

Full Length Research Paper

Evaluation of subchronic dietary fumonisin B₁ on nutrient digestibility and growth performance of rats

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Fumonisin B₁ (FB₁), a toxin produced by *Fusarium verticillioides* (*Fusarium moniliforme*) and other *Fusarium* species which grow on maize worldwide, has been documented to cause various physiological responses in animals. Thirty-nine female Wistar rats randomly assigned to three treatment groups were used to assess the effects of dietary FB₁ on nutrient utilization and growth performance. Each group received one of the three diets containing 0.20, 10.0 and 20.0 mg FB₁/kg constituting diets 1, 2 and 3, respectively. The animals were weighed weekly and proximate chemical compositions of the diets and the faecal samples collected from the rats on each diet were determined using standard methods. Dietary FB₁ significantly ($P < 0.05$) influenced nutrient digestibility, feed intake, feed conversion efficiency and relative weight gain. Rats fed diets 2 and 3 had relative weight gains of 87.2 and 66.2% of the rats fed diet 1, respectively. Rats on diet 1 were about 104.5 and 160.6% more efficient in feed conversion compared to those on diets 2 and 3, respectively. Dietary exposure to FB₁ at a concentration of about 10 mg/kg or higher for a period of 35 days is a potential health risk that reduced nutrient utilization by adversely affecting proper nutrient digestion, absorption and/or metabolism, resulting in poor growth rates in Wistar rats. This study revealed that adverse effects of FB₁ on nutrient digestibility and utilization play a significant contributory role in poor growth performance usually associated with animals exposed to diets containing FB₁.

Key words: Fumonisin B₁, growth performance, mycotoxin, nutrient digestibility, rats.

INTRODUCTION

Maize (*Zea mays* L.), as a main energy source, is the major cereal used in the production of livestock feeds and it is reported to be particularly vulnerable to degradation by mycotoxigenic fungi (Lillehoj, 1987; Munkvold and Carlton, 1997). *Fusarium verticillioides* (Sacc.) Nirenberg (*F. moniliforme* Sheld.) is one of the most prevalent toxigenic fungi associated with dietary staples such as maize for human and animal consumption worldwide (Nelson et

al., 1991; Kedera et al., 1992). The fungus is present in virtually all maize samples (Marasas et al., 2001).

Fumonisin are mycotoxins produced principally by *F. verticillioides* (Gelderblom et al., 1988; Marasas et al., 2001). Worldwide distribution of fumonisins in maize, feeds, and foodstuffs and their implications in human and animal health has been comprehensively reviewed (Dutton, 1996; Marasas, 1993, 1996; Marasas et al., 2001). Pathogenic effects of fumonisins include fatal diseases in farm and laboratory animals such as equine leukoencephalomalacia (ELEM) (Wilson et al., 1990), porcine pulmonary oedema (PPE) (Harrison et al., 1990), hepatotoxicity, hepatocarcinogenicity (Gelderblom et al., 1991, 2001) and nephrotoxicity (Norred et al., 1996; Bucci et al., 1998). Several naturally occurring fumonisins are known; fumonisin B₁ (FB₁) 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, followed by

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Abbreviations: FB₁, Fumonisin B₁; ELEM, equine leukoencephalomalacia; PPE, porcine pulmonary oedema; HPLC, high performance liquid chromatography; DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; CF, crude fibre; NFE, nitrogen-free extract; FCR, feed conversion ratio; TER, transepithelial resistance.

Table 1. Gross composition (%) of the experimental diets.

Ingredient	Diet 1	Diet 2	Diet 3
Non-inoculated maize	60.00	56.00	52.00
Inoculated maize*	-	4.00	8.00
Soybean meal	20.00	20.00	20.00
Wheat offal	12.25	12.25	12.25
Fish meal	5.00	5.00	5.00
Dicalcium phosphate	1.25	1.25	1.25
Vegetable oil	0.50	0.50	0.50
Oyster shell	0.25	0.25	0.25
Minerals/vitamins premix**	0.20	0.20	0.20
Salt	2.50	2.50	2.50
Calculated nutrients			
Crude Fibre (%)	5.23	5.22	5.20
Crude Protein (%)	20.08	20.03	20.01
DE*** (kcal/kg)	2972.48	2972.48	2972.48

*Infected with *Fusarium verticillioides* inoculums; **To provide per kg diet: Vitamin A (10,000 i.u.), vitamin D (20,000 i.u.), vitamin E (5 i.u.), vitamin K (2.5 mg), choline (350 mg), folic acid (1 mg), manganese (56 mg), iodine (1 mg), iron (20 mg), copper (10 mg), zinc (50 mg) and cobalt (1.25 mg); ***calculated values.

fumonisin B₂ (FB₂) and B₃ (Murphy et al., 1993; Norred, 1993). Consequently, toxicological studies on the fumonisins have been concentrated on FB₁ (Gbore, 2009).

