Growth indices and haematological parameters of weanling pigs fed dietary fumonisin B₁

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Abstract

The effects of dietary fumonisin B₁ (FB₁), an environmental mycotoxin produced by fungi of the genus *Fusarium*, on performance and haematological parameters was investigated using 24 male Large White weanling pigs. The animals were divided into four groups, with six animals per group. Each group received one of the four diets containing approximately 0.20, 5.0, 10.0 and 15.0 mg FB₁/kg containing diets 1-4 respectively, in a completely randomized design. Blood sample was collected from the ear vein of each animal at the end of the 4-month feeding trial for haematological analysis. Lower feed intake and a non-significant ($P>0.05$), but FB₁ concentration-dependent decrease in live and daily weight gains in animals fed diets 2, 3 and 4 were observed. Also, dietary FB₁ generally had significant influence on haematological parameters. The daily and the final live weight gains of animals fed diet 4 were 92.3 and 92.6% respectively, of the control values. Animals fed diets containing 10 and 15 mg FB₁/kg suffered significantly ($P<0.05$) reduced erythrogenesis, while only animals fed the diet containing the highest FB₁ concentration suffered significantly ($P<0.05$) reduced concentration of red blood cells, and consequent anaemia, with unpaired respiratory capacity. The investigation demonstrated that dietary FB₁ concentration above 5.0 mg/kg is a potential health risk that may induce adverse physiological response, which can be assessed using haematological parameters in growing pigs. The study suggests that the United States Food and Drug Administration's recommended maximum levels of 10 ppm (=10 mg/kg) total fumonisins in the diet is above the no-observable-effect level (NOEL) for swine.

Key words: Anaemia, Erythrogenesis, Fumonisin B₁, Haematology and Growing Pigs.

Introduction

Fumonisin are a group of environmental mycotoxins produced by fungi of the genus *Fusarium*, especially *F. moniliforme* (= *F. verticillioides* Sacc.) and *F. proliferatum* (Benzaidenhou et al., 1988; Nelson, 1992).

Maize, which is the major cereal used in the production of livestock feeds as the main energy source, has been reported (Lillehoj, 1987) to be particularly vulnerable to degradation by these mycotoxigenic fungi. Shephard et al. (1996) reported that maize is the only commodity that contains significant amounts of fumonisins; hence

the potential for these toxins to be found in feeds and foodstuffs is high. Six fumonisin analogues have so far been reported (Cawood *et al.*, 1991; Gelderblom *et al.*, 1992) but fumonisin B₁ (FB₁) is the major fumonisin produced in culture (Bezuidenhout *et al.*, 1988) and naturally occurring in maize, maize-based feeds and foods (Rheeder *et al.*, 1992). Consequently, toxicological studies on the fumonisins have been concentrated on FB₁.

Fumonisin B₁ is known to be toxic to domestic animals. Equine leukoencephalomalacia (Kellerman *et al.*, 1990) and porcine pulmonary edema (PPE) (Oswiller *et al.*, 1992) are some of the most frequent diseases caused by fumonisins. In parallel with increasing dietary FB₁, body weight and average daily weight gain have been shown to decrease in chicks (Liedink *et al.*, 1992) and rats (Bondy *et al.*, 1998) as well as reduced weight gain and feed intake in rabbits (Fawola *et al.*, 2003). Fumonisin has also been shown to cause hepatic diseases in rats (Gelderblom *et al.*, 2001), reduced weight of organs (Gbere *et al.*, 2007), lowered nutrient utilization (Gbere and Egbunike, 2007), reduced sperm production and reproductive performance (Gbere and Egbunike, 2008a; Gbere, 2009) in growing pigs. Additionally, the occurrence of FB₁ in foods has been associated with a high incidence of human oesophageal cancer (Rheeder *et al.*, 1992; Makula *et al.*, 1996).

Blood is an important index of physiological and pathological changes in an organism, and it is also used in assessing the body's ability to respond to haematological upset. Harper *et al.* (1979) established that blood constituents are

affected by feed components; assessment of blood metabolites therefore provides a milieu for chemical studies of animal nutritional status. Blood contains a myriad of metabolites and other constituents, which provide a valuable medium for clinical investigation and assessment of nutritional status of human beings and animals. Hence, the use of blood and serum biochemical parameters in medical nutritional assessment and survey of animals (Olorode *et al.*, 1991).

