Ameliorative potential of vitamin E on the impact of dietary fumonisins B₁ on reproductive performance of female rabbits

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Abstract

Fumonisin B₁ (FB₁), a contaminant of agricultural products, particularly maize worldwide is known to be consumed by farm animals and has been documented to cause various physiological responses in animals. A 15-week trial on the ameliorative potential of vitamin E on the negative impacts of FB₁ on reproductive performance of rabbits was conducted. Forty-nine female rabbits aged 16 to 18 weeks weighing 1.65 to 2.0 kg body weight were assigned to seven experimental feeding groups: the control group received a diet without FB₁, three groups were fed diets containing different concentrations of FB₁ at 2.5, 5.0 or 7.5 mg kg⁻¹, and three further groups had diets containing FB₁ and vitamin E i.e., 2.5 mg FB₁ kg⁻¹ + 100 mg vitamin E, 5.0 mg FB₁ kg⁻¹ + 100 mg vitamin E, and 7.5 mg FB₁ kg⁻¹ + 100 mg vitamin E. Data obtained on reproductive parameters - gestation length, litter size, kit weight as well as the kit crown-rump length, were analysed using ANOVA. Serum reproductive hormones - luteinizing hormone (LH), follicle stimulating hormone (FSH), prolactin, prostaglandin F₂α (PGF₂α), and estradiol (E₂) levels in rabbits fed diets containing 7.5 mg FB₁ kg⁻¹ were significantly lower than those for all other treatments. Rabbits fed diets containing ≥5 mg FB₁ kg⁻¹ had significantly (p < 0.05) longer gestation lengths and lower kit weights compared to the other treatment groups. The litter sizes of rabbits fed FB₁-contaminated diets supplemented with vitamin E were significantly (p < 0.05) higher compared with those on diets not supplemented, including the control. The 21-day post-partum weight gain of kits of does fed diets containing ≥5 mg FB₁ kg⁻¹ were significantly (p < 0.05) lower than the weight gain observed in the other treatment groups. Does fed diets supplemented with vitamin E had significantly (p < 0.05) higher milk yield compared with does on not supplemented diets, including the control. This study has shown that vitamin E supplementation of does counteracts the adverse impacts of FB₁ on reproductive hormones, gestation length, kit weight, and milk production in rabbits.

Keywords: rabbit, fumonisin B₁, mycotoxin, reproduction, antioxidant

1 Introduction

Rabbit production has potential in many developing countries as a means of supplying cheap, high quality animal protein within the shortest time possible (Oyawoye et al., 1990). Schiere (2004) stated that growth rate of around 15–20 g day⁻¹ are common in the tropics but it is possible to obtain 30–40 g day⁻¹ if rabbits are well fed. Since good growth rate and reproductive performance form the basis of high profit margin in livestock production, good nutrition is essential. However, some low-cost rabbit feed constituents, such as maize-milling waste, under humid tropical environmental conditions, may be infected with moulds and consequently may contain mycotoxins. Also, crops contaminated with high concentrations of mycotoxins are often diverted into animal feeds, thereby posing a serious threat to the growth, health and productivity of the animals (Griessler & Encarnação, 2009).
Fumonisin B$_1$ (FB$_1$), a mycotoxin produced by *Fusarium verticillioides* (= *F. moniliforme*) and other *Fusarium* species that grow on cereals, especially maize, has been documented to cause various physiological responses in humans and animals. The effects of dietary FB$_1$ on reproductive processes in animals have been well documented (Voss et al., 2006; Gbore & Egbunike, 2006; Gbore, 2009a, b; Ewuola & Egbunike, 2010; Gbore et al., 2012).

