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ASSESSMENT OF HAEMATOLOGICAL, SERUM BIOCHEMICAL AND HISTOPATHOLOGICAL EFFECTS OF SUBCHRONIC DIETARY FUMONISIN B_1 IN RATS

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Abstract

Context: Fumonisin B_1 (FB_1), a mycotoxin produced by *Fusarium verticillioides* and other *Fusarium* species that grow on maize worldwide, has been documented to cause various physiological responses in animals. Consumption of lesser amounts of fumonisins at levels below those that cause overt toxicity may exert haematological, serum biochemical and/or histopathological effects in animals.

Objective: The effects of dietary FB_1 on haematology, serum biochemistry and histopathology were assessed in female Wistar rats in a short-term toxicity study.

Materials and Methods: Thirty-nine mature female Wistar rats (*Rattus norvegicus*) weighing between 167.5 – 170.5 g were used in the study. The rats were assigned to diets containing 0.2, 10.0 and 20.0 mg FB_1/kg constituting diets 1, 2 and 3, respectively. After 14 days of feeding, blood samples were obtained from four rats per treatment. The rats were sacrificed by cervical dislocation, eviscerated for organ collections and subsequently processed for histology.

Results: Significant differences in feed consumption and body weight gains were not observed. The final live weight of the rats, however, seemed to decline with an increase in dietary FB_1 levels. Significant ($P<0.05$) alterations were observed in the haematological and serum biochemical parameters with increasing levels of dietary FB_1. Diets containing different FB_1 concentrations, the decreased values of PCV, Hb, erythrocyte and monocyte counts could be attributed to the FB_1 effects on the blood-forming tissues in animals placed on diets 2 and 3 as compared to those fed diet 1. Also, histopathological changes were observed in the livers, kidneys, spleens and hearts of rats fed diets 2 and 3.

Conclusion: This study revealed that the No-observable adverse effect level (NOAEL) of dietary FB_1 above which may cause significant physiological changes without overt toxicity for short-term toxicity study in female Wistar rats is <0.74 mg/kg bw per day.

Keywords: Blood, Rat, Mycotoxin, *Fusarium verticillioides*, Fumonisin B_1, Histopathology.

Introduction

Mycotoxins are a chemically diverse group of fungal secondary metabolites that have a wide variety of toxic effects on human and animal health. Nearly all mycotoxins are reported to be cytotoxic, disrupting various cellular structures such as membranes, and interfering with vital cellular processes including protein synthesis (Guerre *et al*. 2000). In many regions of the world, dietary staples such as cereal grains contain low levels of mycotoxins. The impacts of regular low level intake of mycotoxins on animal health is likely to be significant with a number of possible consequences including impaired growth and development, immune dysfunction and the disease consequences of alterations in DNA metabolism (Voss *et al*. 1996).

Fusarium verticillioides (Sacc.) Nirenberg (=*F. moniliforme* Sheld.), one of the most prevalent toxigenic fungi associated with dietary staples such as maize intended for human and animal consumption worldwide (Nelson *et al*. 1991), produces one of the most potent mycotoxins, fumonisins. *F. verticillioides* is present in virtually all corn samples (Marasas *et al*. 2001). A survey of livestock feed ingredients in several parts of the world revealed that maize has the highest fumonisins level (EHC 2000).

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In general, when mycotoxin-contaminated maize is mixed with the livestock feed and consumed by animals, it may result in an unhealthy situation ranging from decreased nutritive value of feed, poor feed conversion, reduced growth, organ damage and even death. The symptoms depend on the type and dosage of the mycotoxin, age, sex and species of the animal, the period for which the mycotoxin-contaminated feed is ingested and the nutritional status of the feed (Nelson *et al.* 1993).

