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Effect of Dietary Fumonisin B_1 on Histomorphology and Histopathology of Organs of Pubertal Boars

F.A. Gbore

Department of Environmental Biology and Fisheries, Adekunle Ajasin University, Akungba, Akoko, Nigeria

Abstract: The effects of dietary fumonisin B_1 (FB_1) on weight characteristics and pathology of organs of growing pigs were assessed using 24 male weanling pigs of 8-9 weeks of age in a 6-month feeding trial. The animals were randomly assigned to four diets containing 0.2, 5.0, 10.0 and 15.0 mg FB_1/kg as the control diet, diets 1, 2 and 3 respectively. The feeding trial was divided into 3 physiological phases (weanling, peri-pubertal and pubertal). At the end of the feeding trial, all the pubertal boars were sacrificed by stunning and decapitation and carefully eviscerated to collect the organs (the kidneys, liver, spleen and testes) and samples of small intestine. The organs collected from each animal were weighed. Selected organs and tissues collected from sacrificed were processed for histology. Dietary FB_1 significantly ($p<0.05$) reduced the gross and relative weights of livers, spleens and kidneys of boars. Dietary FB_1 also altered the histomorphology of the organs, which was concentration-dependent for all organs and tissues examined. All the animals exposed to the diet containing the highest FB_1 concentration (diet 3) had severe splenic atrophy and/or lymphoid depletion, liver necrosis and/or lesion, intestinal mucosal erosion and testicular necrosis and/or Sertoli cells degeneration as compared to those fed the control. The progressive intestinal mucosal erosion with increased dietary FB_1 observed in this study may be an indication of the role FB_1 can play in non-specific gastrointestinal tract hypofunction in animals. This study has shown that dietary exposure to FB_1 at a concentration of about 5.0 mg/kg or more for a six-month period is a potential health risk that may induce histomorphological and histopathological response in growing pigs.

Key words: Fumonisin · histomorphology · histopathology · organ and boars

INTRODUCTION

Fusarium verticillioides (Sacc.) Nirenberg (= *F. moniliforme* Sheld.), one of the most prevalent fungi associated with dietary staples intended for human and animal consumption throughout the world [1, 2], produces the novel mycotoxins, fumonisins. Maize, a major cereal in livestock feeds, has been reported by Shephard *et al.* [3] to be the only commodity that contains significant amounts of fumonisins. Hence, the potential for fumonisins to be found in feeds and feedstuffs is high.

In general, the consumption of mycotoxin-contaminated feed by animal may result in an unhealthy situation comprising liver damage with marked bile duct proliferation and a decrease production or even death. Fumonisin produces a wide range of biological effects, some of which are specific for particular organs or species and some are common to all investigated animals [4]. Fumonisin is widely distributed in tissues of animals following ingestion; it can thus be transmitted to the human food chain. However, only the liver and kidney have been reported [5-7] to retain small but persistent (and biologically active) amounts of ^{14}C-fumonisin based on measured radioactivity. Elevated serum enzyme levels indicative of liver damage in ponies had been reported by Wilson *et al*. [8].

With the above in mind, coupled with a survey of contemporary literature revealing increasing wave of fumonisin contamination of feeds and feedstuffs [9], this investigation was designed with the aims to assess the histomorphological and histopathological characteristics of organs and tissues of growing pigs fed to dietary FB_1.

MATERIALS AND METHODS

Experimental materials and operations: Fumonisin-contaminated maize grains, cultured with *Fusarium verticillioides*, were generated according to the method

Corresponding Author: Dr. F.A. Gbore, Department of Environmental Biology and Fisheries, Adekunle Ajasin University, Akungba, Akoko, Nigeria

described by Nelson and Ross [10] at the Plant Pathology Laboratory at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Three diets containing 5.0, 10.0 and 15.0 mg FB_1/kg constituting diets 2, 3 and 4 respectively were formulated using ground *Fusarium*-cultured maize substituted for ground, autoclaved non-cultured maize in various proportions. With the control diet, containing 0.2 mg FB_1/kg, the diets were used in a 6-month feeding trial. The FB_1 concentrations were determined using the fumonisin qualitative test kit (Neorgen Corp., USA)

Twenty-four male Large White weanling pigs (about 8-9 weeks of age) were randomly, in a Completely Randomized Design, assigned to each of the 4 dietary treatments, such that each treatment had 6 animals. The feeding trial was divided into 3 physiological phases (weanling, peri-pubertal and pubertal). The gross composition of the treatment diets, fed during weanling, peri-pubertal and pubertal's phases (for 6, 10 and 8 weeks respectively) are shown in Table 1 and satisfied the nutrient requirements of the animals at the various physiological phases as recommended by National Research Council [11]. The animals were fed their respective diets *ad libitum* daily at 0800 and 1600h. Cool, fresh and clean water was made available throughout the experimental period.

Histomorphology and histopathology of organs and tissues: At the end of the feeding trial, all the pubertal boars were sacrificed by stunning and decapitation and carefully eviscerated to collect the organs (the kidneys, liver, spleen and testes) and samples of small intestine.