Considerable research has been directed at finding means to prevent or lessen the toxicity of FB$_1$. The use of feed additives is an approach that is considered to be cheaper than degradation of the mycotoxin. Several studies have shown that a variety of adsorbent materials have a high affinity for binding mycotoxins by the formation of stable linkages (Huwig et al., 2001; Galvano et al., 2001; Diaz et al., 2004; Var et al., 2008). Experimental results obtained with some extensively studied adsorbents, such as hydrated sodium calcium aluminosilicate (HSCAS), are quite satisfactory with respect to aflatoxins, but they are not effective in preventing toxic effects of *Fusarium* mycotoxins, such as fumonisins, trichothecenes or zearalenone (Avantaggiato et al., 2005). In the case of less- and non-absorbable mycotoxins such as these, new strategies have to be applied. Most of the mycotoxins provoke oxygen free radical formation (Balogh et al., 2007; Pál et al., 2009). As a result, the addition of natural or synthetic antioxidants in diets contaminated with these mycotoxins have been reported (Rogers, 2003; Citil et al., 2005; Surai, 2006; Dvorska et al., 2007) to be hypothetically effective in ameliorating the adverse impacts of these mycotoxins on animals due to their superoxide anion scavenging ability.

Dietary concentration of 7.5 mg fumonisin kg$^{-1}$ feed reportedly impaired reproductive performance in rabbits in 175 day studies (Ewuola & Egbunike, 2010). However, consumption of FB$_1$ at levels below this threshold may exert suboptimal reproductive performance in breeding female rabbits. The present study was therefore aimed at evaluating the ameliorative potential of vitamin E on the effects of dietary FB$_1$ below and above the no-observed-adverse-effect-level (NOAEL) of 5 mg kg$^{-1}$ feed on reproductive performance of female rabbits.

## 2 Materials and methods

### 2.1 Animals and experimental site

Forty-nine mixed breeds female rabbits aged 16 to 18 weeks were used in the current study. Rabbits were procured from a reputable commercial farm in Akure, Ondo State, Nigeria. This study was conducted in the Rabbit Unit of the Teaching and Research Farm of the Federal University of Technology, Akure, Nigeria. The farm is located in the humid rain forest zone of western Nigeria with mean rainfall, relative humidity and temperature of 1,500 mm, 75% and 29 °C, respectively. The raining season is usually from March to November yearly. This study was carried out in accordance with “Guide for the care and use of Laboratory Animals” (NRC, 1996), and approved by the local Institutional Animal Ethics Committee.

### 2.2 Fumonisin B$_1$ production and experimental diets

Maize grits cultured with a toxigenic strain of *F. verticillioides* (MRC 286) and quantified in replicates for FB$_1$ and other common *Fusarium* mycotoxins as outlined by Gbore et al. (2016) were combined with non-contaminated maize grits to formulate seven diets consisted as follows: a control diet without FB$_1$ contamination, three diets containing different concentrations of FB$_1$ at 2.5, 5.0 or 7.5 mg FB$_1$ kg$^{-1}$ feed, and three further diets containing FB$_1$ and vitamin E at a fixed rate of 100 mg kg$^{-1}$ contaminated feed (i.e., 2.5 mg FB$_1$ kg$^{-1}$ + 100 mg vitamin E, 5.0 mg FB$_1$ kg$^{-1}$ + 100 mg vitamin E, and 7.5 mg FB$_1$ kg$^{-1}$ + 100 mg vitamin E). The level of FB$_1$ in the control diet was below the detection limit of 0.2 mg kg$^{-1}$ for the mycotoxin. The diets were marked A, B, C, D, E, F, and G, respectively, and provided nearly 15% crude protein, 10% crude fibre and 2600 kcal of digestible energy kg$^{-1}$ (Table 1).

### Table 1: Ingredient and chemical composition (%) of the basal diet.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize †</td>
<td>40.7</td>
</tr>
<tr>
<td>Groundnut cake</td>
<td>11.8</td>
</tr>
<tr>
<td>Wheat offal</td>
<td>22.6</td>
</tr>
<tr>
<td>Palm kernel cake</td>
<td>19.6</td>
</tr>
<tr>
<td>Fish meal</td>
<td>2.0</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>2.0</td>
</tr>
<tr>
<td>Salt (NaCl)</td>
<td>0.2</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.1</td>
</tr>
<tr>
<td>L-Lysine</td>
<td>1.06</td>
</tr>
<tr>
<td>Minerals/vitamins premix</td>
<td>0.1</td>
</tr>
</tbody>
</table>