Fumonisins have been implicated as a causative agent in several animal and human diseases. The toxins have also been associated with different kinds of mycotoxicoses in domestic animals, such as leukoencephalomalacia in equines (Ross *et al*. 1993), pulmonary edema in pigs (Colvin and Harrison 1992), hepatocellular carcinoma in rats (Gelderblom *et al.* 1994) and apoptosis in the livers of rats (Proctor 2000). Several naturally occurring fumonisins are known. Fumonisin B_1 (FB_1) has been reported to be the most abundant and most toxic representing approximately 70% of the total concentration in naturally contaminated foods and feeds, followed by fumonisins B_2 (FB_2) and B_3 (Murphy *et al.* 1993, Norred 1993).

Though histopathologic effects of fumonisins in rats have been reported by several research groups (Gelderblom *et al*. 1988, Voss *et al*. 1995, Bondy *et al.* 1998, Tolleson *et al*. 1996), the experimental models used to date to study fumonisin toxicity on laboratory animals are based mainly on the production of acute mycotoxicoses. However, consumption of lesser amounts of fumonisins at levels below those that cause overt toxicity may exert haematological, serum biochemical and/or histopathological effects in animals.

The no-observable-adverse-effect-level (NOAEL) for female Sprague-Dawley rats was reported to be above 15 mg (Voss *et al*. 1993) and 27 mg dietary fumonisin kg^{-1} for female Fischer rats in 28 day studies (Voss *et al*. 1995). It has been shown that significant differences may exist in response to fumonisins among various rat strains (Voss *et al*. 2001). It is essential, therefore, to assess subchronic haematological, serum biochemical and histopathologic responses in Wistar rats fed FB_1-containing diets below the NOAELs for rats of other strains. The objective of the present study was to assess the effects of varied concentrations of dietary FB_1 above which may cause significant physiological changes without overt toxicity in Wistar rats with a view to suggesting safe dietary FB_1 levels for female Wistar rats which are used for breeding programmes.

Materials and Methods

Experimental site and animals: Thirty-nine mature female Wistar rats (*Rattus norvegicus*) weighing between 167.5 – 170.5 g were obtained from a commercial breeder in Benin City, Edo State, Nigeria. The rats 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 experiment was carried out. Further laboratory analyses were carried out at the Department of Chemical Pathology, University College Hospital, and Department of Veterinary Pathology, all of the University of Ibadan, Ibadan, Nigeria. This study was approved by the local ethics committee and was performed in accordance with "Guide for the Care and Use of Laboratory Animals" (National Research Council 1996).

Fumonisin B_1 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 FB_1 as described previously (Nelson *et al.* 1994). The cultured and uncultured maize grits were used to formulate three diets. The contents of common *Aspergillus* mycotoxin (aflatoxin) and *Fusarium* mycotoxins including deoxynivalenol (DON, vomitoxin), zearalenone, T-2 toxin, and FB_1 in the diets were assayed using mycotoxin quantitative CD-ELISA test kits (Neogen, Lansing, MI, USA) and HPLC as described by Shephard *et al*. (1990). The concentrations of FB_1 in the diets were subsequently adjusted to 0.2, 10.0 and 20.0 mg kg^{-1} and referred to as diets 1 (control diet), 2, and 3, respectively. All other

common mycotoxins determined were found to be negligible. The dietary FB_1 doses used in this study were based on a preliminary dose-range-finding study by Collins *et al*. (1998).

Experimental model: After three weeks of physiological adjustment, the rats were randomly allocated to each of the three diets (n = 13 rats per treatment). 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 the feed portions given and left uneaten after 24 h were determined. After 14 days of feeding, blood samples were obtained by intracardiac puncture from rats from each treatment into two sets of vacutainer tubes. A set containing a calculated amount of ethylene diamine tetraacetic acid (EDTA) was used for haematological study and the other without EDTA were covered and centrifuged at 4000 rpm for 10 minutes. The separated sera were decanted and deep-frozen for serum biochemical analyses.

Determination of food consumption and body weight gain: The animals were individually housed and feed consumption for each animal was measured daily by difference between the daily feed supplied and refusal, and live weight changes of the animals were determined weekly as the weight difference in comparison to the weight in the previous week.

