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Full Length Research Paper

Haematological and serum biochemical response of growing rabbits fed varied levels of dietary fumonisin

***Eguko P. A., Ogunagbe E. B and W. K. Ogbeku**

Department of Medical Science and Pathology, College of Medical Science, Joseph Ayo Babalola University, Ikeji Arakeji, P.M.B 5006, Ilesa, Nigeria.

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An experiment was conducted with 48 crossbred rabbits (49 days old) averaging 757.5 ± 0.50 g to assess haematological and serum biochemical response of growing rabbits exposed to varied levels of dietary fumonisin of 0.1, 5.0, 7.5 and 10 mg fumonisin B_1/kg diet containing in diets 1 (control) 2, 3 and 4 respectively, for a period of 12 weeks. Blood samples were collected from the animals through the ear vein, after the feeding trial, for haematological studies and serum biochemistry. Results showed that packed cell volume, haemoglobin concentration and erythrocytes of rabbits fed diets containing 7.5 and 10.0 mg fumonisin B_1/kg were significantly ($P < 0.05$) lower than those on diets 1 and 2. The white blood cells (WBC) of rabbits fed diet 2 and the control were similar to each other but both were significantly ($P < 0.05$) lower than those fed diets 3 and 4. Among the leukocyte differential counts examined, neutrophils, eosinophils and monocytes were not significantly ($P > 0.05$) different among dietary treatments. However, lymphocytes of animals fed control diet were significantly ($P < 0.05$) lowest (47.83%) as compared to 57.67, 60.00 and 60.50 of animals fed diets 2, 3 and 4, respectively. Platelets and blood constants (MCV, MCH and MCHC) were not significantly ($P > 0.05$) different among the diets. Serum total protein, albumin and albumin-globulin ratio significantly ($P < 0.05$) decreased with increase in the dietary fumonisin levels. Serum total protein of rabbits fed diets 3 (5.20 g/dl) and 4 (5.03 g/dl) were identical but both were significantly ($P < 0.05$) lower than those fed diets 2 (5.60 g/dl) and the control (6.20 g/dl). Urea decreased while creatinine increased apparently with increase in the dietary fumonisin levels. Among the serum enzyme activities examined, AST and ALP were significantly ($P < 0.05$) elevated in the serum of animals fed diets 3 and 4 than those fed diets 2 and the control. ALT, AST and ALP were significantly ($P < 0.05$) highest (94.33, 70.00 and 17.37 i.u/l respectively) in animals fed diet 4 containing 10 mg fumonisin B_1/Kg. These results suggest that fumonisin B_1 above 5.0 mg kg^{-1} in the diet of growing rabbits significantly altered haematological parameters and induced anaemic condition in the animals. It also depressed serum total protein and enhanced abnormal increase in serum enzyme activities, which is an indication of organ toxicity by cellular destruction induced by the toxin most especially when fed at 7.5 and 10.0 mg fumonisin B_1/Kg diet.

Key words: Rabbits, haematology, serum biochemistry, dietary fumonisin B_1.

INTRODUCTION

Moulds, when they grow on agricultural products, produced diverse group of toxic secondary metabolites called mycotoxins (Kedera et al., 1992). They belong to different classes of chemical compounds and they differ in their toxicological effects. The ingestion of food or feed made toxic by these fungal metabolites induced mycotoxicoses. Mycotoxins have been reported to be involved in a chain of physiological disorder in animals (Riley et al., 1996). Among such mycotoxins of significant health concern to both man and animals is fumonisin, produced by *Fusarium verticillioides* (Sacc.) Nirenberg, which grows on every nourishing medium such as cereal crops, most especially maize.

*Corresponding author. E-mail: eguoko.anam13@gmail.com

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

Ingredient	1 (Control)	2 (5.0 mgkg^{-1})	3 (7.5 mgkg^{-1})	4 (10.0 mgkg^{-})
Non-infected maize	30.00	28.26	27.39	26.52
Infected maize*	----	1.74	2.61	3.48
Rice husk	23.00	23.00	23.00	23.00
Wheat offal	27.00	27.00	27.00	27.00
Soybean meal	15.00	15.00	15.00	15,00
Fish meal	2.00	2.00	2.00	2.00
Calcium diphosphate	2.00	2.00	2.00	2.00
Salt	0.50	0.50	0.50	0.50
Premix (grower)**	0.45	0.45	0.45	0.45
Methionine	0.03	0.03	0.03	0.03
Lysine	0.02	0.02	0.02	0.02
Total calculated nutrients	**100.00**	**100.00**	**100.00**	**100.00**
Crude protein (%)	16.11	16.11	16.11	16.11
Crude fibre	10.79	10.79	10.79	10.79
Digestible energy (Kcal/Kg)	2555	2555	2555	2555

*Inoculated with *Fusarium verticillioides*.