### Analysed nutrients

| Crude protein (%)                     | 15.46|
| Crude fiber (%)                       | 9.60 |
| Digestible energy (kcal kg$^{-1}$)    | 2597.96|

†Varied proportion of *Fusarium*-contaminated and non-contaminated grains.
2.3 Treatments and experimental layout

The rabbits were weighed at the end of a two week pre-experimental physiological adjustment period and randomly assigned to each of the diets (n = 7 rabbits per treatment) and housed individually in hutches. The animals were fed with their respective experimental diets for 15 weeks.

2.4 Evaluation of reproductive performance of the does

After eight weeks of feeding the respective experimental diets, the rabbits were mated to intact bucks, at the ratio of one buck to two does. At parturition, gestation length, mean litter size, kit weight, and kit crown rump length were determined. The crown rump length of each of the kits was determined using thread calibrated in cm.

2.5 Determination of serum reproductive hormones

Serum level of each of luteinizing hormone (LH), follicle stimulating hormone (FSH), prolactin, prostaglandin F2α (PGF2α), and estradiol (E2), was determined using appropriate test kits (Endocrines Technologies Inc., Newark, CA). All samples were run in triplicate in a single assay (n = 7 per treatment).

2.6 Evaluation of post-partum performance

Rabbits were maintained on the respective diets through gestation till 21 days post-partum and the following parameters were determined: litter sizes, kit weights, and mortalities of the offspring at birth, 7, 14 and 21 days post partum. Milk yield was calculated using the formula proposed by Lebas et al. (1986) i.e., Milk yield = (Live weight new born at 21 days of age − Live weight of new born) / 1.18.

2.7 Statistical analysis

The design used for this experiment is Completely Randomized Design (CRD). Data obtained were subjected to statistical analysis using one-way ANOVA procedure of Statistical Analysis Systems (SAS, 2008). Duncan’s multiple range tests of the same software was used to separate all means at 5% probability level. Results giving p-values of < 0.05 were considered significantly different.

3 Results

3.1 Reproductive performance

Dietary FB1 generally influenced the concentrations of serum hormones examined (Table 2). The serum LH, FSH, prolactin, PGF2α, and E2 levels in rabbits fed diets containing 7.5 mg FB1 kg⁻¹ were significantly lower than those fed on the other treatment diets.

The reproductive performance of the does fed diets containing FB1 and vitamin E is shown in Table 3. The gestation length of the rabbits was significantly (p < 0.05) influenced by the dietary treatments. Rabbits fed diets containing ≥ 5 mg FB1 kg⁻¹ had significantly longer gestation period compared with does in other treatment groups. The litter sizes of rabbits fed FB1-contaminated diets supplemented with vitamin E were significantly higher (p < 0.05) compared with those on diets not supplemented with vitamin E, including the control. The total and relative weights of the kits of rabbits fed diets containing ≥ 5.0 mg FB1 kg⁻¹ were significantly lower (p < 0.05) than those in other treatment groups. The crown-rump lengths of kits of rabbits fed diets containing 2.5 mg FB1 kg⁻¹ were significantly (p < 0.05) longer than those of the kits of rabbits in the control group and those on the diet containing 5.0 mg FB1 kg⁻¹.

3.2 Pre-weaning performance

Table 4 shows the pre-weaning performance of kits of does fed different diets. Weight gained by the kits was significantly (p < 0.05) influenced by the dietary treatments. The mean weight of kits of does fed diets containing 7.5 mg FB1 kg⁻¹ (Diet D) was significantly lower compared to those fed the control diet (Diet A), diets containing 2.5 mg FB1 kg⁻¹ (Diet B) and ≤ 5.0 mg FB1 kg⁻¹ supplemented with vitamin E (Diets E and F) at day 7 post-partum. At 21-day post-partum, weight gained by kits of does fed diets containing ≥ 5.0 mg FB1 kg⁻¹ (Diets C and D) were significantly lower than the weights gained by kits of does in the other treatment groups, with the kits of does fed diets containing 2.5 mg FB1 kg⁻¹ supplemented with vitamin E having the highest gain. The gain in weights of kits of does fed the control diet, diet containing 2.5 mg FB1 kg⁻¹ and diets supplemented with vitamin E were significantly higher than those of kits of does fed diets containing 5.0 and 7.5 mg FB1 kg⁻¹ without vitamin E supplementation.