Haematological and serum biochemical measurements: Haematological parameters (erythrocyte counts, total leukocyte counts, packed cell volume (PCV), haemoglobin (Hb) concentrations and the blood constants - mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC)) and biochemical parameters (total serum protein, albumin, globulin and albumin - globulin ratio) were determined as described by Ewuola and Egbunike (2008). The serum total protein, albumin, globulin and albumin-globulin ratio were determined as earlier described (Gbore and Egbunike 2009). All determinations were carried out in duplicate.

Examination of tissues: Four randomly selected rats from each treatment were killed by cervical dislocation. The sacrificed rats were carefully eviscerated to collect the organs (kidney, liver, spleen, and heart). For examination by light microscopy, the collected tissues were fixed in 10% neutral buffered formalin (pH 7.2) before dehydration in ten changes in ethanol of different concentrations ranging from 70 to 100% at 1-hr intervals. After dehydration, the tissues were cleared in two changes of chloroform before infiltration and embedding in molten wax (60°C) for 12 h. Thereafter, the tissues were blocked in paraffin wax and later sectioned using a microtome. Paraffin sections (4µm) of the tissue samples were stained with haematoxylin and eosin.

Statistical evaluation: Data from these studies were analyzed by one-way analysis of variance procedure of SAS (2001). The treatment means were compared using the Duncan procedure of the same software and results giving P values of < 0.05 were considered significantly different.

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

Ingredient	Diet 1	Diet 2	Diet 3	Ingredient	Diet 1	Diet 2	Diet 3
1. Non-inoculated maize	60.00	56.00	52.00	8. Oyster shell	0.25	0.25	0.25
2. Inoculated maize*	-	4.00	8.00	9. Minerals/vitamins premix**	0.20	0.20	0.20
3. Soybean meal	20.00	20.00	20.00	10. Salt	2.50	2.50	2.50
4. Wheat offal	12.25	12.25	12.25	*Analysed nutrients:*			
5. Fish meal	5.00	5.00	5.00	1. Crude Fibre	5.23	5.22	5.20
6. Dicalcium phosphate	1.25	1.25	1.25	2. Crude Protein	20.08	20.03	20.01
7. Vegetable oil	0.50	0.50	0.50	3. 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), cobalt (1.25 mg). ***Calculated values.

Results

Effects on feed consumption, body weight and feed conversion efficiency: Daily observations for 14 days indicated no readily detectable alterations in the general state of any of the rats. Significant differences in feed consumption and body weight gains were not observed. The average feed consumption for the period

were 17.6, 16.0, and 18.8 g, respectively, for animals fed diets 1, 2, and 3, respectively. The final live weight of the rats, however, seemed to decline with an increase in dietary FB_1 levels. The animals fed diets 1, 2, and 3 had final live weights of 187.7, 183.0, and 178.7 g, respectively. However, the average daily weight gain and feed conversion ratio of the rats were significantly ($P < 0.05$) influenced by the dietary FB_1 levels. The rats fed diets 2 and 3 had live weight changes of about 75.7 and 46.9 % of those on diet 1, respectively. The animals on diet 1 were about 120.6 and 171.8 % more efficient in feed conversion compared with those on diets 2 and 3, respectively.

Effects on haematology and serum biochemistry: The haematological indices of rats fed diets containing different concentrations of FB_1 are shown in Table 2. The PCV and Hb concentrations of rats fed diet 1 were not significantly different from those of the rats fed diet 2 but significantly higher ($P<0.05$) than those fed diet 3. The erythrocyte counts of rats fed diets 2 and 3 were significantly ($P<0.05$) lower than those fed the control diet. The mean leucocytes and monocyte counts of the rats fed diet 3 were significantly ($P<0.05$) higher than the leucocytes counts of those on the control diet and diet 2.

