diets 2 to 4 were significantly (P<0.05) reduced when compared with cocks on the control diet. However, the sperm viability (SV) and sperm concentration (SC) of the cocks fed the diets were not significantly (P>0.05) influenced by the varied levels of FB_1 contamination. The mass activity grade ranged from very turbulent motions for the cocks on the Diets 1 to rapid wave motion for the cocks on Diets 2 and 3 to slow-wave motion for those on Diet 4. Furthermore, fortification of Diets 2 to 4 with equal quantity of Vitamin E, respectively, resulted in significant (P<0.05) restorative improvement of the semen parameters of cocks on Diet 6 when compared with those on Diet 2. This improvement in semen characteristics made them comparable to those of the cocks on the control diet. However, significant (P>0.05) improvements were not observed when the parameters recorded among the cocks on Diets 7 and 8 were compared with those on Diets 3 and 4. The mass activity grade of the cocks on Diets 6 and 8 were equally improved by Vitamin E inclusion when compared with those on Diets 2 and 4.

3.3. Morphological characteristics of spermatozoa of the cocks

The results of the effect of feeding diets containing FB_1 are presented in Table 4. Sperm cell abnormalities such as curved tail (CT), headless tail (HT), and rudimentary tail (RT) were significantly (P<0.05) increased in the corresponding trend to the increase in the inclusions of FB_1 in the experimental diets. However, the addition of Vitamin E to the diet that contained 10 mg FB_1 (Diet 6) offset the damaging effect of the mycotoxin as a percentage of these abnormalities were significantly (P<0.05) reduced when compared with birds on Diet 2 and were statistically (P>0.05) comparable with the same parameters in the control diet. Vitamin E inclusion did not significantly (P>0.05) improve on the abnormalities recorded among cocks on Diets 3 and 4 as the parameters recorded among the cocks on Diets 7 and 8 were statistically similar (P>0.05) to those on Diets 3 and 4 but significantly (P<0.05) higher than what were recorded among the cocks on the control diet. Other morphological abnormalities such as double tail (DT), tailless head (TH), double head (DH), and bent mid-piece (BMP) were equally studied. The varied inclusion levels of FB_1 were observed not to have caused any significant (P>0.05)

Table 3. Spermiogram of the experimental cocks

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8	SEM	P-Value
EV (ml)	0.50^{a}	0.42^{ab}	0.34^{b}	0.31^{b}	0.52^{a}	0.49^{a}	0.35^{b}	0.33^{b}	0.15	0.048*
SM (%)	73.41^{a}	72.18^{b}	72.27^{b}	71.50^{bc}	73.11^{a}	73.16^{a}	72.15^{b}	71.51^{bc}	1.03	0.046*
SV (%)	74.95	75.53	74.68	73.88	74.87	75.84	75.17	74.91	1.06	0.635^{ns}
SC (x10^{8} ml^{-1})	2.39	2.34	2.42	2.55	2.42	2.63	2.8	2.27	0.21	0.474^{ns}
TSC/E (x10^{8} ml^{-1})	1.34^{a}	1.21^{b}	1.20^{b}	1.16^{b}	1.36^{a}	1.31^{a}	1.22^{b}	1.20^{b}	0.03	0.032*
TLC ml^{-1} (x10^{8} ml^{-1})	2.09^{a}	1.95^{b}	1.16^{c}	1.18^{c}	2.11^{a}	2.08^{a}	1.17^{c}	1.16^{c}	0.05	0.045*
TMC ml^{-1} (x10^{8} ml^{-1})	2.05^{a}	1.89^{b}	1.61^{c}	1.62^{c}	2.07^{a}	2.01^{a}	1.71^{bc}	1.64^{c}	0.05	0.049*
MAG	+++	++	++	+	+++	+++	++	++	0.00	

Means in a row without common superscripts are significantly (P<0.05) different. Level of significance= ns (not significant)= P>0.05; *= P<0.05. SEM= Standard Error of Means, Diet 1= Control, Diet 2= (10 mg kg^{-1} FB1), Diet 3= (20 mg kg^{-1} FB_1), Diet 4= (30 mg kg^{-1} FB_1), Diet 5= (10 mg kg^{-1} FB_1+ 200 mg kg^{-1} Vit E), Diet 6= (20 mg kg^{-1} FB_1+200 mg kg^{-1} Vit E), Diet 7= (30 mg kg^{-1} FB_1+ 200 mg kg^{-1} Vit E). EV= Ejaculate Volume, SM = Sperm Motility, SV = Sperm Viability, SC= Sperm Concentration, TSC/E= Total Sperm Cells/Ejaculate, TLC ml^{-1} = Total Live Cells per ml, TMC/ml= Total Motile Cells per ml, MAG= Mass Activity Grade, + + +: Very turbulent motion; + +: Rapid wave motion; +: Slow wave motion.