The estimated milk yield of does fed diets containing FB1 or with vitamin E is shown in Fig. 1. Milk yield of the does was significantly (p < 0.05) influenced by the dietary treatments. Does fed diets supplemented with vitamin E had significantly (p < 0.05) higher milk yield compared with those on diets not supplemented, including the control. The milk yield of the does significantly decreased with increase in the concentrations of FB1 in the diets.
Table 2: Serum hormones of female rabbits fed diets containing FB1 with or without vitamin E.

<table>
<thead>
<tr>
<th>Parameters (ng ml⁻¹)</th>
<th>Diet A</th>
<th>Diet B</th>
<th>Diet C</th>
<th>Diet D</th>
<th>Diet E</th>
<th>Diet F</th>
<th>Diet G ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>2.5 mg FB1</td>
<td>5.0 mg FB1</td>
<td>7.5 mg FB1</td>
<td>2.5 mg FB1 + Vitamin E</td>
<td>5.0 mg FB1 + Vitamin E</td>
<td>7.5 mg FB1 + Vitamin E</td>
</tr>
<tr>
<td>Luteinizing hormone</td>
<td>5.23a</td>
<td>5.20a</td>
<td>5.20a</td>
<td>4.53b</td>
<td>5.00a</td>
<td>5.27a</td>
<td>5.00a ± 0.15</td>
</tr>
<tr>
<td>Follicle stimulating hormone</td>
<td>17.37a</td>
<td>17.80a</td>
<td>17.00a</td>
<td>14.20b</td>
<td>17.00a</td>
<td>17.23a</td>
<td>17.93a ± 0.69</td>
</tr>
<tr>
<td>Prolactin</td>
<td>2.03a</td>
<td>2.00a</td>
<td>1.90a</td>
<td>1.53b</td>
<td>2.10a</td>
<td>2.03a</td>
<td>2.07a ± 0.15</td>
</tr>
<tr>
<td>Prostaglandin F₂α</td>
<td>5.77a</td>
<td>5.70a</td>
<td>5.83a</td>
<td>4.33b</td>
<td>5.93a</td>
<td>5.93a</td>
<td>5.77a ± 0.28</td>
</tr>
<tr>
<td>Estradiol</td>
<td>9.11a</td>
<td>9.75a</td>
<td>9.38a</td>
<td>6.52b</td>
<td>9.47a</td>
<td>9.42a</td>
<td>9.33a ± 0.66</td>
</tr>
</tbody>
</table>

a,b: Means on the same row with different superscripts differ significantly (p < 0.05).

4 Discussion

Although, previous studies (Voss et al., 1996; LaBorde et al., 1997; Collins et al., 1998a,b) provided no evidence that FB1 is a reproductive toxicant, more recent observations and experimental findings have however shown that FB1 is a possible risk factor for birth defects (Merrill et al., 2001; Marasas et al., 2004; Voss et al., 2006) and impaired reproductive capacity in animals (Gbore & Egbunike, 2008; Gbore, 2009a, b; Ewuola & Egbunike, 2010; Gbore et al., 2012; Cortinovis et al., 2014; Albonico et al., 2016). In the present study, results show that > 5 mg dietary FB1 kg⁻¹ feed had observable adverse effect on gestation length in rabbits. However, the inclusion of vitamin E at 100 mg kg⁻¹ diet counteracted this effect by reducing the gestation length. This could be attributed to the fact that vitamin E plays an important role in reproductive performance in animals due to its anti-oxidative properties which enabled its participation in metabolism of all cells as reported by Gutteridge & Halliwell (1994). Seemingly elongated gestation lengths was observed in rats fed F. moniliforme culture material containing 10 ppm FB1 from two
weeks before mating by Voss et al. (1996). In a previous study, Gbore et al. (2012) observed significantly elongated gestation length from 21.60 ± 0.53 days in rats fed diets containing ≥10.0 mg FB1 kg⁻¹ to 23.33 ± 0.51 days. These reports and the results from this study, further lead credibility to the fact that FB1 potentially affects reproductive development in rabbits. The result of this study on gestation length shows that FB1 at low concentration of 2.5 mg kg⁻¹ had no detrimental effect on the performance of rabbits but at higher concentrations of 5.0 and 7.5 mg kg⁻¹ the detrimental effects were observable.