**To provide per Kg diet: Vit. A (10,000 i.u), Vit. D (20,000 i.u.), Vit E (5 i.u), Vit. 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).

Maize has been reported to be vulnerable to mycotoxigenic fungi (e.g. *Fusarium* spp.) and the only commodity that contains significant amount of fumonisin (Shephard et al., 1996). This toxin has been implicated by association as the cause of various human and animal diseases (Riley et al*.,* 1996). Consumption of *F. verticillioides* culture materials have induced Equine leucoencephalomalacia (ELEM), Porcine pulmonary edema and hepatotoxic, carcinogenic and weight reduction in rats and rabbits (Marasas et al., 1984; Jaskiewicz et al., 1987; Kellerman et al., 1990; Colvin and Harrison, 1992; Ewuola et al., 2003).

The haematological indices are an index and reflection of the effects of dietary treatments on the animals in terms of the type and amount of feed ingested and were available for the animal to meet its physiological, biochemical and metabolical necessities (Ewuola et al., 2004). The blood contains a myriad of metabolites and other constituents, which provide a valuable medium for clinical investigation and nutritional status for human beings and animals. Dietary components have measurable effects on blood components; hence, blood constituents are widely used in nutritional evaluation and survey of animals (Church et al., 1984; Olorode et al*.,* 1995). Application of laboratory tests can be used to evaluate the functional status of several organs notably the liver, pancreas, and kidney of animals. In view of this, haematological and serum biochemical response of growing rabbits fed varied levels of dietary fumonisin were studied.

MATERIALS AND METHODS

Experimental materials and feeding trial

F. verticillioides cultured maize grains containing fumonisin B_1 was generated at the Plant Pathology Laboratory, International Institute of Tropical Agriculture (IITA), Ibadan, according to the method of Nelson and Ross (1992). 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 mg Kg^{-1} fumonisin, using fumonisin qualitative test kit (Neorgen Corp., USA), constituting diets 1 (control), 2, 3 and 4, respectively.

Forty-eight, 49-day old New Zealand white x Chinchilla male rabbits weighing averagely 757.50 ± 0.50 g were assigned randomly by weight, to the 4 diets in a 12-week feeding trial. The gross compositions of the diets are shown in Table 1. The animals were fed their respective diets *ad libitum* daily at 0800 and 1600 h. Potable water was made available throughout the experimental period. At the end of the feeding trial, blood samples were collected from the animals

Blood collection

The rabbits were bled through the ear vein and the blood collected into two vaccutainer tubes for each animal, one containing a calculated amount of ethylene diamine tetraacetic acid (EDTA) for haematological study and the other sterile vaccutainer tubes without EDTA. The second set of tubes were covered and centrifuged, serum separated out, decanted, deep-frozen for serum biochemical analyses.

Estimation of haematological variables

Packed cell volume (PCV) was estimated from the blood samples collected in bottles containing EDTA by gently mixing and drawing up in a micro haematocrit capillary tube to ¾ of its length. One end of tube was sealed with platicine. The capillary tube was placed in micro-haematocrit centrifuge ensuring that the plasticine end is outward. After closing, it was centrifuged at 12,000 rpm for 4 min. The tubes were then read in the haematocrit reader. The reading expressed the packed red blood cells as a percentage (%) of the total volume of the blood. Haemoglobin concentration was deter-

Table 2. Haematological parameters of growing bucks fed varied level of dietary fumonisin B_1.