Table 4. Sperm morphology of cocks fed FB_1 contaminated and Vit. E fortified diets

Parameters (%)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8	SEM	P-Value
CT	9.15^{c}	15.91^{b}	18.55^{b}	31.98^{a}	9.11^{c}	10.29^{c}	12.22^{bc}	25.71^{ab}	29.59	0.012*
HT	7.05^{c}	10.73^{b}	15.00^{a}	16.67^{a}	7.01^{c}	7.11^{c}	12.53^{ab}	12.87^{ab}	19.86	0.022*
RT	3.33^{c}	6.67^{b}	11.43^{ab}	14.47^{a}	3.33^{c}	4.13^{c}	10.27^{ab}	11.45^{ab}	18.67	0.014*
DT	1.02	1.09	1.07	1.03	1.18	1.04	1.05	1.08	1.09	0.662^{ns}
TH	1.12	1.37	1.18	1.15	1.14	1.12	1.19	1.13	1.47	0.623^{ns}
DH	3.33	3.15	2.22	2.67	3.35	2.79	2.43	3.43	3.51	0.552^{ns}
BMP	0.00	2.14	1.43	1.65	0.00	0.82	1.99	1.25	2.13	0.091^{ns}

Means in a row without common superscripts are significantly (P<0.05) different. Level of significance= ns (not significant)= P>0.05; *= P<0.05. SEM= Standard Error of Means Diet 1= Control, Diet 2= (10 mg kg^{-1} FB1), Diet 3= (20 mg kg^{-1} FB_1), Diet 4= (30 mg kg^{-1} FB_1), Diet 5= (10 mg kg^{-1} FB_1+ 200 mg kg^{-1} Vit E), Diet 6= (20 mg kg^{-1} FB_1+200 mg kg^{-1} Vit E), Diet 7= (30 mg kg^{-1} FB_1+ 200 mg kg^{-1} Vit E). CT= Curved Tail, HT= Headless Tail, RT= Rudimentary Tail, DT= Double Tail, TH = Tailess Head, DH = Double Head, BMP = Bent Mid-piece.

spermatozoa abnormalities such as DT, TH, DH, and BMP, respectively. Vitamin E supplementation in Diets 6 to 8 did not also cause any significant (P>0.05) change in these parameters when compared with those on Diets 1 to 4, respectively.

4. Discussion

The most important factor that underscores efficient cock production is reproductive efficiency which is expressed in the form of quantity and quality of sperm production and efficiency (Olarotimi and Adu 2020). The quality and quantity of testicular sperm production is an important tool in the selection for breeding purposes. Hence, parameters such as testicular weight, volume, and density as well as the epididymal weight are used in assessing semen quality and quantity to detect any abnormality resulting from experimental procedures and these parameters have been established to have a direct bearing with the spermatogenic activity of the testis (Olarotimi and Adu 2020). The adverse effects of FB_1 on reproductive potentials of animals have been severally stressed (Gbore 2009; Ewuola and Egbunike 2010; Ogunlade 2015). Negative effects such as reduced sperm characteristics and morphological abnormalities have been reported. However, the antioxidant properties of vitamin E in alleviating the adverse effects of FB_1 have also been reported (Adu and Gbore 2015). The significant reductions observed in the left, right, and paired epididymal weights among the cocks fed Diets 2 to 4 in the present study suggests that FB_1 contamination from 10 to 30 mg kg^{-1} diet might have a structural toxic and negative effects on the epididymides of the cocks resulting in reduced sperm count in the epididymides and may be responsible for the acceleration of the sperm transit time through the epididymides. This result agreed with Ogunlade (2015) and Supriya et al. (2014) who equally recorded significant reductions in the reproductive organ weights when more than 10 mg/kg FB_1 and AFB_1 (Aflatoxin B1) were included in cocks' and rats' diets respectively. Furthermore, vitamin E supplementation of 200 mg kg^{-1} diet as used in diets 6 to 8 has proved to have a restorative effect on epididymal weights among the cocks on Diet 6, whereas, those on Diets 7 and 8 did not show any ameliorative effect of vitamin E respectively. This is indicative that the negative impacts of contamination of cocks' diets with FB_1 above 10 mg kg^{-1} could not be restored with the quantity of vitamin E used in the present study. It is, however, possible that an increase in the inclusion of vitamin E above 200 mg kg^{-1} diet might bring about ameliorative effects of contamination of the diet above 10 mg kg^{-1} FB_1. This study as shown by the results of the paired testicular and epididymal weights as well as paired testicular volume agreed with Ogunlade (2019) who revealed that exposure of cocks to be used for breeding purpose to dietary FB_1 higher than 10 mg/kg will impede the reproductive efficiency of the cocks.