Pigs are particularly susceptible to fumonisins, as demonstrated by data from several experimental and naturally acquired outbreaks (Haschek *et al.*, 1992; Colvin and Harrison, 1992; Rotter *et al.*, 1996). In view of the physiological effects of this mycotoxin in animals, this study was designed to assess the effects of varied levels of dietary FB₁ on haematological parameters of growing pigs with a view to suggesting safe dietary FB₁ level below which may cause significant immunosuppression without overt toxicity in growing pigs.

Materials and Methods

Autoclaved maize grains were cultured with a toxigenic strain of *F. verticillioides* (MRC 286) inoculum obtained from the Plant Pathology Laboratory of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, to produce FB₁ as described by Nelson *et al.* (1994). The ground cultured maize was substituted for ground autoclaved, noncultured maize in various proportions to formulate four diets containing 0.2, 5.0, 10.0 and 15.0 mg FB₁/kg, as determined using the fumonisin quantitative CD-ELISA test kit (Neogen, Lansing, MI, USA), constituting diet 1 (control diet) and diets 2, 3 and 4, respectively.

Twenty-four clinically normal male Large White weaned piglets of about 8–9 weeks of age averaging 6.94 ± 0.26 kg were sourced from the Piggery Unit of the Teaching and Research Farm, University of Ibadan, Ibadan, Nigeria. The animals were housed individually in concrete-floor indoor pens, and were randomly divided into four groups (six replicates per group), with each group receiving one of the four diets after a 2-week physiological adjustment period.

The feeding trial, which was conducted at the Animal Physiology Unit of the Teaching and Research Farm, University of Ibadan, Ibadan, Nigeria, lasted 4 months. The feeding was divided into weanling (starter) and peri-pubertal (grower) phases. The composition of the diets fed for 6 and 10 weeks during the weanling and peri-pubertal phases are shown in tables 1 and 2 respectively. The diets satisfied the nutrient requirements of the animals at the two physiological phases as recommended by the National Research Council (NRC, 1998).

The use of *Escherichia coli* culture material, frequently employed by other workers (Colvin and Harrison, 1992; Osweiler *et al.*, 1992) for toxicological studies in swine was chosen for this study due to the large amount of FB₁ necessary to run the experiment.

The animals were fed their respective diets *ad libitum* and fresh water was made available throughout the experimental period. Feed consumption for each animal was monitored daily and live weight changes of the animals were taken weekly throughout the experimental

period. The dietary FB₁ levels in diets 1 (control), 2, 3 and 4 resulted in average daily FB₁ intake of 0.2, 6.0, 11.5 and 17.0 mg/kg live weight respectively based on consumption by animals. At the end of the feeding trial, blood sample was collected from the ear vein of each animal into Monoject® vacutainer containing Ethylenediaminetetraacetic acid (EDTA) for haematological analysis.

The packed cell volume (PCV) was determined by spinning about 75 µl of each blood sample in heparinised capillary tube in a haematocrit centrifuge for about 5 minutes, and was then read on haematocrit reader as described by Jensen *et al.* (1989). Erythrocyte (RBC), leukocyte (WBC) and the differential counts were determined using haemocytometer as described by Laub (1981). The haemoglobin (Hb) concentration and the blood constants: mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were determined using cyanmethaemoglobin method and appropriate formula respectively as described by Jain (1986).

The data obtained were subjected to one-way ANOVA procedure of SAS® (1999) and the Duncan's multiple range tests of the same software was used to separate all means at 5% probability level.