Parameter	1 (Control)	2 (5.0 mgkg^{-1})	3 (7.5 mgkg^{-1})	4 (10.0 mgkg^{-1})	SEM*
Packed cell volume %	53.83^{a}	52.33^{a}	49.17^{b}	47.33^{b}	0.93
Haemoglobin (g/dl)	17.22^{a}	17.12^{a}	16.13^{ab}	15.60^{b}	0.39
Red blood cells (x10^{6}/l)	8.64^{a}	8.54^{a}	7.46^{b}	7.33^{b}	0.08
White blood cells (x10^{3}/l)	9.83^{b}	9.21^{b}	13.90^{a}	13.32^{a}	1.13
Platelets (x10^{3}/l)	178.67	174.33	180.67	176.67	15.89
Mean cell volume ($\propto^{3}$)	62.29	61.17	64.45	63.94	2.93
Mean cell haemoglobin ($\propto\propto$g)	19.92	20.03	22.51	21.11	1.13
MCHC** (%)	31.99	32.73	32.81	32.96	0.55
Lymphocyte (%)	47.83^{b}	57.67^{a}	60.00^{a}	60.50^{a}	1.51
Neutrophil (%)	40.00	37.00	37.33	36.17	1.71
Eosinophil (%)	0.67	1.33	2.00	1.67	0.26
Monocyte (%)	2.33	1.67	1.33	1.17	0.37

ab: Means in the same row with different superscripts are significantly (P < 0.05) different.
*SEM: Standard error of mean.
**MCHC: Mean cell haemoglobin concentration.

determined by a cyanmethaemoglobin method using Drabkin's solution as diluent. Blood indices and corpuscular constants, mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) were determined using appropriate formulae as described by Jain (1986). Red blood cells were estimated by taking 0.02 ml of the blood sample from the bottle containing EDTA and mixing with 4 ml of diluting fluid (3 g sodium citrate, 1 ml formaldehyde in 100 ml distilled water) by shaking for about half a minute. About a quarter of the content was expelled before filling the haemocytometer counting chamber and allowed to settle by leaving to stand for about a minute after filling. All the red cells were then counted using the x 40 objective lens and x 8 eyepiece of the microscope, with the aid of a counter. RBC total counts were estimated using the formula below:

RBC Total count = RBC counts x 10 x 5 x dilution factor (200) = RBC counts x 10,000

Total leukocyte counts were determined using Neubauer haemocytometer after appropriate dilution, and differential leukocyte counts was performed using the oil - immersion objective examination of blood films stained with the modified Romanovsky's Giemsa stain.

Determination of serum parameters

Biuret method of total serum protein determination was employed in this assay 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. The albumin/globulin ratio was obtained by dividing the calculated albumin value by the calculated globulin value. Aspartate amino transferase (AST) and alanine amino transferase (ALT) activities were determined using spectrophotometric methods as described by Rej and Hoder (1983) and Hoder and Rej (1983), respectively.

Data analysis

All data obtained were subjected to statistical analysis using analysis of variance of statistical analysis software (SAS, 1999) program. Treatment means were compared using Duncan's option of the same software.

RESULTS

Haematological indices of the growing rabbits

The response of haematological indices of growing rabbits to the dietary treatments containing varied levels of fumonisin is as shown in Table 2. The haematological indices examined were significantly (P < 0.05) influenced by dietary treatments. PCV of rabbits fed diets containing 7.5 and 10.0 mg kg^{-1} fumonisin B_1 was not significantly different from each other but significantly (P < 0.05) lower than those rabbits fed control diet and 5.0 mg kg^{-1} fumonisin B_1. Haemoglobin concentration of rabbits fed diets 1, 2 and 3 were not significantly different. However, the haemoglobin concentration of rabbits fed diet 4 was significantly (P < 0.05) lower than those fed diets 1, 2 and 3. The red blood cell (RBC) of rabbits fed control diet and diet 2 were not significantly different but were significantly (P < 0.05) higher than those fed diets 3 and 4 with 7.46 and 7.33 x 10^{6}/l, respectively. The white blood cell values were inversely related to RBC. The value increased with increase in the fumonisin level in the diets. The WBC of rabbits fed diets 2 and control were not statistically different from each other, but were significantly (P < 0.05) lower than those fed diet 3, which was not significantly different from diet 4. Among all the leukocytes differential counts, only lymphocytes were significantly (p < 0.05) influenced with rabbits fed diets 2, 3 and 4 being statistically similar but were significantly (P < 0.05) higher than the control. Neutrophils, eosinophils and monocytes examined were not significantly influenced by the dietary treatments. The platelets and blood constants (MCV, MCH and MCHC) examined were not statistically different among the treatments (Table 2).

Table 3. Serum proteins of growing bucks fed varied levels of dietary fumonisin B_1.