Table 1: Gross composition (%) of the test diets for the various physiological phases

	Physiological phase		
Ingredient	Weanling	Peri-pubertal	Pubertal
*Maize	40.00	30.00	20.00
Soybean meal	20.00	15.00	8.50
Palm kernel cake	20.00	25.00	25.00
Wheat offal	14.00	14.30	5.00
Rice husk	-	11.00	17.80
Fish meal	3.00	2.00	1.00
**Fixed ingredients	2.70	2.70	2.70
Total	100.00	100.00	100.00
Analysed nutrients:			
Crude fibre (%)	5.35	9.82	10.83
Crude protein (%)	20.38	17.97	15.30
DE (Kcal/kg)	2701.80	2269.11	2240.61

*Mixture of *Fusarium*-cultured and non-cultured maize in various proportions to achieve desired dietary FB_1 levels for each treatment

**Contained Dicalcium phosphate (1.50), Oyster shell (0.05), Salt (0.45) Minerals/Vitamins premix (0.20), Methionine (0.01) and Lysine (0.04)

The organs collected from each animal were weighed. Selected organs and tissues collected from sacrificed boars exposed to dietary fumonisin B_1 were processed routinely for histology [12]. Slides of the spleen, liver, kidney, small intestine and testis of individual animals were read with the assistance of Pathologists at the Department of Veterinary Pathology, University of Ibadan, Ibadan, Nigeria.

Statistical analysis: The design used for this experiment is Complete Randomization Design (CRD). Data collected were subjected to statistical analysis using analysis of variance procedure of SAS [13]. The treatment means were compared using the Duncan procedure of the same software.

RESULTS

Table 2 showed the summary of the organ characteristics of pubertal boars exposed to varied dietary FB_1. The results revealed that the gross and relative

Table 2: Organ characteristics of pubertal boars exposed to varied levels of dietary FB_1

Parameter	Control 0.2mg FB_1	Diet 1 5mg FB_1	Diet 2 10mg FB_1	Diet 3 15mg FB_1	±Sem
Liver weight (g)	1217.90^{a}	1149.06^{ab}	1145.40^{ab}	981.44^{b}	25.90
Rel.* liver weight (%)	1.98^{a}	1.81^{ab}	1.79^{ab}	1.57^{b}	0.04
Spleen weight (g)	156.73^{ab}	205.70^{a}	86.54^{bc}	66.04^{c}	6.66
Rel.* Spleen weight (%)	0.26^{ab}	0.32^{a}	0.14^{bc}	0.11^{c}	0.02
Right kidney weight (g)	95.88^{a}	89.02^{ab}	88.98^{ab}	77.38^{b}	1.91
Rel.* right kidney wt. (%)	0.16^{a}	0.14^{ab}	0.14^{ab}	0.12^{b}	0.03
Left kidney weight (g)	104.58^{a}	93.08^{ab}	96.13^{ab}	83.90^{b}	2.71
Rel.* left kidney wt. (%)	0.17^{a}	0.15^{ab}	0.15^{ab}	0.13^{b}	0.04
Paired kidney weight (g)	200.46^{a}	182.04^{ab}	185.02^{ab}	161.28^{b}	4.62
Rel.* paired kidney wt. (%)	0.33^{a}	0.29^{ab}	0.29^{ab}	0.25^{b}	0.01
Right testis weight (g)	175.38	186.80	157.56	167.86	5.61
Rel* right testis weight (%)	0.28	0.29	0.25	0.27	0.01
Left testis weight (g)	178.76	188.46	159.09	169.85	5.73
Rel* left testis weight (%)	0.29	0.30	0.25	0.28	0.01
Paired testes weight (g)	354.15	375.25	316.64	337.71	11.33
Rel* paired testes weight (%)	0.57	0.59	0.50	0.55	0.02

^{abc}: Means on the same row with different superscripts differ significantly (p0.05) *Relative to live weight

Table 3: Histopathology of organs and tissue of pubertal boars exposed to dietary FB_1 [no (%)]

Parameter	Control 0.2mg FB_1	Diet 1 5mg FB_1	Diet 2 10mg FB_1	Diet 3 15mg FB_1
Splenic atrophy / lymphoid depletion	0 (0)	2 (33.33)	1 (16.67)	6 (100)
Liver necrosis /lesion	0 (0)	0 (0)	4 (66.67)	6 (100)
Kidney lesion/ necrosis	0 (0)	0 (0)	0 (0)	3 (50)
Intestinal mucosal erosion	1 (16.67)	2 (33.33)	5 (83.33)	6 (100)
Testicular necrosis/ Sertoli cells degeneration	0 (0)	1 (16.67)	6 (100)	6 (100)

weights of livers, spleens and kidneys of boars were significantly ($p<0.05$) influenced by dietary FB_1. Table 3 shows the histopathology of organs and tissue of pubertal boars exposed to dietary FB_1. The results showed that dietary FB_1 altered the histomorphology of the organs, which was concentration-dependent for all organs and tissues examined. All the animals exposed to the diet containing the highest FB_1 concentration (diet 3) had severe splenic atrophy and/or lymphoid depletion, liver necrosis and/or lesion, intestinal mucosal erosion and testicular necrosis and/or Sertoli cells degeneration as compared to those fed the control.

