



## Toxicological Influence of Dietary Fumonisin B<sub>1</sub> on Blood Profile of Adult Rabbits

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### SUMMARY

An experiment was conducted to assess the toxicological effects of dietary fumonisin B<sub>1</sub>, a mycotoxin produced by *F. verticillioides* in the culture, on haematology and serum biochemistry of rabbits exposed to fumonisin-contaminated diets. Forty-eight 7 to 8 weeks old male crossbred rabbits were used. The animals were randomly assigned to four treatment diets formulated with *F. verticillioides* cultured maize that produced approximately 5.0, 7.5 and 10.0 mg/kg fumonisin B<sub>1</sub> (FB<sub>1</sub>) constituting diets 2, 3 and 4 respectively, while diet 1 (control) contained no cultured maize in a 210 days feeding trial. Blood samples were collected from the animals through the ear vein, after the feeding trial, for haematology and serum biochemistry. Results revealed that rabbits fed 7.5 and 10.0 mg/kg FB<sub>1</sub> showed significantly ( $P < 0.05$ ) reduced erythropoiesis and concentration of red blood cells, and consequent anaemia. Their leukocyte counts also indicate a condition of leukocytosis. Serum protein synthesis in some animals indicates both hypoproteinemia and hypoalbuminemia conditions. Cholesterol level significantly increases among dietary treatments with increase in the FB<sub>1</sub> level in the diets. Rabbits exposed to 7.5 and 10.0 mg/kg FB<sub>1</sub> showed a hypercholesterolemia condition. Activities of aspartate amino transferase, alanine amino transferase and alkaline phosphatase were significantly ( $P < 0.05$ ) elevated in the serum of treated animals, which

is an indication of liver and kidney toxicity and cell damage. This study indicates that prolonged exposure to a concentration of at least 7.5 mg FB<sub>1</sub>/kg in rabbit diets will depress erythropoiesis and protein synthesis; altered serum biochemical variables and induced leukocytosis, liver and kidney toxicity as well as hypercholesterolemia in rabbits.

Key words: Dietary fumonisin B<sub>1</sub>, toxicity, blood profile, rabbits.

### INTRODUCTION

Maize, a feed ingredient of high caloric value used for ration formulation in livestock industry has been implicated to be vulnerable to degradation by mycotoxigenic fungi. One of such fungi reported to be associated with maize intended for human and animal consumption worldwide is *Fusarium verticillioides* (Sacc. Nirenberg), which produces a mycotoxin known as fumonisin.

Fumonisin is a novel, naturally occurring toxin with hepatocarcinogenic activities (Gelderblom *et al.*, 1994, Mezes and Balogh, 2009). By association, the toxin has been implicated as the cause of various human and animal diseases (Riley *et al.*, 1996). The discovery of this toxin was as a result of home grown maize contaminated with *F. verticillioides* that was associated with the high incidence of human oesophageal cancer in the

southeastern part of Transkei, South Africa (Marasas *et al.*, 1988) that led to the isolation of the toxin. Fumonisin B<sub>1</sub> is the major toxin produced by *F. verticillioides* in the culture (Bezuidenhout *et al.*, 1988; Ross *et al.*, 1992; Rheeder *et al.*, 1992).

A survey of contemporary literature shows increasing wave of fumonisin contamination of feeds and feedstuffs. The consumption of feed contaminated with the toxin by an animal may result in an unhealthy situation and physiological imbalance such as poor feed conversion and efficiency, retarded growth, hormonal changes and even cause mortality depending on the amount of the toxin produced in the feed, the period for which the feed is ingested, the nutritional status of the feed and consumption of sufficient quantities of toxin-containing plant material by susceptible animals (Marasas and Nelson, 1987). Rabbits have been reported to be sensitive to fumonisin intoxication (Mezes and Balogh, 2009) and because of its proximity to humans; they are used as animal model to judge the effect of mycotoxin on humans by inference.

Blood contained a myriad of metabolites and other constituents, which provide a valuable medium for clinical investigation and nutritional status for humans and animals. Blood constituents have been reported to be influenced by dietary components (Olorode *et al.*, 1995); hence, blood constituents are widely used in nutritional evaluation and survey of animals. Reports by Aletor and Egberongbe (1992) and Aletor (1989) indicated that the blood variables most consistently affected by dietary influences include RBC counts, PCV, plasma protein and glucose.