Parameters	1 (Control)	2 (5.0 mgkg^{-1})	3 (7.5 mgkg^{-1})	4 (10.0mgkg^{-1})	SEM
Total protein (g/dl)	6.20^{a}	5.60^{b}	5.20^{c}	5.03^{c}	0.19
Globulin (g/dl)	2.49^{b}	2.53^{b}	3.00^{a}	3.02^{a}	0.06
Albumin (g/dl)	3.71^{a}	3.07^{b}	2.20^{c}	2.01^{c}	0.07
Albumin/globulin ratio	1.49^{a}	1.21^{b}	0.73^{c}	0.65^{c}	0.04

abc: Means in the same row with different superscripts are significantly ($P < 0.05$) different.
SEM: Standard error of mean.

Table 4. Serum biochemical test and enzymes activities of growing bucks fed varied levels of dietary fumonisin B_1.

Parameters	1 (Control)	2 (5.0 mgkg^{-1}	3 (7.5 mgkg^{-1}	4 (10.0 mgkg^{-1})	SEM
Urea (Mg/dl)	24.10	23.40	22.00	18.53	2.41
Creatinine (µg/dl)	1.30	1.57	1.64	1.92	0.03
Alanine amino transferase (ALT-i.u/l)	74.33	83.00	85.00	94.33	6.79
Aspartate amino transferase (AST-i.u/l)	55.00^{b}	57.83^{b}	60.00^{b}	70.00^{a}	3.23
Alkaline phosphatase(ALP-i.u/l)	8.80^{c}	13.42^{b}	16.48^{ab}	17.37^{a}	1.13

abc: Means in the same row with different superscripts are significantly ($P < 0.05$) different.
SEM: Standard error of mean.

Serum biochemical variables of the growing rabbits

The response of serum biochemical variables of growing rabbits fed varied levels of dietary fumonisin B_1 is as shown in Tables 3 and 4. The serum proteins of growing rabbits were significantly ($P < 0.05$) influenced by dietary treatments. Serum total protein significantly ($P < 0.05$) decreased with increase in the fumonisin level in the diets. Serum total protein of rabbits fed diets 3 and 4 were identical but significantly ($P < 0.05$) lower than those fed diets 2 and the control. Serum albumin significantly ($P < 0.05$) decreased with increase in the dietary fumonisin levels in the diets with serum albumin of rabbits that fed diets 3 and 4 being significantly ($P < 0.05$) lower than those rabbits fed control diet and diet 2. Serum globulin was inversely related with serum albumin of the rabbits fed the dietary treatments. The serum globulin of the animals fed diets 3 and 4, which was not significantly different from each other, were significantly ($P < 0.05$) higher than those fed diets 2 and 1. Albumin - globulin ratio also declined with increase in the fumonisin level in the diets.

Urea apparently decreased while creatinine increased apparently with increase in the fumonisin levels but were not statistically different among the diets. All serum enzyme activities examined were significantly ($P < 0.05$) influenced except ALT, among the dietary treatments. AST of rabbits fed control diet and diets 2 and 3 were significantly ($P < 0.05$) lower than those fed diet 4 containing the highest fumonisin level in this study. Alkaline phosphatase (ALP) of rabbits fed diet 2 was not significantly different from those rabbits fed diet 3 but was significantly ($P < 0.05$) higher than those fed control diet. AST and ALP increased with increase in the fumonisin levels in the diets with those on diet 4 having highest values, 70.00 i.u./l and 17.37 i.u./l, respectively.

DISCUSSION

Haematological indices are an index and a reflection of the effects of dietary treatments on the animal in terms of the type, quality and amounts of the feed ingested and were available for the animal to meet its physiological, biochemical and metabolic necessities (Ewuola et al., 2004). 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. In this study, in which *F. verticillioides* - cultured maize was used to formulate diets for rabbits at varied fumonisin concentrations, PCV, Hb and RBC were significantly influenced by the dietary treatments. The values obtained from these parameters decreased significantly with increase in fumonisin levels in the diets. This could be probably attributed to fumonisin effects on the blood profile that depressed the parameters in animals placed on diets 3 and 4 significantly as compared to control. This may have likely induced anaemic condition/disease in those rabbits although; the values were still within the reported normal physiological range of rabbits as reported by Mitruka and Rawnsley (1977) for temperate New Zealand White male adult rabbits.

