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# HAEMATOTOXICITY OF DIETARY FUMONISIN $B_1$ IN GROWING PIGS

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## ABSTRACT

Investigation was carried out with 24 male Large White weanling pigs of 8-9 weeks of age averaging 6.94±0.26 kg to study the haematotoxicity of dietary fumonisin $B_1$ ($FB_1$) in pigs. The animals were randomly assigned to four treatment diets containing approximately 0.2 (control), 5.0 (diet 1), 10.0 (diet 2) and 15.0 ppm (diet 3) $FB_1$ in a 6-month feeding trial. Blood sample was collected from the ear vein of each animal at the end of the feeding trial for haematological analyses. Dietary $FB_1$ had significant influence on haematological parameters. Animals fed diets 2 and 3 suffered significantly ($p<0.05$) reduced erythrogenesis and concentration of red blood cells, resulting in anaemia, with impaired respiratory capacity. The animals exposed to diet 3 (containing the highest $FB_1$ concentration) suffered leukocytosis. Dietary $FB_1$ concentration above 5.0 ppm is haematotoxic in pigs. The study suggests that the USFDA recommended maximum level of 10 ppm total fumonisins is above the no observable effect level (NOEL) for swine.

**Key words:** Erythrogenesis, fumonisin $B_1$, haematotoxicity, leukocytosis, pig.

## INTRODUCTION

The contamination of feeds and feedstuffs with mutagenic and carcinogenic mycotoxins is a major concern for animal and human health. Mycotoxins are formed where environmental factors are conducive, during the growth of frequently occurring mycomycetes on foodstuffs and animal feeds, the process taking place during the secondary metabolism.

Fumonisins are a group of environmental mycotoxins produced by various species of *Fusarium*, especially *Fusarium verticillioides* (= *F. moniliforme*), one of the most prevalent seed-borne fungi associated with maize intended for human and animal consumption throughout the world (Nelson *et al.*, 1991; Kedera *et al.*, 1992). The major types of fumonisins are $B_1$, $B_2$ and $B_3$, and have been implicated in modification of immune response. Shephard *et al.* (1996) reported that maize, which is the major cereal utilized in the formulation of livestock feeds as well as a major dietary staple in several parts of the world, is the only commodity that contains significant amounts of fumonisins; hence the potential for fumonisins to be found in feeds and foodstuffs is high. A survey of contemporary literature reveals increasing wave of fumonisin contamination of feeds and feeding stuffs (Fazekas *et al.*, 1997) and conse-

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quent poisoning of a large number of animals in several parts of the world.

In general, the consumption of mycotoxin-contaminated feed by an animal may result in an unhealthy situation ranging from decreased nutritive value of feeds, poor feed conversion, reduced growth, hormonal changes to occasional organ damage or even death depending on the type of mycotoxin. The effects depend on the amount of toxin in the feed, the period for which the feed is ingested, the nutritional status of the feed and the consumption of sufficient quantities of toxins–containing plant material (Marasas and Nelson, 1987).

The blood profile was reported (Wilson and Medd, 1978) to be a guide to nutritional status in feeds. The 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*., 1995). Fumonisin $B_1$ ($FB_1$) is the major fumonisin produced in culture (Bezuidenhout *et al*., 1988; Ross *et al*., 1992) as well as naturally occurring in maize and maize-based feeds and foods (Rheeder *et al*., 1992; Ross *et al*., 1992). Consequently, toxicological studies on the fumonisins have concentrated on $FB_1$. There are numerous reports that weanling piglets (Friend *et al*., 1982; Döll *et al*., 2003) and growing and finishing pigs (House *et al*., 2002; Dänike *et al*., 2004) are particularly sensitive to feed-borne *Fusarium* mycotoxins. With these in mind, coupled with the fact that fumonisins have been reported to cause different physiological responses in animals, this study was designed to assess the effect of varied dietary $FB_1$ on haematological parameters in growing pig.

## MATERIALS AND METHODS

Fumonisin–contaminated maize grains, cultured with a toxigenic strain of *F. verticillioides* (MRC 286), was generated according to the method described by Nelson and Ross (1992) at the Plant Pathology Laboratory, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Three treatment diets containing approximately 5.0, 10.0 and 15.0 ppm $FB_1$ constituting diets 1, 2 and 3, respectively, were formulated. With the control diet containing approximately 0.2ppm $FB_1$, the treatment diets were used in a 6-month feeding trial. The varied dietary $FB_1$ concentrations were achieved by substituting ground -cultured maize for ground, autoclaved noncultured maize in various proportions. The $FB_1$ concentrations were determined using the fumonisin qualitative test kit (Neorgen Corp., USA).