Results

The performance of growing pigs exposed to varied dietary FB₁ levels are shown in Table 3. The mean dry matter intake (DMI), and the final

Table 1: Gross composition (%) of the weanling pigs' test diets

	Control (1)	Diet 2	Diet 3	Diet 4
<i>Ingredient</i>	<i>0.2mg FB₁</i>	<i>5.0mg FB₁</i>	<i>10.0mg FB₁</i>	<i>15.0mg FB₁</i>
Non-inoculated maize	49.00	59.26	56.52	54.78
Inoculated maize*	-	1.74	3.48	5.22
Soybean meal	20.00	20.00	20.00	20.00
Palm kernel cake	20.00	20.00	20.00	20.00
Wheat offal	14.30	14.30	14.30	14.30
Fish meal	3.00	3.00	3.00	3.00
Bone meal	1.50	1.50	1.50	1.50
Oyster shell	0.50	0.50	0.50	0.50
Salt	0.45	0.45	0.45	0.45
Minerals/Vitamins premix	0.20	0.20	0.20	0.20
Melchiorine	0.01	0.01	0.01	0.01
Lysine	0.04	0.04	0.04	0.04
Total	100.00	100.00	100.00	100.00
Calculated nutrients:				
Crude Fibre (%)	5.35	5.35	5.35	5.35
Crude Protein (%)	20.38	20.38	20.38	20.38
DE** (Kcal/Kg)	2701.80	2701.80	2701.80	2701.80

* Inoculated with *Fusarium verticillioides*; ** Digestible Energy.

Table 2: Gross composition (%) of the perinatal pigs' test diets

	Control (1)	Diet 2	Diet 3	Diet 4
<i>Ingredient</i>	<i>0.2mg FB₁</i>	<i>5.0mg FB₁</i>	<i>10.0mg FB₁</i>	<i>15.0mg FB₁</i>
Non-inoculated maize	30.00	28.26	26.52	24.78
Inoculated maize*	-	1.74	3.48	5.22
Soybean meal	15.00	15.00	15.00	15.00
Palm kernel cake	25.00	25.00	25.00	25.00
Wheat offal	14.30	14.30	14.30	14.30
Rice husk	11.00	11.00	11.00	11.00
Fish meal	2.00	2.00	2.00	2.00
Bone meal	1.50	1.50	1.50	1.50
Oyster shell	0.50	0.50	0.50	0.50
Salt	0.45	0.45	0.45	0.45
Minerals/Vitamins premix	0.20	0.20	0.20	0.20
Melchiorine	0.01	0.01	0.01	0.01
Lysine	0.04	0.04	0.04	0.04
Total	100.00	100.00	100.00	100.00
Calculated nutrients:				
Crude Fibre (%)	9.82	9.82	9.82	9.82
Crude Protein (%)	17.97	17.97	17.97	17.97
DE** (Kcal/Kg)	2269.11	2269.11	2269.11	2269.11

* Inoculated with *Fusarium verticillioides*; ** Digestible Energy.

live and daily weight gains (DWG) were dietary FB₁ concentration-dependent. These parameters decreased with increased dietary FB₁. Although there was no significant difference in the daily DM of the animals for all treatment groups, the efficiency of feed conversion of the animals fed diets 3 and 4 were significantly ($P < 0.05$) lower than those fed diets 1 and 2. The DWG of the animals fed diet 4 was significantly ($P < 0.05$) lower than those fed diets 1, 2 and 3. The animals fed diets 3 and 4 had significantly ($P < 0.05$) lower feed conversion efficiency than those fed diets 1 and 2. The efficiency of feed conversion for animals on diets 2, 3 and 4 were 4.2, 14.5 and 8.8% lower than those on diet 1 respectively.

The haematological indices of growing pigs fed varied levels of dietary FB₁ for 16 weeks are as shown in Table 4. The erythrocyte, Hb, and PCV values decreased significantly ($P < 0.05$) with increased dietary FB₁ concentrations while the leukocyte and MCV values significantly ($P < 0.05$) increased with increased dietary FB₁ concentrations. The monocytes, MCH and MCHC did not follow any particular trend, while the differential leukocyte counts were not influenced by dietary FB₁.