The serum biochemical parameters of rats fed different concentrations of dietary FB_1 showed that dietary FB_1 significantly ($P<0.05$) altered the total serum protein values. The serum protein profiles were FB_1 concentration-dependent. The mean serum total protein and albumin/globulin values of rats fed diets 1 and 2 were significantly ($P<0.05$) higher than the serum protein and albumin/globulin values of those fed diet 3. The serum albumin showed an inverse relationship, while the serum globulin exhibited a direct relationship with the dietary FB_1 concentrations. The serum urea significantly ($P<0.05$) increased with an increase in the FB_1 concentrations in the diets. The serum urea of rats fed the control diet was 12.6 and 42.4 % lower than the serum urea of those fed diets 2 and 3. However, the serum creatinine did not follow any particular trend. These results are as shown in Table 3.

Effects on organs: The histopathological examinations of the rats showed modifications by the dietary FB_1. The liver samples of rats fed diet 2 (Fig. 1b), in comparison with those fed diet 1 (Fig. 1a), showed diffuse hydropic degeneration, portal congestion and cellular infiltration by mononuclear cells, while diffuse fatty degeneration, necrosis of hepatocytes and portal cellular infiltration by mononuclear cells were observed in the liver samples of rats fed diet 3 (Fig. 1c). In the kidneys of the rats fed diet 3, cortical congestion, interstitial haemorrhage and protein casts in renal lumen (Fig. 2c) were observed compared with the kidney samples of those fed diet 1 (Fig. 2a) and those fed diet 2 (Fig. 2b) that showed no visible lesion. Marked splenic congestion and moderate depletion of the lymphoid cell population in the spleens of the rats fed diet 2 (Fig. 3b), and marked splenic congestion and depletion of lymphoid cell population in the rats fed diet 3 (Fig. 3c) were observed compared with the spleens of rats fed diet 1 (Fig. 3a). However, no visible lesion was observed in the hearts of rats fed diets 1 and 2 (Figs. 4a and b, respectively), but diffuse muscle fiber disintegration was observed in the hearts of rats fed diet 3 (Fig. 4c).

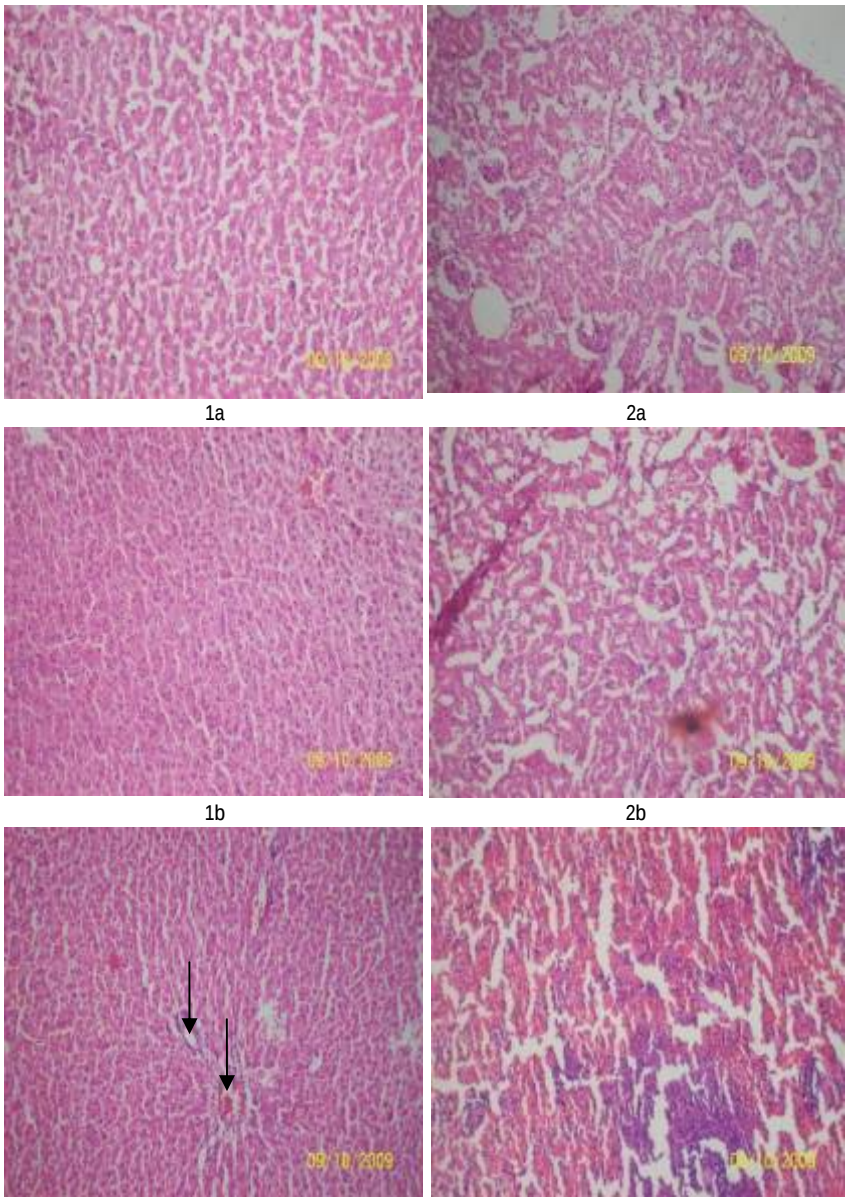
Table 2. Haematological indices of rats fed dietary different concentrations of FB_1