Twenty four clinically normal male Large White weaned piglets of about 8 - 9 weeks of age averaging 6.94 $\pm$ 0.26kg 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 assigned into one of the 4 diets (6 per treatment) 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 ($7^{o}$20'N, $3^{o}$50'E, 200m above sea level with an average day-

time temperature of 24-25°C and relative humidity 80-85%) during the early dry season, lasted 6 months. The feeding was divided into 3 physiological phases [weanling (starter), pre-pubertal (grower) and pubertal (finisher)]. The composition of the diets fed for 6, 10 and 8 weeks during the weanling, pre-pubertal and pubertal phases, respectively are shown in Table 1. The diets satisfied the nutrient requirements of the animals at the various physiological phases as recommended by National Research Council (NRC, 1998).

The animals were fed their respective diets *ad libitum* daily at 0800h and 1600h. Potable water was made available throughout the experimental period. At the end of the feeding trial, blood sample was collected from the ear vein of each animal into Monoject® vacutainer containing Ethylene diaminetetraacetic acid (EDTA) for haematological analyses.

A portion of each blood sample was centrifuged in a capillary tube for 5 minutes in a haematocrit centrifuge and read in a haematocrit reader to determine the packed cell volume (PCV) while the erythrocyte, leukocyte and the differential leukocyte counts were determined using the new improved Neubauer haemocytometer. The haemoglobin concentration and the blood constants: mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were determined using cyanethaemoglobin method and appropriate formula respectively as described by Jain (1986).

**Table 1: Gross composition (%) of the test diets for the various physiological phases**

| | Physiological Phase | | |
|---|---|---|---|
| Ingredient | Weanling | Pre-pubertal | Pubertal |
| *Maize | 40.00 | 30.00 | 20.00 |
| Soybean meal | 20.00 | 15.00 | 8.50 |
| Palm kernel cake | 20.00 | 25.00 | 45.00 |
| Wheat offal | 14.00 | 14.30 | 5.00 |
| Rice husk | - | 11.00 | 17.80 |
| Fish meal | 3.00 | 2.00 | 1.00 |
| **Fixed ingredients | 2.70 | 2.70 | 2.70 |
| Total | 100.00 | 100.00 | 100.00 |
| Analysed nutrients: | | | |
| Crude Fibre (%) | 5.35 | 9.82 | 10.83 |
| Crude Protein (%) | 20.38 | 17.97 | 15.30 |
| DE (Kcal/kg) | 2701.80 | 2269.11 | 2240.61 |

*Mixture of *Fusarium*-cultured and non-cultured maize in various proportions to achieve desired dietary $FB_1$ levels for each treatment.
**Contained Dicalcium phosphate (1.50), Oyster shell (0.50), Salt (0.45) Minerals/Vitamins premix (0.20), Methionine (0.01) and Lysine (0.04).

Data obtained were subjected to one-way ANOVA procedure of SAS® (1999) and the significant treatment means separated by Duncan option of the same software.

## RESULTS

The haematological indices of pubertal boars exposed to varied levels of dietary $FB_1$ for 24 weeks are shown in Table 2. All the parameters monitored, except the blood constants and the platelets, were significantly ($p<0.05$) influenced, with the erythrocytes, haemoglobin, PCV and the leukocytes revealing a dose-dependent significant variations among the treatments. The erythrocytes, haemoglobin, MCV, MCH, and the PCV values generally decreased with increase in the dietary $FB_1$. The platelets and the leukocyte values, on the other hand, increased while all other parameters did not follow any particular trend with increases in the dietary $FB_1$ concentrations.

## DISCUSSION

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 insult. The results revealed that the animals exposed to feeds containing Fusarium–inoculated maize (diets 1, 2 and 3) suffered significantly from the synthesis (erythrogenesis) and concentration of red blood cells (RBCs). The corresponding statistical decrease in PCV of the animals exposed to diets 1, 2 and 3 revealed that the animals were anaemic. The significantly reduced PCV values were directly related to the $FB_1$ concentration in the diets, with the animals that consumed the diet containing the highest dietary $FB_1$ concentration recording the lowest value.