The Leukocytes examined for rabbits fed diets containing 7.5 and 10.0 mg kg^{-1} fumonisin levels were significantly higher than those on diets 2 and the control

and were outside the reported physiological range of 5.50 - 12.50x10^3/mm^3 for normal rabbits (Mitruka and Rawnsley, 1977). This may probably be attributed to dietary fumonisin effect. The increase in the leukocyte counts outside the normal range may be an indication that the blood cell production increases in attempt to combat the toxin assault in the diets, since leukocytes are known to be among body defense mechanisms that fight against non-self or pathogenic organisms. Besides, it also implies that the animals suffered from leukocytosis as a result of the excessive production of the WBC in order to get rid of the toxin from the animal body system with increase in lymphocytes. The platelets and blood constants (MCV, MCH and MCHC) were within the reported physiological range for normal rabbits reported by Mitruka and Rawnsley (1977).

The results corroborate the report of relevant literatures. Espada et al*.* (1994) reported altered heamatological parameters of chicks fed dietary fumonisin. Pigs fed 1 mg fumonisin/kg diet was reported to have induced signifi-cant effects on the heamatological variables (Rotter et al*.,* 1996). Similar evidence was reported by Powell et al*.* (1996) that dietary fumonisin B_1 caused several alterations to haematological parameters of minks fed diets contaminated with fumonisin.

The serum total protein and albumin that significantly decreased with increase in the fumonisin level in the diets could be attributed to the toxin in the experimental diets. Serum biochemical analysis is used to determine the level of heart attack, liver damage and to evaluate protein quality and amino acid requirements in animals as reported by Harper et al*.* (1979). Reduced level of total protein below the normal standard mean value of 5.5 - 8.0 g/dm reported by Mitruka and Rawnsley (1977) was observed for animals fed diets 3 and 4 containing 7.5 and 10.0 $mgkg^{-1}$ fumonisin B_1 respectively. The observed serum total protein and albumin values below the standard physiological range were an indication that the animals suffered from hypoproteinemia and hypoalbuminemia. A reading of albumin less than the normal physiological value for albumin usually indicates hypoalbuminemia (Altman, 1979). The low level of serum protein in rabbits fed 7.5 mg kg^{-1} and 10.0 mg kg^{-1} fumonisin indicated some alteration in their protein 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) or protein absorption. Merrill et al*.* (1993) reported fumonisin to cause adverse effects on normal epithelial morphology, which may be responsible for poor protein absorption and utilization from the gastro-intestinal tract of the animals. The increase in the level of serum globulin with increased dietary fumonisin levels may be a corresponding response of the body defense mechanism to combat the antigen (the toxin).

Serum urea, creatinine and ALT were similar among the dietary treatments, which may probably be an indication that the toxin did not influence them. However, ALT concentration for rabbits fed diets containing 5.0, 7.5 and 10.0 mg kg^{-1} was outside the reported physiological range of 48.5 - 78.91.u/l for normal rabbits (Mitruka and Rawnsley, 1977). AST and ALP significantly increased with increase in fumonisin level in the diets. ALP of rabbits fed diets 3 and 4 containing 7.5 and 10.0 mg fumonisin/kg respectively were outside the reported physiological range of 4.10 - 16.20 i.u./l by Mitruka and Rawnsley (1977).

In serum enzymology, the concentration of the enzymes used in diagnosis of heart, liver and kidney damage give valuable information as regards their state of damage (Harper et al*.*, 1979). The observed enzyme activities above the physiological range is an indication that the animals may have suffered heart, kidney or liver damage since the serum concentration of AST is an index to measure the state of heart and liver in animals. 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 the cellular destruction. Restum et al. (1995) reported that ALT, AST and ALP were greater in minks fed 119 ppm fumonisin B_1 diet as compared to the control. Similar evidence of abnormal increases in serum enzymes activities; probably due to cellular destruction induced by fumonisin has been reported (Voss et al*.*, 1993; Gelderblom et al*.*, 1994; Mehta et al., 1998).

Conclusion

This study investigated that exposure of rabbit bucks to diet containing fumonisin- contaminated grains above 5.0 mg fumonisin B_1/Kg will significantly alter haematological variables and probably induce anaemic condition in the animals. Dietary fumonisin level up to 7.5 mg fumonisin/Kg diet also increased serum enzyme activities, which is an indication of organ toxicity, and reduced serum proteins.

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