Gbore et al. (2012) reported depressed serum gonadotropins levels in rats fed diets containing ≥10 mg FB1 kg⁻¹. The authors postulated that this might be due to an increase in gonadal steroid inhibition or suppression of the hypothalamus and/or pituitary gland with resultant decline in serum gonadotropins levels. Adverse effects of mycotoxins on sexual and reproductive developments have been reported. Dietary zearalenone levels as low as 0.05–0.06 mg kg⁻¹ DM have been shown to increase the number of ovarian follicles and to decrease the serum concentration of the gonadotropic hormone (FSH) in female piglets (Döll et al., 2003), thus potentially affecting their sexual development. Also, dietary FB1 was reported to delay attainment of sexual maturity in growing pigs (Gbore, 2009a) and rabbits (Ewuola & Egbonike, 2010). Fertility disturbances and other reproductive pathologies, particularly suppressive effect on testosterone secretion in mice, was reported (Yang et al., 2007 a,b) following ingestion of cereals contaminated with Fusarium fungi. Also, reduced progesterone synthesis due to inhibition of the follicle stimulating hormone secretion (FSH) by Fusarium mycotoxins in cultured granulosa cells (GCs) from porcine ovaries was reported by Tiemann et al. (2003).

Improved litter size in rabbits fed diets supplemented with vitamin E over other treatment groups in this study may be attributed to the role vitamin E plays in reproduction. In the present study, it is evident that the dose-dependent significant decline in litter weight of does fed diets B, C, and D was countered by vitamin E supplementation. In a study, LaBorde et al. (1997) reported a reduced foetal weight from rabbits dosed daily by gavage on gestation days (GD) 3–19 with purified FB1 at 0.5–1 mg kg⁻¹ day⁻¹. The authors ascribed this weight reduction to maternal toxicity, rather than any developmental toxicity produced by FB1. However, FB1 dose-responsive significant decrease in foetal weight observed in this study correlates with reports of other studies. Pregnant rats dosed by gavage on GD 8–
12 with a semi-purified extract of culture material containing FB₁ with a purity of 80% resulted in lower foetal weight at dose of 60 mg kg⁻¹ (Lebepe-Mazur et al., 1995). Similarly, decreased body weight of live foetuses obtained from pregnant Syrian hamsters dosed with FB₁ in a dose-dependent manner was reported in a study by Penner et al. (1998). Voss et al. (1996) reported lower litter weights from rats fed F. moniliforme culture material providing 1–55 ppm FB₁ from two weeks before mating compared to the control group.

The significantly reduced litter weights at concentration of 7.5 mg FB₁ kg⁻¹ in this study corroborates the report of Voss et al. (1996) that FB₁ at high concentrations of 10 and 55 mg kg⁻¹ significantly reduced the weight of dams fed diets contaminated with F. verticilloides. It is also similar to the report of Lebepe-Mazur et al. (1995) that FB₁ at high dose significantly suppressed growth in rats. In the present study, vitamin E countered this effect by increasing the litter weight. The significant positive impact of vitamin E could be a result of the crucial role it plays in the growth of animals. It has been reported that FB₁ is not transferred through the placenta or into the milk in several animal species (Scott et al., 1994; Becker et al., 1995; Voss et al., 1996; LaBorde et al., 1997; Collins et al., 1998b), nor are its metabolites found in animal products such as milk, meat and eggs (Jonker & van Egmond, 1999). Therefore, the resultant depressed growth rate of litters of does fed diets containing ≥ 2.5 mg FB₁ kg⁻¹ without vitamin E supplementation may be attributed to the reduced milk yields in does fed diets B, C, and D (Fig. 1).