The semen volumes in this study were significantly affected only among the cocks fed above 10 mg kg^{-1} diet FB_1 inclusion rate. This agreed with the report of Ewuola and Egbunike (2010) who did not observe any adverse effect in semen volume in rabbit bucks fed 10 mg FB1 per kg diet but disagreed with Ogunlade (2015) who opined that dietary FB_1 did not significantly influence the ejaculate volume. Our results, however, agreed with earlier findings (Ewuola and Egbunike 2010; Ogunlade 2015) that varied inclusion levels of FB_1 did not adversely influence spermatozoa concentration and viability. This could have been because the quantitative semen characteristics were determined not considering whether the sperm cells are normal or abnormal, alive or dead. The significant reduction in mass activity, total live sperm, and sperm motility in cocks fed 10 mg kg^{-1} FB_1 and above also aligned with the findings of Ewuola and Egbunike (2010) who

also attributed this to the toxin effect which may have played an inhibitory role to cyclic 3'5' AMP activity and calcium ion, which are believed, among other factors, to initiate motile ability of spermatozoa in the caput epididymis. Ewuola et al. (2007) also opined that FB_1 can impede protein absorption and utilization in the epididymis. This protein is required for sperm maturation and to maintain the motility of sperm cells from the caput epididymis. The trends of influence of dietary FB_1 on total motile cells per ejaculate and total live cells per ejaculate were similar. The adverse effect of dietary FB_1 on spermatozoa motility may be linked with the significantly lower total motile cells and total live cells of the cocks fed a diet containing above 10 mg kg^{-1} dietary FB_1. The consistent reduction in the sperm wave motion to slow swirling characteristics in the mass activity of the cocks fed 10 to 30 mg kg^{-1} dietary FB_1 is directly related to the motility of the spermatozoa as determined by FB_1 concentration.

Sperm morphology, as an essential semen parameter that indicates the degree of normality and maturity of sperm cells in an ejaculate, directly correlates with fertility (Omirinde et al. 2014). Our result further revealed that varying inclusion levels of FB1 led to secondary sperm morphological abnormalities such as curved, headless, and rudimentary tails. Similar results in sperm abnormalities were earlier reported by Yang et al. (2010) and Qingqing et al. (2012) when above 10 mg kg^{-1} diet citrinin and trichothecene-2 were administered to mice for up to 7 days. Unlike primary abnormalities that are generated during spermatogenesis and tertiary abnormalities that are due to handling techniques like cold or heat shock, osmotic effects, the toxicity of stains, or changes in pH during collection and processing of semen, Secondary abnormalities are due to changes that take place in the excurrent duct of the testis while (Olukole et al. 2014). The changes observed in sperm morphology in this study could be attributed to the direct effect of FB1 on testicular tissue which further leads to reproductive dysfunction such as reduced ejaculate volume, sperm motility, and total live cells. Abnormalities in sperm development could be linked to major testicular damage destroying the seminiferous tubules and progressive sloughing of immature germ cells (Zhang et al. 2010). The high occurrence of secondary abnormalities (curved tail, headless tail, and rudimentary tail) observed in this study indicated that high contamination of cocks' diet with FB_1 adversely affected the high functional integrity of the epididymis. The implication of cocks with rudimentary tails is that sperm cells will be immotile and are unable to fertilize mature ovum (Saba et al. 2009). This could be responsible for a progressive reduction in total motile sperms recorded in this study.