Parameters	Diet 1	Diet 2	Diet 3
Erythrocytes ($x10^{12}$/l)	12.85 ± 0.30^a	9.55 ± 0.49^b	7.70 ± 0.27^b
Haemoglobin (g/l)	175.0 ± 6.10^a	133.0 ± 19.4^{ab}	117.5 ± 17.7^b
PCV (%)	51.50 ± 0.61^a	39.50 ± 1.06^{ab}	31.50 ± 0.61^b
MCV (fl)	40.10 ± 0.10	41.30 ± 4.80	40.90 ± 1.25
MCH (pg)	132.80 ± 0.40	139.10 ± 1.90	152.20 ± 0.05
MCHC (g/dl)	33.15 ± 0.40	33.65 ± 10.30	37.20 ± 2.20
Leucocytes ($\times 10^9$/l)	4.80 ± 1.22^b	5.90 ± 1.50^b	9.75 ± 0.93^a
Neutrophils (%)	31.50 ± 1.70	30.50 ± 0.35	29.00 ± 1.12
Lymphocytes (%)	64.50 ± 1.70	66.00 ± 0.50	66.00 ± 1.00
Basophils (%)	1.00 ± 0.00	1.00 ± 0.00	0.50 ± 0.35
Monocytes (%)	2.00 ± 0.00^b	2.00 ± 0.00^b	3.50 ± 0.35^a
Eosinophils (%)	1.00 ± 0.35	0.50 ± 0.35	1.00 ± 0.35

Means on same row with different superscripts differ significantly ($P<0.05$).

Table 3. Serum biochemistry of rats fed dietary different concentrations of FB_1

Parameters	Diet 1	Diet 2	Diet 3
Total protein (g/l)	8.60 ± 0.45^a	8.60 ± 0.16^a	7.85 ± 0.11^b
Albumin (g/l)	4.32 ± 0.45	4.25 ± 0.35	3.25 ± 0.11
Globulin (g/l)	4.28 ± 0.01	4.35 ± 0.39	4.60 ± 0.01
Albumin/globulin	1.01 ± 0.01^a	0.98 ± 0.01^a	0.71 ± 0.02^b
Urea (µmol/l)	41.50 ± 0.61^c	47.50 ± 1.17^b	72.00 ± 2.40^a
Creatinine (µmol/l)	45.0 ± 1.10	40.0 ± 0.90	45.0 ± 1.11