The mode of action of fumonisins is primarily explained by interference with the *de novo* synthesis of complex glyco-sphingolipids (Wang et al., 1991) which results in disturbances of cellular processes such as cell growth, cell differentiation and cell morphology, endothelial cell permeability and apoptosis (EC, 2000). Significant adverse effects of FB₁ on food consumption, body weights and body weight gains in animals, especially rats, have been well documented (Bondy et al., 1998; Voss et al., 1998; Gelderblom et al., 1988, 1994; NTP, 2001; Swamy et al., 2002; Theumer et al., 2002). However, the impact of FB₁ on nutrient digestibility and its role in the observed depressed feed consumption, body weights and body weight gains in rats exposed to FB₁ have not been reported. This study was designed because fumonisins are known to be consumed by farm animals and is the causative agents or suspected contributing factor in farm animals' diseases and poor growth performances. The objective of this study was to assess the effects of dietary exposure to FB₁ on nutrient digestibility by rats and its contributory role in poor growth performance which is usually associated with exposure to fumonisins.

MATERIALS AND METHODS

Experimental site and animals

Thirty-nine mature female Wistar rats (*Rattus norvegicus*) obtained from a commercial breeder in Benin City, Edo State, Nigeria, were housed in wire mesh rat cages at the Animal House of the Department of Biochemistry, Adekunle Ajasin University, Akungba

Akoko, Nigeria, where the feeding trial was carried out between May and July, 2009. The study was approved by the Institutional Committee on the care and use of laboratory animals.

FB₁ production and experimental diets

Maize grits in 500 g quantities were placed into autoclavable polypropylene bags and soaked with 200 ml of distilled water for 2 h, then autoclaved for 1 h at 121°C and 120 kPa. The autoclaved maize grits were then cultured with a toxigenic strain of *F. verticillioides* (MRC 286) obtained from the Plant Pathology Laboratory of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria to produce FB₁ as described previously (Nelson et al., 1994). Uncultured maize grits and the cultured maize grits were used to formulate three diets. In addition to FB₁, dietary contents of aflatoxin and other common *Fusarium* mycotoxins including deoxynivalenol (DON, vomitoxin), T-2 toxin and zearalenone were also checked by using mycotoxin quantitative CD-ELISA test kits (Neogen, Lansing, MI, USA) and reconfirmed by using High Performance Liquid Chromatography (HPLC) analyses as described by Shephard et al. (1990). All other common mycotoxins screened were found to be negligible. The concentrations of FB₁ in the diets were adjusted to 0.2, 10.0 and 20.0 mg/kg constituting diets 1 (control diet), 2 and 3, respectively.

Experimental model

After 3 weeks of physiological adjustment period, the rats were allocated by weight to each of the three diets (n = 13 rats per treatment) such that the initial body weights of the rats were uniform across the dietary groups. The gross compositions of the pelleted diets are shown in Table 1. The rats were provided with fresh clean water and appropriately weighed feed daily, and the weights of feed portions given and left uneaten after 24 h were determined. The body weight was determined weekly on a weighing scale (Ohaus Corp., Pine Brook, NJ, USA) with a precision of 0.05 g. The body weight gain of each rat was determined weekly as the weight difference in comparison to the weight in the previous week.

Table 2. Apparent nutrient digestibility (%) of Wistar rats fed with varied levels of dietary fumonisin B₁.

Nutrient	Diet 1	Diet 2	Diet 3
Dry matter	50.00 ± 0.28 ^a	41.93 ± 0.23 ^{ab}	40.95 ± 0.35 ^b
Crude protein	53.29 ± 0.30 ^a	48.08 ± 0.40 ^a	35.96 ± 0.20 ^b
Crude fibre	77.43 ± 0.44 ^a	72.92 ± 0.62 ^a	55.11 ± 0.30 ^b
Ether extract	51.92 ± 0.30 ^a	48.26 ± 0.53 ^b	32.13 ± 0.18 ^c
Ash	63.44 ± 0.36	57.76 ± 0.31	55.96 ± 0.47
Nitrogen-free extract	98.41 ± 0.08 ^a	97.68 ± 0.13 ^b	97.69 ± 0.20 ^b

^{abc}: Means on same row with different superscripts differ significantly ($P < 0.05$).

During the last seven days of the experimental period, faecal droppings from nine rats on each diet were collected, weighed, mixed and aliquots taken daily. The daily aliquots and the respective feed samples for each animal were dried in an air-circulatory oven at 105°C for 24 h (to determine their moisture contents) and stored for further analysis.