Supplementation of vitamin E, as used in this study as an antioxidant, has been reported to reduce the toxic effects of mycotoxins in livestock (Adu and Gbore 2015). Mycotoxins are noted for generating free radicals in living cells, thereby, causing oxidative stress. The addition of antioxidants capable of acting as free radical scavengers has been recommended (Citil et al. 2005). However, there is limited information on the potential of vitamin E supplementation in restoring the damaging effects of FB_1 on the reproductive characteristics of cocks. From the present study, the significant reductions observed in the sperm morphological abnormalities among the cocks fed vitamin E supplemented diets (Diets 6 to 8) when compared with those on Diets 2 to 4 respectively showed the ameliorative effect of vitamin E as a potent synthetic antioxidant in combating the damaging effects of FB_1 in cocks. Cocks on Diet 6 recorded comparable sperm morphological parameters with those on the control diet. Vitamin E equally significantly improved the testicular weights and volumes, ejaculate volume, sperm motility, total live cells, total motile cells, and mass activity of cocks on Diet 6 to be comparable with those on the control while those on Diets 7 and 8 were only marginally increased in values. This trend was equally observed by Khan et al. (2014) who reported significant ameliorative effects of vitamin E against lower aflatoxin-B_1 doses while these were no longer apparent in the highest doses. Our results further strengthened the report of Alpsoy et al. (2009) who documented the ameliorative effects of vitamins A, C, and E on aflatoxin B1-induced oxidative stress in human lymphocytes. The present study further revealed that extremely high contamination of cocks' diets with FB_1 was capable of masking the restorative effects of vitamin E or any other antioxidants as observed among cocks on Diets 7 and 8. Though their values were slightly improved by vitamin E, these were not statistically similar to those on the control.

5. Conclusion

This study has demonstrated the restorative potentials of vitamin E on the damaging effects of FB_1 on cock testicular parameters, semen characteristics, and sperm morphology. Dietary inclusion of vitamin E at 200 mg kg^{-1} diet is capable of masking the deleterious effects of FB_1, especially, if the contamination level does not exceed 10 mg kg^{-1} diet. However, further study to ascertain the required inclusion level of vitamin E to counter the damaging effects of high contamination level is recommended as a nutritional strategy.

References

Adu OA, Gbore FA (2015) Vitamin E ameliorates the impacts of dietary fumonisin B1 on growth and blood profile of rabbits. Annals of Biological Sciences 3(3):12-19.

Alkan S, Baran A, Ozdas OB, Evesen M (2002) Morphological defects in turkey semen. Turkish. Journal of Veterinary and Animal Science 26: 1087-1092.

Alpsoy L, Yildirim A, Agar G (2009) The antioxidant effects of vitamin A, C, and E on aflatoxin B1-induced oxidative stress in human lymphocytes. Toxicology and Industrial Health 25: 121-127.

Citil M, Gunes V, Atakisi O, Ozcan A, Tuzcu M, Dogan A (2005) Protective effect of L-carnitine against oxidative damage caused by experimental chronic aflatoxicosis in quail (Coturnix coturnix). Acta Veterinaria Hungarica 53(3): 319-324. doi: 10.1556/avet.53.2005.3.5.

Ewuola EO, Gbore FA, Ogunlade JT, Egbunike GN (2007) Growth response of rabbit bucks fed dietary fumonisin B1. Tropical Journal of Animal Science 10: 257-262.

Ewuola EO, Gbore FA, Ogunlade JT, Bandyopadhyay R, Niezen J, Egbunike GN (2008) Physiological response of rabbit bucks to dietary fumonisin: performance, haematology and serum biochemistry. Mycopathologia 165(2): 99-104. doi: 10.1007/s11046-007-9083-y.

Ewuola EO, Egbunike GN (2010) Effects of dietary fumonisin B1 on the onset of puberty, semen quality, fertility rates and testicular morphology in male rabbits. Reproduction 139: 439-445.

Gbore FA (2009) Reproductive organs weight and semen quality of pubertal boars fed dietary fumonisin B1. Animal 3(8): 1133-1137.