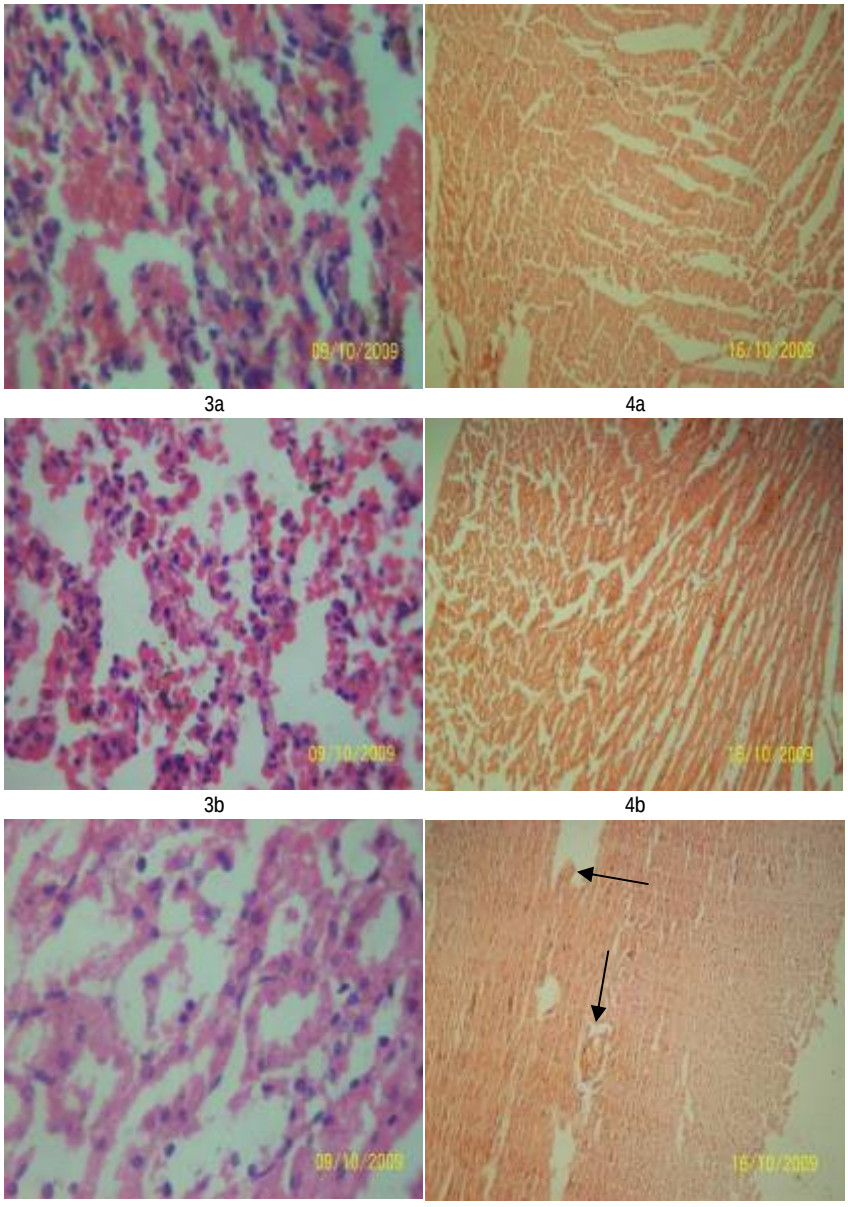


1a 2a

1b 2b

1c 2c

Fig. 1 and 2. Haematoxylin-and-eosin stained section of rat organs exposed to dietary FB_1. Fig. 1b is liver sample of rats fed diet 2, in comparison with those fed diet 1 (Fig. 1a), showed diffuse hydropic degeneration, portal congestion and cellular infiltration by mononuclear cells, while diffuse fatty degeneration, necrosis of hepatocytes and portal cellular infiltration by mononuclear cells were observed in the liver samples of rats fed diet 3 (Fig. 1c). Cortical congestion, interstitial haemorrhage and protein casts in renal lumen were observed in rats fed diet 3 (Fig. 2c) compared with the kidney samples of those fed diet 1 (Fig. 2a) and those fed diet 2 (Fig 2b) that showed no visible lesion.