**Table 2: Haematological indices of pubertal boars exposed to varied levels of dietary $FB_1$.**

| | Dietary fumonisin level | | | | |
|---|---|---|---|---|---|
| Parameter | 1(0.10ppm) | 2(5.10ppm) | 3(10.10ppm) | 4(15.10ppm) | SEm |
| Erythrocytes (1012/L) | 7.42a | 7.28a | 6.13b | 6.68ab | 0.13 |
| Haemoglobin (g/L) | 136.10a | 133.60a | 112.60b | 122.90ab | 2.30 |
| Mean Cell Volume (fl) | 50.12 | 50.48 | 57.43 | 51.53 | 1.19 |
| MCH * (μμ/g) | 18.35 | 18.35 | 18.36 | 18.40 | 0.01 |
| MCHC** (%) | 36.96 | 37.13 | 32.89 | 36.52 | 0.72 |
| PCV*** (%) | 36.83a | 36.00ab | 34.33b | 33.83b | 0.32 |
| Platelets (109/L) | 20.03 | 21.68 | 20.42 | 20.60 | 0.38 |
| Leukocytes (109/L) | 15.28b | 15.42b | 18.37b | 22.52a | 0.52 |
| Neutrophils (109/L) | 2.01ab | 2.91a | 1.87b | 2.99a | 0.24 |
| Eosinophils (109/L) | 0.37ab | 0.46ab | 0.27b | 0.69a | 0.03 |
| Lymphocytes (109/L) | 12.69b | 11.81b | 16.34a | 18.41a | 3.00 |
| Monocytes (109/L) | 0.21b | 0.23ab | 0.09b | 0.44a | 0.02 |

[ab] Means in the same row with different superscripts differ significantly ($P<0.05$).
*Mean Corpuscular Haemoglobin; **Mean Corpuscular Haemoglobin Concentration; ***Packed Cell Volume.

Haemoglobin (Hb), an iron-containing conjugated protein, has been described (Mitruka and Rawnsley, 1977) to have physiological function of transporting oxygen and carbon dioxide. Although the significantly lower Hb values of 112.6 and 122.9 g/L recorded for animals on diets 2

and 3 compared to those on diet 1 and the control were within the 100 – 160 g/L reference values for young adult boars reported by Blood (1995) and Thorn (2000), the results suggested that the animals fed diets 2 and 3 suffered normocytic normochromic anaemia.

The values of the haematological indices-MCV and MCH, were within the reported reference ranges of 50 – 68 fl and 17.8 – 18.8 μμ/g 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 diets 1, 2 and 3 indicated normocytic normochromic anaemia. This further confirms the low respiratory and gas-exchange capacity of the RBCs in animals exposed to diets 2 and 3, which have been suggested earlier in this report to suffer from normocytic normochromic anaemia.

The non-significant difference observed for the platelets among the treatments revealed that the consumption of the dietary $FB_1$, the concentration not withstanding, did not cause splenic atrophy– a characteristic pathologic thrombocytosis, neither did the animals suffer bone marrow damage nor marrow failure which, among other factors, was reported by Coles (1986) to cause thrombocytopenia.

The circulating leukocyte count of animals fed diet 3 was higher than the 11.0 – $22.0x10^9$/L reference values reported by Blood (1995) and Thorn (2000) for swine. This indicates that the animals fed diet 3, containing the highest concentration of $FB_1$, may have suffered from leukocytosis. Coles (1986) described leukocytosis as a consequence of an increase in the total number of circulating neutrophils, which is highest for the animals on diet 3 in this study. Leukocytosis, according to Coles (1986), may result from intoxications including those produced by metabolic disturbances among others.

The generally significant influence of the dietary treatment on most of the haematological parameters investigated agrees 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 $B_1$. These results, however, contradict the report of Parent–Masin and Parchment (1998), Zomborszky–Kovács et al. (2002) and Ogunlade et al. (2004) that considered fumonisins as non–haematotoxic mycotoxins. The variability in haematology of livestock fed fumonisin-contaminated 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.

The dietary $FB_1$ concentrations used in this study bracketed the published FDA recommended maximum level of 10 ppm total fumonisins for swine (USFDA, 2001). This study has shown that dietary exposure to $FB_1$ at a concentration of about 10 ppm or more for a six-month period may result in significantly reduced synthesis and concentration of RBC and consequent anaemia with impaired respiratory capacity in boars. The results in this study suggest that the FDA recommended maximum levels of 10

ppm are above the no observable effect level (NOEL) for swine. The study also demonstrates that boars exposed to dietary fumonisin concentration of about 15 ppm will suffer leukocytosis.

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