Gbore FA, Adu OA (2017) Ameliorative potential of vitamin E on the impact of dietary fumonisin B1 on reproductive performance of female rabbits. Journal of Agriculture and Rural Development in the Tropics and Subtropics 118(2): 161-169.

Khan WA, Khan MZ, Khan A, Hassan ZU, Saleemi MK (2014) Potential for amelioration of aflatoxin B1-induced immunotoxic effects in progeny of white leghorn breeder hens co-exposed to vitamin. Journal of Immunotoxicology 11(2): 116-125. doi: 10.3109/1547691X.2013.804134.

Ogunlade JT, Egbunike GN (2013) Fumonisin mycotoxicity in laying hens: haematology, serum biochemistry and organ characteristics. Global Advances and Research Journal of Environment, Science and Toxicology 2(2): 47-53.

Ogunlade JT (2015) Effects of dietary fumonisin B1 on reproductive organs and semen quality indices of breeder cocks. Journal of Biology Agriculture and Health Care 6: 28-33.

Ogunlade JT (2019) Gonadal, extra gonadal sperm reserve and daily sperm production of breeder cocks fed graded levels of dietary fumonisin B1. International Journal of Animal Research 3(22): 1-7.

Olarotimi OJ, Sokunbi OA, Abdullah AR (2015) Determination of daily sperm production (DSP) in rabbit (*Oryctolagus cuniculus*) bucks using testicular parameters. Greener Journal of Agricultural Sciences 5(4): 141-148.

Olarotimi OJ, Adu OA (2020) Semen characteristics, gonadal and extragonadal sperm reserves in cocks fed diets containing different inclusion levels of monosodium glutamate. Slovak Journal of Animal Science 53(1): 1-11.

Olukole SG, Oyeyemi MO, Oke BO (2014) Semen characteristics and spermiogram of the African greater cane rat (*thryonomys swinderianus*, temminick). Slovak Journal of Animal Science 47 (3): 125-131.

Omirinde JO, Ozegbe PC, Oyeyemi MO (2014) Comparative evaluation of the sperm characteristics and morphology of adult Wistar rats fed either low or normal protein-energy diets and orally dosed with aqueous *Cuscuta australis* extracts. Nigerian Journal of Physiological Science 29: 055-061.

Qingqing H, Linbo Y, Yunqian G, Shuqiang L (2012) Toxic effects of citrinin on the male reproductive system in mice. Experimental and Toxicologic Pathology 64: 465-469.

Saba AB, Oridupa OA, Oyeyemi MO, Osanyigbe OD (2008) Spermatozoa morphology and characteristics of male wistar rats administered with ethanolic extract of Lagenaria Breviflora Roberts. African Journal of Biotechnology 8(7): 1170-1175.

Supriya C, Girish BP, Reddy PS (2014) Aflatoxin B1-Induced reproductive toxicity in male rats: possible mechanism of action. International Journal of Toxicology 33: 155-161.

Surai PF, Kochish II, Romanov MN, Griffin DK (2019) Nutritional modulation of the antioxidant capacities in poultry: the case of vitamin E. Poultry Science 98(9): 4030-4041. doi: 10.3382/ps/pez072. PMID: 30805637.

Tardieu D, Travel A, Metayer JP, Le Bourhis C, Guerre P (2019) Fumonisin B1, B2 and B3 in muscle and liver of broiler chickens and turkey poults fed with diets containing fusariotoxins at the EU maximum tolerable level. Toxins 11(10): 590. doi: doi.org/10.3390/toxins11100590.

Verma RJ, Nair A (2001) Vitamin E ameliorates aflatoxin-induced biochemical changes in the testis of mice. Asian Journal Andrology 3: 305-309.

Yang JY, Zhang YF, Liang AM, Kong XF, Li YX, Ma KW, Jing AH, Feng SY, Qiao XL (2010) Toxic effects of T-2 toxin on reproductive system in male mice. Toxicology and Industrial Health 26: 25-31.

Zhang M, He Z, Wen L, Wu J, Yuan L, Lu Y, Guo C, Zhu L, Deng S, Yuan H (2010) Cadmium suppresses the proliferation of piglet Sertoli cells and causes their DNA damage, cell apoptosis and aberrant ultrastructure. Reproductive Biology and Endocrinology 8: 97.