The chemical compositions of the experimental diets and faecal samples collected, which were used to calculate the apparent digestibility of dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), crude fibre (CF), ash and nitrogen-free extract (NFE), were determined by the methods of AOAC (1995).

Statistical evaluation

Data from this study were analyzed by one-way analysis of variance procedure of SAS (1999). The treatment means were compared using the Duncan procedure of the same software and results giving $P < 0.05$ were considered significantly different.

RESULTS

The apparent nutrient digestibility of Wistar rats fed with different concentrations of dietary FB₁ (Table 2) showed that the apparent digestibility values for rats on diet 1 were generally higher than those on diets 2 and 3 for each variable. The CP and CF digestibility values of rats on diets 1 and 2 were significantly ($P < 0.05$) higher than the nutrient digestibility values of those on diet 3, containing the highest FB₁ concentration. The DM and NFE digestibility values of the rats on diet 3 were significantly ($P < 0.05$) lower than those on diet 1, which were however not statistically ($P > 0.05$) different from those on diet 2. The significantly lower EE digestibility values obtained for rats on diets 2 and 3 were about 7.1 and 38.1% lower than the digestibility value of EE of those on diet 1, respectively. The apparent ash digestibility values, though not significantly different across the treatments ($P < 0.05$), appeared to decline with increased dietary FB₁.

The performance variables of rats exposed to different concentrations of dietary FB₁ are shown in Table 3. The final live weights, relative weight gains (expressed as percent of initial live weights), daily feed intake and the feed conversion ratio (FCR) were significantly ($P < 0.05$) influenced by the dietary FB₁ concentrations. The daily weight gain was, however, not significantly ($P > 0.05$)

influenced by the dietary FB₁ but seemed to decline with increase in dietary FB₁ concentrations. The rats fed diets 2 and 3 had relative weight gains of about 87.2 and 66.2% of the rats fed diet 1, respectively. The mean daily feed intake of rats on diet 3 was significantly ($P < 0.05$) higher than the daily feed intake of those on diet 2, which was not different from those on diet 1. Rats fed diet 1 were about 104.5 and 160.6% more efficient in feed conversion compared to those on diets 2 and 3, respectively.

DISCUSSION

The significant decline in digestibility values for all nutrients, apart from ash, with increased dietary FB₁ by the Wistar rats in this study suggested altered normal digestive and nutrient absorptive functions of the epithelial lining of the gastrointestinal tract. Adverse influences of dietary fumonisin on normal epithelial morphology were observed by Yoo et al. (1992) and Merrill et al. (1993). Similarly, progressive erosion of the epithelial lining of the small intestine resulting from chronic exposure to *F. verticillioides* culture material containing 1.69 - 1.90 mg fumonisin/kg was observed in rabbits by Ewuola et al. (2003), while Gbore (2007) observed progressive erosion of the intestinal mucosa in experimental pigs fed with increased concentrations of dietary FB₁. Mahfoud et al. (2002) reported that the mycotoxin patulin altered the barrier function of the intestinal epithelium by inducing a rapid and dramatic decrease of transepithelial resistance (TER) in two distinct human intestinal cell lines, which have been widely used as *in vitro* models for the human intestinal epithelium in transport and toxicity studies by Maresca et al. (2001). Mahfoud et al. (2002) reported that unrelated mycotoxins can induce a similar deleterious effect on the intestinal barrier function as demonstrated by Maresca et al. (2001) using ochratoxin A. All these observations are an indication of the role mycotoxins, including FB₁, can play in non-specific gastrointestinal tract hypofunction in animals. These observations might have been responsible for the decline digestibility values of the nutrients with increased dietary FB₁. The inability of the rats fed diets 2 and 3 to efficiently utilize the essential nutrients in their feeds might have

Table 3. Performance of female Wistar rats exposed to different levels of dietary fumonisin B₁.

Variable	Diet 1	Diet 2	Diet 3
Initial weight (g)	168.93 ± 1.41	168.83 ± 1.51	169.87 ± 1.32
Final weight (g)	228.09 ± 1.10 ^a	220.43 ± 1.76 ^b	209.05 ± 1.50 ^b
Relative weight gain* (%)	35.02 ± 0.93 ^a	30.56 ± 1.16 ^b	23.06 ± 1.09 ^c
Daily weight gain (g)	1.69 ± 0.08	1.47 ± 0.07	1.12 ± 0.08
Daily feed intake (g)	17.64 ± 0.31 ^{ab}	16.03 ± 0.06 ^b	18.77 ± 0.68 ^a
FCR** (g/g)	10.43 ± 1.86 ^b	10.90 ± 0.86 ^b	16.75 ± 0.85 ^a

*Expressed as percent of initial live weight; **feed conversion ratio; ^{abc}means on same row with different superscripts differ significantly (P < 0.05).

accounted for the observed significantly lower relative weight gains for these rats in this study.