Exposure to Fusarium mycotoxins has been linked to reproductive disorders in pigs (Cortinovis et al., 2014; Gbore & Egbughike, 2008; Gbore, 2009a,b), mice (Yang et al., 2007a,b), and rabbits (Ewuola & Egbughike, 2010). In a study to determine the potential reproductive effect of FB₁ on granulosa cell (GC) proliferation and steroid production in swine, Cortinovis et al. (2014) reported that this mycotoxin alone or with other Fusarium mycotoxins, including deoxynivalenol (DON) and zearalenone, influenced porcine GC proliferation and steroid production, thereby demonstrating their potential reproductive effects on swine. GC is reported to be crucial in the process of normal folliculogenesis and oocyte growth and development as they provide essential nutrients to the oocyte and establish a link between the oocyte and the surrounding ovarian tissue in which the follicle is embedded (Petro et al., 2012). Also, GC is responsible for ovarian steroidogenesis that can be altered at any level leading to changes in the rate of hormone production and concentration (Ndossi et al., 2012; Petro et al., 2012). Progesterone is an essential regulator of the reproductive events and plays key roles in ovulation, zygote implantation, and subsequent maintenance of pregnancy (Graham & Clarke, 1997). The influence of FB₁ on porcine GC function as noted by Cortinovis et al. (2014) could be one mechanism whereby this mycotoxin may impair reproductive activity in animals, particularly inhibiting effect on synthesis and secretion of gonadotropins. In rats, FB₁ concentrations at ≥ 10 mg kg⁻¹ diet were found to significantly reduce serum gonadotropin levels without inducing histopathological changes in the ovaries (Gbore et al., 2012).

There exists a high correlation between the milk production by the doe and the growth of the kits because rabbit kits do not show significant feed intake before the age of 18–19 days (Fortun-Lamothe & Gidenne, 2000). Although Pascual et al. (1996) reported increase in milk yield with increase in litter size, the significantly decreased milk yield with increase in the concentrations of FB₁ in does fed diets supplemented with vitamin E, which had similar litter size, could be attributed to the mycotoxin in the diets. FB₁ bears a remarkable structural resemblance to sphinganine and sphingosine and the mycotoxin inhibits sphingosine metabolism in tissues, leading to an accumulation of sphingoid bases, which are intermediates in sphingolipid biosynthesis (Wang et al., 1991). Sphingolipids are critical components for cell survival and homeostasis (Hirabayashi, 2012). High levels of sphingolipids have been reported in brains (Assi et al., 2013) and its metabolism has a key neuropathological impact (Mencarelli & Martinez-Martinez, 2013). The disruption of sphingosine metabolism and alterations in the amounts of any of these intermediates by FB₁, which could potentially alter neuronal function (Fonteh et al., 2006) and result in a variety of biological effects (Penner et al., 1998), may be responsible for the significant decline in serum gonadotropins and consequently reduced milk yield of does, with increased dietary FB₁ as observed in this study.

5 Conclusion

Economic loss in livestock production due to mycotoxins in animal feeds is a major problem in the tropics, including Nigeria. This study revealed that reproductive and pre-weaning performances of rabbits exposed to diets containing FB₁ could be impaired at concentrations above 5.0 mg kg⁻¹ of feed. Inclusion of vitamin E at 100 mg kg⁻¹ of feed in FB₁-contaminated feeds is recommended as a nutritional strategy to alleviate the reproductive-depressing effect of the mycotoxin in female rabbits, especially at concentrations of ≥ 5.0 mg FB₁ kg⁻¹.
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