Discussion

There are numerous reports on the weaning piglets (Umoru *et al.*, 2007; Odeh *et al.*, 2009) and growing and finishing pigs (Hassan *et al.*, 2002; Damiye *et al.*, 2004) are particularly sensitive to feed-borne *Fusarium* mycotoxins. In the present study, the growth of the pigs, expressed as DWG and the daily DM generally declined with increase in the dietary FB₁ over the 4 months period of experimental feeding across the groups. The results also showed a dietary FB₁ concentration-dependent decline in final live weights. The overall feed efficiency over the 4 months generally declined with increased dietary FB₁, implying that the main effect of fumonisin was to reduce feed utilization in addition to reduced feed consumption as reported by Rortter *et al.* (1994) and Gboye and Eghunfide (2007). These suggest that fumonisin could play adverse effects in feed intake and nutrient utilization and the resultant growth performance in animals, especially growing pigs.

The negative effect of FB₁ sometimes observed on feed intake could be due to repellant taste or odour of contaminated feeds or altered brain neurochemistry as observed by Swamy *et*

Table 3: Performance of growing pigs exposed to varied levels of dietary FB₁

Parameter	Control (1) 0.2mg FB ₁	Diet 2 5.0mg FB ₁	Diet 3 10.0mg FB ₁	Diet 4 15.0mg FB ₁	SEM
Initial Live Weight (kg)	6.8	7.0	6.9	7.1	0.26
Final Live Weight (kg)	60.7	58.5	55.9	56.2	1.27
Daily Dry Matter Intake (g)	1159.0	1142.6 ¹	1208.8	1202.8	12.12
Daily Weight Gain (g)	559.6 ¹	529.9 ²	527.1 ²	516.0 ²	14.47
Feed Conversion Ratio	2.07 ¹	2.16 ¹	2.42 ²	2.27 ²	0.11

^{1,2} Means on same row with different superscripts differ significantly ($P < 0.05$).

Table 4: Haematological indices of growing pigs exposed to various levels of dietary FB₁

Parameter	Control (I) 0.2mg FB ₁	Diet 2 5.0mg FB ₁	Diet 3 10.0mg FB ₁	Diet 4 15.9mg FB ₁	SEM
Erythrocytes (10 ¹² /L)	7.75 ^a	7.25 ^{ab}	7.17 ^b	6.67 ^c	0.07
Haemoglobin (g/L)	131.89 ^a	127.20 ^{ab}	123.00 ^{ab}	119.50 ^c	1.40
Mean Cell Volume (fl)	47.18 ^a	49.85 ^a	51.49 ^a	53.38 ^a	0.57
MCH [*] (pg)	17.08 ^a	17.68 ^a	18.54 ^a	17.90 ^a	0.11
MCHC ^{**} (%)	36.15 ^a	34.29 ^a	36.46 ^a	33.02 ^a	0.39
PCV ^{***} (%)	36.67 ^a	36.53 ^a	36.50 ^a	34.17 ^a	0.24
Platelets (10 ⁹ /L)	20.63	22.28	21.02	21.30	0.38
Leucocytes (10 ⁹ /L)	14.98 ^a	14.98 ^a	17.35 ^a	19.47 ^a	0.35
Neutrophils (10 ⁹ /L)	1.90	2.22	2.83	2.53	0.08
Eosinophils (10 ⁹ /L)	0.50	0.42	0.51	0.52	0.13
Lymphocytes (10 ⁹ /L)	12.38	12.16	13.85 ^a	15.10 ^a	0.92
Monocytes (10 ⁹ /L)	0.42 ^b	0.30 ^a	0.26 ^{bc}	0.52 ^a	0.11

^{ab} Means in the same row with different superscripts differ significantly ($P < 0.05$).

* Mean Corpuscular Haemoglobin; ** Mean Corpuscular Haemoglobin Concentration; *** Packed Cell Volume.

et al. (2002) in pigs consuming deoxynivalenol-contaminated feed. In addition, digestive and metabolic disorders caused by FB₁ are probably involved in reducing voluntary intake. Fawcra *et al.* (2003), for example, observed mild cell proliferations in the cortex, stomach and small intestinal tunica mucosa and caecal mucosa erosion induced in rabbits fed *B. verticilloides* culture material containing micro doses of fumonisin for eight weeks. This result is an indication of the role that FB₁ can play in cell proliferation and growth, and by extension, feed intake and the resultant less weight gain in animals, especially growing pigs.