Haematological and serum biochemical indices are index and a reflection of the effects of dietary treatments on the animal in terms of the types, quality and amounts of the feed ingested and were available for the animal to meet its physiological, biochemical and

metabolic necessities (Church *et al.*, 1984; Ewuola *et al.*, 2004). In view of this, the study was designed to assess the effects of *Fusarium*-infected maize-based diets containing different levels of fumonisin B<sub>1</sub> on blood profile of rabbits.

## MATERIALS AND METHODS

### *Experimental animals and diets*

Fumonisin-contaminated maize grains, cultured with *Fusarium verticillioides*, were generated at the Plant Pathology Laboratory, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, according to the method described by Nelson *et al.* (1991) and Ewuola *et al.* (2008). The ground-cultured maize was substituted for autoclaved, noncultured maize in various proportions to formulate four treatment diets containing approximately 0.1, 5.0, 7.5, and 10.0 ppm FB<sub>1</sub>, as quantified by using the fumonisin qualitative text kit (Neorgen Corp., USA) – a competitive direct enzyme-linked immunosorbent assay (CD-ELISA), constituting diets 1 (control), 2, 3 and 4 respectively.

Forty-eight male New Zealand White x Chinchilla rabbits (7 - 8 weeks old, average weight = 757.5±50.0g) were randomly assigned by weight, to the four diets. Each diet has 12 animals in a completely randomized design during the feeding trial lasted 210 days. The animals were housed individually in metal cages, each of 75 x 36 x 30cm in dimension and were fed fumonisin – contaminated diets containing corn, 30%; Rice husk, 30%; Wheat offal, 25%; Soybean meal, 10%; Fish meal, 2%; Calcium diphosphate, 2%; Salt, 0.5%, vitamin-premix, 0.45%; Methionine, 0.03% and lysine, 0.02% as basal diet *ad libitum* daily at 08.00h and 16.00h. Potable water was made available to the animals throughout the experimental period.

### *Blood collection and evaluation*

At the end of the feeding trial, blood sample was collected, through the ear vein, from each of the



animals per treatment into 2 set of separate tubes. One set of tubes contain anticoagulant (EDTA) for haematological study and the second set without EDTA, was centrifuged and serum decanted for serum biochemistry. Packed cell volume (PCV) was determined using haematocrit centrifuge and haematocrit reader. Erythrocyte counts and total leukocyte counts were determined using Neubauer haemocytometer after appropriate dilution. Mean cell volume (MCV), Mean cell haemoglobin (MCH) and Mean cell haemoglobin concentration (MCHC) were determined using appropriate formulae as described by Jain (1986). Serum total protein was determined using Biuret method as described by Kohn and Allen (1995). Albumin was determined using Bromocresol Green (BCG) method as described by Peter *et al.* (1982). The globulin concentration was obtained by subtracting albumin from the total protein while the albumin/globulin ratio was obtained by dividing the albumin value by the calculated globulin value. Cholesterol was determined according to the method of Coles (1987). Aspartate amino transferase (AST), Alanine amino transferase (ALT) and alkaline phosphatase (ALP) activities were determined using spectrophotometric methods as described by Rej and Hoder (1983) and Hoder and Rej (1983).

#### Data analysis

All data obtained from this investigation were subjected to one way analysis of variance of

completely randomized design using statistical software package SAS (1999). Significant means were separated using Duncan multiple range test of the same software. Data are presented as mean  $\pm$  SEM. A *p* value  $<0.05$  was considered significant.

## RESULTS

### *Haematological parameters of the rabbits.*

The haematological response of adult rabbits fed varied levels of dietary  $FB_1$  is shown in Table 1. The haematological variables of rabbits were significantly ( $p<0.05$ ) altered by the dietary  $FB_1$  except for haemoglobin concentration, which was not significant among the group fed diets containing different amount of  $FB_1$ . PCV was significantly ( $p<0.05$ ) lower in rabbits fed diet 4 containing 10.0mg  $FB_1$ /kg as compared to others, while erythrocytes and leukocytes trend were inversely related. Erythrocyte counts were highest in animals fed control diet and significantly ( $p<0.05$ ) lower in rabbits fed diets 3 and 4 containing 7.5 and 10.0 mg  $FB_1$ /kg below the lower limit of reported physiological range for normal rabbits. Leukocyte counts significantly ( $p<0.05$ ) increased with increase in  $FB_1$  level in the diets with animals on diet 4 recording the highest value compared to others. The MCV, MCH and MCHC of rabbits fed diets 3 and 4 were significantly ( $p<0.05$ ) higher than those rabbits that fed the control diet.