3a 4a

3b 4b

3c 4c

Figures 3 and 4: Haematoxylin-and-eosin stained section of rat organs exposed to dietary FB_1. Marked splenic congestion and moderate depletion of the lymphoid cell population in the spleens of rats fed diet 2 (Fig. 3b), and marked splenic congestion and depletion of lymphoid cell population in rats fed diet 3 (Fig. 3c) were observed compared with the spleens of rats fed diet 1 (Fig. 3a). No visible lesion was however observed in the hearts of rats fed diets 1 and 2 (Figs. 4a and b, respectively), but diffuse muscle fiber disintegration was observed in the hearts of rats fed diet 3 (Fig. 4c).

Discussion

The lower relative change in the body weight of rats fed on diets 2 and 3, compared with those fed the control diet, may be attributed to the adverse effects of the mycotoxin on feed intake and nutrient utilization as observed in pigs fed dietary FB_1 by Gbore and Egbunike (2007). The general decline in the weight gain over the 14 days is in agreement with results from similar studies that dietary FB_1 depressed live weight gain in animals. Gelderblom *et al*. (1988) reported that the mean body weights of BD IX rats consuming a diet containing 1 g FB_1 kg^{-1} during a 4-week promotion treatment were 50% lower than those of the controls. Also, a declining relative change in body weights with an increase in dietary fumonisin was reported in rabbits fed ≥ 12.30 mg fumonisin kg^{-1} for five weeks (Gbore *et al*. 2006). The change in live weight of 14.17 and 8.79 g for rats fed diets 2 and 3, respectively, compared with 18.73 g for rats fed the control diet for 14 days, was similar to a recent report by Gbore and Akele (2010) that the relative live weight gains decline with an increase in dietary fumonisin in pregnant rabbits exposed to a ≥5 mg fumonisin kg^{-1} diet. In this study, since all the rats were fed *ad libitum* and all the diets were isocaloric and isonitrogenous, the concentration-dependent decline in final live weights indicates the role that FB_1 can play in animal nutrition and subsequent growth performance.

Haematological and serum chemical indices are becoming increasingly important diagnostic tools in veterinary medicine. Haematological indices are a reflection of the effects of dietary treatments on animals in terms of the type, quality and amounts of feed ingested and nutrients available to an animal to meet its physiological and metabolic requirements (Gbore and Akele 2010). In this study, in which *F. verticillioides*-cultured maize was used to formulate diets containing different FB_1 concentrations, the decreased values of PCV, Hb, erythrocyte and monocyte counts could be attributed to the FB_1 effects on the blood-forming tissues in animals placed on diets 2 and 3 as compared to those fed diet 1. The results also revealed that the rats exposed to diets containing *Fusarium*–inoculated maize (diets 2 and 3) suffered significantly from the synthesis (erythropoiesis) and concentration of erythrocyte.

The Hb value of 117.5 g/l observed for rats fed diet 3 in this study was below the normal value of 129.8 g/L reported by Oyewale (1987) for normal rats and 139 g/l reported by Lillie *et al*. (1996) for young normal female rats. Haemoglobin, an iron-containing conjugated protein, has the physiological function of transporting oxygen and carbondioxide. The significantly low Hb concentration observed for rats fed diet 3 in this study confirmed that the animals might have suffered depressed respiratory capability.

The significantly higher leucocytes counts obtained for the rats fed diets 2 and 3 compared to those fed diet 1 were higher than the mean reference value of 5.09 x 10^9/l for young normal female rats (Lillie *et al*. 1996). The results indicate that the animals fed diets 2 and 3 might have suffered leucocytosis. According to Coles (1986), leucocytosis may result from intoxications, including those produced by metabolic disturbance. The leucocytosis observed in rats fed diets 2 and 3 may be attributed to dietary FB_1 effect. The significant dose-dependent increased leucocytes and monocyte counts for rats fed diets 2 and 3 in this study suggest physiological response by the animals to the dietary mycotoxin insult.