The results in this study revealed that the animals exposed to diet 4 suffered significantly from the synthesis of red blood cells (erythropoiesis). The mean MCV and MCH values of the animals fed diet 4 were within the reported reference ranges of 50–68 fl and 17.8–18.8 pg by Thorn (2000) and Mitruka and Rawnsley (1977) respectively for young adult boars. The characteristic normal MCV and MCH

with decreased number of erythrocytes and low PCV observed for animals fed diet 4 indicated normocytic anaemia. This might have contributed to the significantly lower daily weight gain of animals fed diet 4.

Haemoglobin (Hb), an iron-containing conjugated protein, has been described (Mitruka and Rawnsley, 1977) to have physiological function of transporting oxygen and carbon dioxide. The value of Hb for animals fed diet 4 was significantly lower than for the control but comparable to values obtained for animals on diets 2 and 3. However, the values obtained across the groups were within the 100–160 g/L reference values for young adult boars reported by Blund (1995) and Thorn (2000). The results however, suggested that the animals fed diet 4 suffered significantly depressed respiratory capability and gas-exchange capacity of the RBCs.

The comparable values observed for the platelets across the groups revealed that dietary FB₁ levels between 5–15 mg/kg were not

sufficient to cause splenic atrophy – a characteristic pathologic thrombocytopaenia which, among other factors, was reported by Coles (1986) to cause thrombocytopenia.

The circulating leukocyte counts of all the experimental animals were within the $11.0 - 22.0 \times 10^9/L$ reference values reported by Blood (1995) and Horn (2000) for swine. This indicates that all the experimental animals in this study did not suffer from leukocytosis, although the animals fed diet 4 had the highest leukocyte counts ($P < 0.05$). Leukocytosis, according to Coles (1986), may result from intoxications including those produced by metabolic disturbances among others. This suggests that the animals exposed to the diet containing 15.0 mg FB/kg might have suffered intoxication from altered metabolism as reported by Ghore and Eghonike (2007) thereby contributing to the significantly lower daily weight gain of the animals compared to other groups.

The generally significant influence of the dietary FB₁ treatment on some of the haematological parameters investigated agreed with the findings of Rotter *et al.* (1996) that reported changes in selected haematological parameters in pigs at dietary levels as low as 1mg/kg pure fumonisin and those of Espada *et al.* (1997) who revealed altered haematological parameters in broilers fed diets formulated from *F. verticillioides* culture material containing 10 mg/kg and 30 mg/kg fumonisin B₁. Also, altered haematological parameters have been reported in milks (Powell *et al.*, 1996), rabbit bucks (Iwunola and Eghonike, 2008) and pigs (Ghore and Eghonike, 2008b) fed dietary fumonisin.

The results of this present study, however,

contradict the report of Rotter *et al.* (1996) and Parchment (1998). Zamborczy *et al.* (2002) and Ogunlade *et al.* (2004), that considered fumonisins as non-haematotoxic mycotoxins. The variability in haematological parameters of livestock fed fumonisin-containing diets might be due to different doses of fumonisin administered, the species of livestock used, or the length of exposure of such livestock to the mycotoxin. In general, the symptoms observed in an animal following the consumption of mycotoxin-contaminated feed, according to Marasas and Nelson (1987), depend on the amount of toxin in the feed, the period for which the feed is ingested and the nutritional status of the feeds.

The dietary FB₁ concentrations used in this study bracketed the published United States Food and Drug Administration's recommended maximum level of 10 ppm (= 10 mg/kg diet) total fumonisins for swine (USFDA, 2001). The results in this present study suggest that the USFDA recommended maximum level is above the no-observable-adverse-effect level (NOAEL) for swine.

Conclusion

This study has shown that dietary exposure to FB₁ at a concentration of about 10 mg/kg or more for a four-month period may result in significantly reduced synthesis and concentration of RBC and consequent anaemia with impaired respiratory capacity in boars. The study also demonstrates that growing pigs exposed to chronic dietary fumonisin concentration of about 10 mg/kg and above may suffer depressed feed utilization and consequently reduced weight gain.

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