**Table 1. Haematological parameters of pubertal rabbits fed varied levels of dietary  $FB_1$  (Mean $\pm$ SEM)**

Parameters	Dietary fumonisin levels			
	0.1mg/kg	5.0mg/kg	7.5mg/kg	10.0mg/kg
Packed cell volume (%)	35.70 $\pm$ 1.04 <sup>a</sup>	36.20 $\pm$ 1.16 <sup>a</sup>	36.20 $\pm$ 1.37 <sup>a</sup>	32.20 $\pm$ 1.02 <sup>b</sup>
Haemoglobin (mmol/L)	6.93 $\pm$ 0.57	7.50 $\pm$ 0.40	7.76 $\pm$ 0.63	7.68 $\pm$ 0.33
Erythrocytes ( $\times 10^6$ /mm <sup>3</sup> )	9.12 $\pm$ 1.16 <sup>a</sup>	7.01 $\pm$ 0.65 <sup>ab</sup>	5.20 $\pm$ 0.27 <sup>b</sup>	4.06 $\pm$ 0.74 <sup>b</sup>
Leukocytes ( $\times 10^3$ /mm <sup>3</sup> )	5.23 $\pm$ 0.47 <sup>b</sup>	5.68 $\pm$ 0.68 <sup>b</sup>	7.73 $\pm$ 0.64 <sup>a</sup>	8.12 $\pm$ 1.18 <sup>a</sup>
Mean corpuscular volume ( $\mu^3$ )	42.23 $\pm$ 0.43 <sup>b</sup>	53.68 $\pm$ 1.24 <sup>ab</sup>	70.37 $\pm$ 0.36 <sup>a</sup>	71.23 $\pm$ 1.21 <sup>a</sup>
MCH (fmol)	0.83 $\pm$ 0.01 <sup>c</sup>	1.10 $\pm$ 0.04 <sup>bc</sup>	1.52 $\pm$ 0.03 <sup>b</sup>	1.69 $\pm$ 0.10 <sup>a</sup>
MCHC (%)	31.49 $\pm$ 0.82 <sup>b</sup>	33.48 $\pm$ 0.37 <sup>ab</sup>	34.56 $\pm$ 0.68 <sup>ab</sup>	38.63 $\pm$ 0.76 <sup>a</sup>

abc: Means in the same row with different superscripts are significantly ( $P < 0.05$ ) different.

SEM: Standard Error of Mean

### ***Serum biochemical and enzymes activities of the rabbits.***

The serum biochemical parameters and enzymes activities of experimental rabbits fed varied levels of dietary FB<sub>1</sub> are shown in Table 2. Serum total protein was depressed significantly ( $p<0.05$ ) with increase in the FB<sub>1</sub> level in the diets, with the highest (66.10g/L) and the least (45.00g/L) values being recorded for rabbits fed control diet and diet 4 respectively. Albumin content of blood serum of rabbits fed diets 1 and 2 was not significantly different from each other but was significantly ( $p<0.05$ ) higher than those fed diets 3 and 4. Calculated globulin content of blood serum was significantly ( $p<0.05$ ) higher in rabbits fed diets 3 and 4 than those fed diet 2 and the control diet.

Serum cholesterol concentration was significantly ( $p<0.05$ ) influenced by the dietary FB<sub>1</sub> in the diets. The cholesterol level was significantly ( $p<0.05$ ) highest in rabbits that consumed diets containing 7.5 and 10.0 mg FB<sub>1</sub>/kg above the upper limit of physiological range (0.52 – 2.40 mmol/L, Mitruka and Rawnsley, 1977) for normal rabbits compared to the control group. Serum enzyme activities of the experimental animals were significantly ( $p<0.05$ ) influenced by the dietary treatments. Serum ALT, AST and ALP were significantly ( $p<0.05$ ) elevated with increase in the FB<sub>1</sub> level in the diets. The enzymes concentration was significantly ( $p<0.05$ ) higher in rabbits fed diets 3 and 4 than those that fed diets 2 and the control.