In this study, the final live weights and relative weight gains of the rats declined significantly (P < 0.05) with increase in the dietary FB₁ after the 35-day feeding experiment. The general decline in the weight gain is an indication of the role that FB₁ could play in animal nutrition and subsequent weight gain. The lower relative change in live weights of rats fed diets 2 and 3 compared to controls are in agreement with results from similar studies that dietary FB₁ depressed feed consumption and live weight gain in rats (Bondy et al., 1998; Voss et al., 1998; Gelderblom et al., 1988, 1994; NTP, 2001; Swamy et al., 2002; Theumer et al., 2002) and lowered feed conversion efficiency in animals (Gbore, 2009).

Drastic alterations in serum protein values are often observed, as reported by Coles (1986), in association with either kidney and liver diseases or gastrointestinal diseases involving interference with protein digestion and absorption. However, Bauer et al. (1974) reported that a low albumin level might be due to increased loss of albumin in the urine, decreased formation in the liver or insufficient protein intake. The significantly lower protein digestibility values by rats on diets 2 and 3 containing *Fusarium*-inoculated grains might be an indication of the role FB₁ could play in serum protein alterations as earlier reported in animals (Ewuola and Egbunike, 2008; Gbore and Egbunike, 2009) and fish (Gbore and Akele, 2010) exposed to dietary fumonisin. Serum protein and albumin syntheses have been reported to be related to the amount of available protein in the diet (Iyayi and Tewe, 1998).

The nutritive state of the animal may be dependent not only on the proper and adequate intake of protein building materials in the diet but may also be a reflection of the nutritive state existing within the animal body, reflecting alterations in metabolism. Reduced serum protein profiles and tissue protein synthesis often observed in animals exposed to *Fusarium* mycotoxins (Dänicke et al., 2006; Ewuola and Egbunike, 2008; Gbore and Egbunike, 2009) might be a reflection of altered dietary protein metabolism, including digestibility and subsequent absorption of the nutrients in the intestine of the animals as well as biosynthesis in the body systems in animals. Patulin, a secondary metabolite of a number

of fungal species, has also been reported to interfere with protein biosynthesis (Arafat and Musa, 1995). The mycotoxin was found to inhibit several key biosynthetic enzymes including RNA polymerase and aminoacyl-tRNA synthetases (Arafat et al., 1985). In a study on channel catfish fed FB₁, Lumlerdacha et al. (1995) reported that osmium staining indicated that the vacuoles in the hepatocytes of the catfish contained lipid, which was suggestive of impaired lipid metabolism. The results of nutrient digestibility in this study revealed that besides altering the digestive and absorptive functions of the intestinal epithelium, dietary FB₁ can as well perturb protein and lipid metabolism in animal systems.

The significantly reduced feed efficiency of rats fed diet 3 compared to those on diet 1 despite the higher feed intake by rats on diet 3, could be attributed to immunological response by the animals as a result of the dietary FB₁. This finding corroborates the study of Gbore and Egbunike (2007) that reported reduced feed utilization in growing pigs fed diets containing ≥5 mg FB₁/kg. The finding suggests that FB₁ could have adverse effects on feed intake and nutrient utilization and the resultant weight gain in animals as earlier reported (Theumer et al., 2002; Gbore and Egbunike, 2007; Gbore, 2009), which were probably due to adverse effects of FB₁ on intestinal function in nutrient digestibility and absorption in rats.

Diets containing ≥10.0 mg FB₁/kg, generally reduced nutrient utilization by adversely affecting proper nutrient digestion, absorption or metabolism and subsequent growth performance in Wistar rats. Further studies are however warranted to clarify how dietary FB₁ contributes to lowered serum proteins profiles commonly observed in exposed animals by altering dietary nutrient digestion and absorption from the intestinal tract or interferes with protein and lipid metabolism in animal systems.

Conclusion

Fumonisin B₁ is a potent inhibitor of ceramide synthase, an enzyme critical to the metabolism of sphingolipids (Wang et al., 1991; Merrill et al., 1993). A multitude of biological activities and cellular functions has also been reported for sphingolipids (Wang et al., 1992; Merrill et

al., 1996). It is reasonable, therefore, to assume that many of the physiological effects of FB₁ are related to disruption of these pathways. However, this study revealed that dietary FB₁ concentrations of ≥ 10.0 mg/kg had adverse effects on nutrient utilization and subsequently contributed to reduced growth performance in the Wistar rats. The implication of this is that the adverse effects of dietary FB₁ on nutrient digestibility and utilization also play a significant contributory role in poor growth performance usually observed in animals exposed to FB₁.

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