An estimation of the total quantity of serum proteins may be utilized as an estimation of the nutritive state of an animal. According to Gbore and Egbunike (2009), the nutritive state may be dependent not only on 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. The significantly decreased serum total protein values and albumin - globulin ratios with increased FB_1 concentrations within 14 days revealed a dietary FB_1 concentration-dependent response to the toxin. The results may indicate the toxin as a protein metabolism inhibitor as reported for sphingolipid synthesis (Riley and Norred 1996). Since all the rats were fed isonitrogenous diets which contain only varied levels of FB_1, the result revealed the roles which dietary FB_1 could play in serum protein alterations, as previously observed in rabbits (Ewuola and Egbunike 2008),

growing swine (Gbore and Egbunike 2009), and fingerlings (Gbore *et al*. 2010) fed different concentrations of dietary FB_1. The results from this study suggest that FB_1 could perturb protein biosynthesis in animal body systems as well.

Liver has been reported to be the primary target organ for toxicity caused by fumonisins in all species tested thus far (EHC 2000). Histopathological changes in livers of rats fed dietary fumonisin characterized by scattered single cell hepatocellular necrosis and variability in nuclear size have been reported (Voss *et al*. 1993). Similarly, Bondy et al. (1998) reported increased incidence of randomly distributed single-cell necrosis in rats gavaged 5 – 75 mg FB_1/kg for 11 consecutive days. These observations are in agreement with the diffuse hydropic degeneration, portal congestion and cellular infiltration by mono-cellular cells and necrosis of the hepatocyte and portal infiltration by mononuclear cell observed in livers of rats exposed to diets 2 and 3 in this study.

Cortical congestion, interstitial haemorrhage and protein casts in renal lumen observed in the kidneys of rats exposed to diet 3 correlate with the reports of Voss *et al.* (1993) that observed apoptotic bodies and necrotic alteration in the tubular epithelial cell in the kidneys of rats fed dietary fumonisin. As observed in this present study, Voss *et al*. (1993) also reported an increase in the capsular space and presence of proteinogenous material in the tubular lumen which was not found in the control rats. Coles (1986) reported that serum creatinine determination has a reputation for being a specific test for the diagnosis and prognosis of progressive renal disease as there are fewer non-renal factors that may influence creatinine. The serum creatinine values obtained in this study across the treatments were, however, not above the reference value of 48.3 µmol/l reported by Lillie *et al*. (1996) for young female rats. The implication of this finding is that the rats might not have suffered from impaired kidneys within the short period of their exposure to dietary FB_1.

Splenic alterations in rats fed diets containing 10 and 20 mg FB_1 kg^{-1} are in contrast to the report of Bondy *et al.* (1998) that observed no histopathological lesions in spleens and hearts of rats gavaged with 75mg FB_1 $kg^{-1}$for 11 consecutive days. Colvin *et al*. (1993) reported cardiotoxic effect as an indirect consequence of fumonisin-induced hepatotoxicity which could cause cardiac failure that altered pulmonary haemodynamics, thereby resulting in pulmonary oedema. The report correlates with the observation in this study in which diffuse muscle fiber disintegration was observed in the hearts of the rats fed diet 3. The different organ response to dietary FB_1 in different species observed by researchers seemed to depend on the dosage of the toxin, the age, species, body weight and sex of the animals, the period for which the feed is ingested and the nutritional status of the feed (Gelderblom *et al*. 1996, Voss *et al.* 1996, NTP 1999).

Conclusion

Results from this study revealed that the exposure of rats to the dietary FB_1 concentrations of ≥10 over a short period lead to leucocytosis, significant reduction in erythrocytes and serum protein syntheses, and histopathological changes in some organs of rats. Based upon feed consumption and body weight data, the diets used for this study provided 0.08, 0.74 and 1.74 mg FB_1/kg BW per day to the rats on diets 1, 2, and 3, respectively. From this present study, the dietary FB_1 concentrations above which may cause significant physiological changes in female Wistar rats without overt toxicity is similar to NOAEL of <0.75 mg/kg BW per day established by Gelderblom *et al*. (1994) for short-term toxicity study in rats.

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