**Table 2. Serum biochemical and enzyme activities of pubertal rabbits fed varied levels of dietary fumonisin B<sub>1</sub> (Mean±SEM)**

Parameters	Dietary fumonisin levels			
	0.1mg/kg	5.0mg/kg	7.5mg/kg	10.0mg/kg
Total protein (g/L)	60.11±1.27 <sup>a</sup>	60.10±1.91 <sup>ab</sup>	50.40±3.35 <sup>ab</sup>	45.00±1.18 <sup>b</sup>
Globulin (g/L)	12.60±0.64 <sup>b</sup>	15.70±0.52 <sup>b</sup>	22.10±0.59 <sup>ab</sup>	22.40±1.33 <sup>a</sup>
Albumin (g/L)	48.50±1.02 <sup>a</sup>	45.30±1.05 <sup>a</sup>	28.30±0.64 <sup>b</sup>	22.60±0.98 <sup>b</sup>
Albumin/Globulin Ratio	3.85±0.48 <sup>a</sup>	2.83±0.07 <sup>a</sup>	1.28±0.05 <sup>b</sup>	1.01±0.02 <sup>b</sup>
Cholesterol (mmol/L)	1.16±0.05 <sup>c</sup>	1.75±0.08 <sup>bc</sup>	2.49±0.21 <sup>b</sup>	2.90±0.25 <sup>a</sup>
Alanine Amino Transferase (ALT- I.U/L)	69.39±0.74 <sup>b</sup>	67.02±0.35 <sup>b</sup>	80.62±1.05 <sup>ab</sup>	94.06±2.47 <sup>a</sup>
Aspartate Amino Transferase (AST- I.U/L)	50.20±0.59 <sup>b</sup>	58.30±0.69 <sup>b</sup>	86.20±1.28 <sup>a</sup>	102.40±1.18 <sup>a</sup>
Alkaline Phosphatase (ALP- I.U/L)	10.68±0.29 <sup>b</sup>	12.48±0.62 <sup>ab</sup>	14.76±1.20 <sup>a</sup>	17.18±0.10 <sup>a</sup>

abc: Means in the same row with different superscripts are significantly ( $P < 0.05$ ) different.

SEM: Standard Error of Mean

### **DISCUSSION**

The significant reduction in the concentration of erythrocytes and corresponding packed cell volume with increase in the dietary FB<sub>1</sub> in rabbits fed 7.5 and 10.0mg/kg observed in this study was below the lower limit of normal physiological range ( $5.46 - 7.94 \times 10^6/\text{mm}^3$  and 33.0 – 50.0% respectively) reported by Mitruka

and Rawnsley (1977) for rabbits. This is an indication of depressed erythropoiesis and consequent macrocytic anaemic condition in the animals as compared to the control rabbits. The circulating leukocyte counts which were significantly higher in rabbits fed 7.5 and 10.0 mg FB<sub>1</sub>/kg indicated that the animals suffered from leukocytosis. Leukocytosis, as reported



by Coles (1986) may result from intoxications including those produced by metabolic disturbances among others. It could also be as a result of resistance of the animals to toxin assault. This result corroborates the report of Mezes and Balogh (2009) who reported the toxic effect of mycotoxin most especially fumonisin B<sub>1</sub> in rabbits in their review. However, this result was at variance with the reports of Parent-Massin and Parchment (1998), Zomborszky-Kovács *et al.* (2002) and Ogunlade *et al.* (2004) that fumonisins are considered as non-haematotoxic mycotoxins. The variability in the haematology of animals fed fumonisin contaminated diets might be as a result of different doses of the toxin and the length of exposure of animals used to the mycotoxin.

Serum total protein and albumin that significantly decreased with increase in the FB<sub>1</sub> levels in the diets could be attributed to the toxin in the experimental diets. Serum biochemical analysis is used to determine the level of heart, kidney and liver damage and to evaluate protein quality and amino acid requirements in animals (Harper *et al.*, 1999). Reduced level of total protein and albumin below the normal standard value of 5.0 - 8.0 g/dl and 2.8 - 4.0 g/dl respectively (Mitruka and Rawnsley, 1977; CCAC, 1980) were observed for animals exposed to 10.0 mg/kg FB<sub>1</sub> respectively. This indicates that the animals suffered from hypoproteinemia and hypoalbuminemia, which Altman (1979) described as a consequence of abnormal decrease in the concentration of serum albumin. The low level of serum protein indicates some alteration in protein synthesis and metabolism, since serum protein and albumin syntheses are related to the amount of available protein (Iyayi and Tewe, 1998) in the diet. There are two possibilities, probably the toxin had inhibited protein metabolism as reported for sphingolipid synthesis (Riley *et al.*, 1996). Besides, Merrill *et al.* (1993b) reported fumonisin to cause adverse effects on normal epithelial morphology, which may be

responsible for poor amino acid absorption and utilization from the gastro-intestinal tract (Ewuola *et al.*, 2003). The increase in the level of serum globulin with increased dietary FB<sub>1</sub> levels may be a corresponding response to combat the toxin assault as part of body defensive mechanism.