DISCUSSION

Liver has been reported [14, 15] to be a target organ for fumonisin in both rats and mice. The significant reduction in both the absolute and relative weights of liver and kidneys in this study are in agreement with Pollman *et al*. [16] who found linear decline in these organ weights in starter pigs fed 0, 1.2, 2.4 and 3.6ppm deoxynivalenol (a *Fusarium* mycotoxin) from contaminated wheat. These researchers, however, did not find significant differences in the absolute spleen weights, but reduced spleen weights in broiler chicks fed diets containing 10mg pure FB_1/kg, or diets formulated from *F. verticillioides* culture material had been reported by Espada *et al*. [17]. Recently, Swamy *et al*. [18] reported that the weights of liver and kidney, expressed as a percentage of body weight, were lower in pigs fed diets containing grains naturally contaminated with *Fusarium* mycotoxins than in those fed the control diet.

The result of the histopathology of organs of pubertal boars exposed to dietary FB_1 revealed that the severity of liver and kidney necrosis/lesion, intestinal mucosal erosion, splenic atrophy, as well as testicular necrosis/Sertoli cells degeneration increased with increased dietary FB_1 in animals fed diets containing maize grains inoculated with *F. verticillioides.* These findings corroborate the findings reported in relevant literatures. Histopathological changes in the liver characterized by scattered single-cell hepatocellular necrosis [14, 19] and variability in nuclear size [20] have been reported in rats. Also, histopathological abnormalities in liver and kidney have been reported in horses orally dosed with pure fumonisins, maize screenings naturally contaminated with fumonisins, or culture material containing known amounts of fumonisins [8, 21-23].

A 4-week exposure of Sprague–Dawley rats to aqueous extracts of *F. verticillioides* cultures (containing fumonisins) resulted in decreased relative liver weights and microscopic liver lesions [24] and Voss *et al*. [25] reported renal lesions accompanied by decreased relative kidney weight in male Fischer-344 rats fed $\geq$ 27mg/kg diet for 4 weeks. Inhibition of hepatocyte proliferation was also observed in rats after dietary exposure to $\geq$ 50mg FB_1/kg diet by Gelderblom *et al*. [26].

The lower organ weights of pubertal boars fed diets containing maize grains cultured with *F. verticillioides* compared to the controls may be combined adverse effects of dietary FB_1 on DMI, nutrient digestibility and absorption by the growing animals. Similarly, the concentration-dependent severity in histopathological abnormalities of the organs as the dietary FB_1 increased may be attributed to the systemic toxicity of the toxin. The reduced organ weights observed in this study are contradictory to the report of Trenholm *et al*. [27], who observed a significant increase in liver and kidney weights in pigs fed 3.9, 5.0 and 8.7 ppm deoxynivalenol (DON) (a *Fusarium* mycotoxin) from contaminated wheat for 7 weeks.

The effect of mycotoxins on organ weights seems to be dependent on the age of animals, duration of exposure of animals to the mycotoxins and dose of the mycotoxins. In short-term studies with rats, rabbits and mice, disruption of sphingolipid metabolism occurs at or below the fumonisin dosages that cause liver or kidney lesions [28-30]. In rats and mice dosed with fumonisins, the increase in free sphinganine concentration in the kidney and/or liver is closely correlated with the extent of severity of lesions [28-30]. This suggests that the effect of FB_1 on organ weights and the severity of the pathology in organs or animals seem to correlate well with disruption of sphingolipid biosynthesis and inhibition of which Yoo *et al*. [31] have shown a concentration-dependent association with fumonisin.

It is evident from this study that testes weights appeared not to be influenced by dietary FB_1. It has been suggested [32] that differences in tissue specificity may

be due to differing susceptibility to the adverse cellular effects of disrupted sphingolipid metabolism. For example, the testis may have different abilities to metabolise or eliminate free sphinganine or to compensate for depletion of complex sphingolipids, such as the blood-testis barrier.

The progressive erosion of the intestinal mucosa of the experimental pigs with increased dietary FB_1 is in agreement with the finding of Ewuola *et al*. [33] that observed caecal mucosal erosion induced by fumonisin in rabbits. These findings may be attributed to the inhibition of sphingolipid synthesis, a condition that has been reported [31] to adversely influence normal epithelial morphology and inhibition of cell proliferation [34]. The progressive intestinal mucosal erosion with increased dietary FB_1 observed in this study may be an indication of the role FB_1 can play in non-specific gastrointestinal tract hypofunction in animals.

CONCLUSIONS

Based on the findings in this study, diets containing about 5.0mg FB_1/kg and above may alter organ weight characteristics with severe pathological response indicating systemic toxicity of the toxin. The adverse influence of dietary FB_1 on normal epithelial morphology of the small intestine suggests that chronic ingestion of dietary FB_1 by growing pigs may result in progressive intestinal mucosal erosion.

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