Rabbits fed 7.5 and 10.0 mg/kg FB<sub>1</sub> showed an indication of hypercholesterolemia. Similar increases in serum cholesterol concentration were also observed in most mammalian species administered fumonisin B<sub>1</sub> including rats at 55 mg/kg (Voss *et al.*, 1996), pigs at 1 mg/kg (Rotter *et al.*, 1996), lambs at 273 mg/kg (Edrington *et al.*, 1995), and calves at 105 mg/kg (Osweiler *et al.*, 1993). The mechanism for fumonisin-induced hypercholesterolemia is currently unclear, although studies in treated calve that observed hypercholesterolemia indicated that fumonisin had an early and marked effect on hepatic function (Mathur *et al.*, 2001). However, Ridgway (1995) reported that hypercholesterolemia is not the result of sphinganine- or sphingosine-mediated inhibition of cholesterol esterification. Some authors have reported serum cholesterol to be inversely related to sphingolipids and that increases in sphingolipid lower serum cholesterol level most especially low-density lipoprotein (Hubert *et al.*, 1999). Similar evidence of increase in serum cholesterol level in experimental animals induced by the toxin probably because of inhibition of sphingolipid biosynthesis by the toxin has been reported (Fincham *et al.*, 1992; Yoo *et al.*, 1992; Merrill *et al.*, 1993a; Voss *et al.*, 1993; Wang *et al.*, 1993).

Serum AST, ALT and ALP activities significantly increased with increase in FB<sub>1</sub> levels in the diets. The ALT of rabbits fed 7.5 and 10.0 mg/kg FB<sub>1</sub> were outside the reported physiological range of 48.5 - 78.91 U/L for normal rabbits (Mitruka and Rawnsley, 1977; CCAC, 1980). The AST and ALP of rabbits exposed to 10.0 mg/kg FB<sub>1</sub> exceeded the upper



limit of physiological range (42.5 - 98.0 U/L and 4.10 - 16.20 U/L respectively, Mitruka and Rawnsley, 1977) for normal rabbits. Severe liver and bile duct injury was evident in these fumonisin B<sub>1</sub>-treated rabbits as indicated by increased serum AST and ALP activities, and increased serum ALT activity relative to rabbits in the control group. Serum AST activity also increased in fumonisin-treated rabbits, probably as a result of hepatocellular necrosis observed in same animals (Ewuola, 2009). This result is in agreement with the report of Voss *et al.* (1990) that increase in serum alanine amino transferase, aspartate amino transferase and alkaline phosphates is an indication of damage cause to the liver and kidney by the toxin which involves in cellular destruction. Restum *et al.* (1995) reported that ALT, AST and ALP were greater in minks fed 119 mg/kg FB<sub>1</sub> diet compared to the control. Similar evidence of abnormal increase in serum enzymes activities; probably due to cellular destruction (e.g. hepatocytes) induced by fumonisin has been reported (Voss *et al.*, 1993; Gelderblom *et al.*, 1994 and Mehta *et al.*, 1998). It has been reported that damage done to visceral organs such as heart, kidney and liver by toxic substances usually lead to a diagnostic increase in serum enzymes concentration (Harper *et al.*, 1979).

## CONCLUSION

This study has demonstrated that feeding a diet formulated with an ingredient infected with *Fusarium verticillioides* that would liberate up to 7.50 mg/kg in the diet could be deleterious to the welfare and health of rabbits, as this will depress protein synthesis and metabolism, induce leukocytosis, and elevate serum enzymes (AST, ALT and ALP) activities which result in physiological disorder in the animals. Therefore, effort should be geared towards planting resistant maize varieties to *Fusarium* spp., proper crop storage to avoid mould growth and discarding *Fusarium*- infected or fumonisin-contaminated grains in ration formulation for farm animals.

## ACKNOWLEDGEMENT

The authors wish to thank Dr. R. Bandyopadhyay and Mr. O. Ayinde, Plant Pathologist, International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria for their technical assistance in generating *F. verticillioides* inoculums and the fumonisin quantification in the diets used for this experiment.

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