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Effect of Aflatoxin-B1 Exposure on Performance and Haematological Stress Indicators in Male Sprague Dawley Rats

[1,2*]Sikiru, A. B., [1]Egena, S. S. A., [1]Alemede, I. C., [1]Alabi, J. O., [3]Makinde, J. O and [4]Behera, H.

[1]Department of Animal Production, Federal University of Technology, Minna, Nigeria.
[2]Department of Animal Science, Federal University of Agriculture Zuru, Nigeria.
[3]Department of Animal Science, Federal University Gashua, Nigeria.
[4]Department of Animal Reproduction, Gynaecology and Obstetrics, Odisha University of Agriculture and Technology, Bhubaneswar, India.

Abstract

Aflatoxin-B1 is a ubiquitous mycotoxin usually accumulated in food and feeds, thereby posing a critical health risk to humans and animals. This study was carried out to determine its effects on performance characteristics and haematology indicators of stress in male Sprague Dawley rats. The rats were randomly distributed into 4 groups (n=10 per group) including an unexposed group that served as control and other groups which were orally exposed to 150 µg of aflatoxin-B1 per kg body weight on a varied basis for 28 days. The records of food intake and body weight changes were obtained while blood was collected at the end of the study for downstream analyses. There was a significant difference observed in food intake, body weight, kidney weight, liver weight, lung weight, and testes weight of the rats ($p<0.05$). There was a significant difference observed in the heterophil-lymphocytes ratio and bilirubin concentration as haematoolgy biomarkers of stress in the rats ($p<0.001$). The study concluded that aflatoxin-B1 exposure in rats is capable of inducing stress-related performance dysfunction such as reduced food intake and body weight loss, while haematoolgy biomarkers of stress heterophil-lymphocyte ratio and bilirubin concentrations could be used as potential biomarkers suitable for measuring these performance dysfunctions. There is a need for further studies to establish the possible use of heterophil-lymphocyte ratio and bilirubin as measures of performance compromise in response to aflatoxin-B1 exposure in rats.

Keywords: Aflatoxin-B1, Stress, Heterophil-lymphocyte ratio, Bilirubin concentration

Introduction

Aflatoxin-B1 is a secondary metabolite of the fungi *Aspergillus flavus*; it is a mycotoxin capable of poisoning foods and feeds of humans and animals (Awapak *et al*., 2021; El-Sayed et al., 2022). It is a reported carcinogen representing one of the most toxic principles of foods and feeds that could pose serious health consequences for humans and animals (Shabeer et al., 2022). It is found in crop harvests including maize, cassava, rice, wheat, and barley which are the commonest foods of people living in developing countries (Mahato et al., 2019). Therefore, it is a huge burden on food security and well-being because, it is almost inevitable to avoid aflatoxin-B1 contamination in many foods and feeds since it is capable of

* Corresponding Author: +2348160942976
Email address: akeembaba01@gmail.com

contaminating crops from cultivation to harvest, storage, processing, and even after processing (Gallo et al., 2020).

Meanwhile, there are pieces of evidence supporting that aflatoxin-B1 causes molecular complications such as changes in chromosomal components, DNA strands breakage, and the formation of molecular adducts which was the basis of its categorization as a human carcinogen (Kumari et al., 2021; Marchese et al., 2018; Alahlah et al., 2020; IARC, 1993). Furthermore, identified mechanisms associated with toxic bio-transformation of aflatoxin-B1 include induction of stress but this has not been fully elucidated (Guerre, 2020; Marin *et al*., 2020; Navale *et al*., 2021). Identification of simple blood markers associated with stress induction can contribute to the filling of this research gap and the heterophil-lymphocyte ratio is a suitable candidate.

The heterophil-lymphocyte (H/L) ratio has been described as a haematoolgy indicator of stress because of its roles in the adrenal-corticoid activity, metabolic regulations, immune system functions, blood pressure, and other essential body functions associated with stress compromise of performance characteristics in animals (Khan, 2000; Price, 2016). Furthermore, the heterophil-lymphocyte ratio is related to the rapid secretion of catecholamine from the adrenal medulla and glucocorticoids from the adrenal cortex which could make it a simple measure of physiological stress using blood samples. However, despite the suitability and the simplicity of the heterophil-lymphocyte ratio as a measure of stress, its usage for measuring aflatoxin-B1-induced stress-related performance compromise is rarely reported. Hence, this present study was conducted to understand the relationships between performance characteristics and heterophil-lymphocyte ratio in rats orally exposed to aflatoxin-B1.

Materials and Methods

The Rat Models and The Aflatoxin-B1 (Afb1) Samples

Male Sprague Dawley rats (n=40, average initial body weight of 102.92±5.69 g) were procured from a reputable laboratory animal facility in Nigeria for use in the study. The animals were randomized equally using their body weight and distributed as 2 animals per replicate of 5 replicates per group, giving 10 rats per experimental group. The aflatoxin-B1 sample used was obtained as an isolate of the fungus *Aspergillus flavus* maintained at the Laboratory of Pathology and Mycotoxin Unit of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. The aflatoxin-B1 sample was supplied as a dried extract containing 28.34 mg of aflatoxin-B1 per kg of the extract.

The Rat Housing and Management

The rats were housed individually in plastic transparent cages arranged on a rack; the laboratory temperature throughout the experimental study period was 25±2º C, while the rats were provided with light and dark regimes of 12 h each. The food served to the rats was produced in the laboratory using natural ingredients and then pelletized for easy consumption by the rats. The food was formulated to meet the protein requirement of the rats, according to NIH 31M Rodent Diet, while the energy composition of the food followed the requirement of 3.8–4.1 kCal ME/g according to the guidelines of the National Research Council (Benevenga et al., 1995). The ingredients and nutritional composition of the rat food are presented in Table 1.

Aflatoxin-B1 Exposure and Recording of the Rat's Performance Parameters

The rats were exposed to aflatoxin-B1 through food contamination, the procedure involved contamination of a known quantity of food with the required quantity of the aflatoxin-B1 per kg body weight of each rat, then the food is served to the rat at 08:00 h in the morning. After complete consumption of the served contaminated food, a known quantity of uncontaminated food is then served *ad-libitum*; while the remnants are weighed the following day to account for the previous day's food intake. This procedure was repeated every day for each rat throughout the study period; the food intake was recorded daily, while records of body weight changes were recorded weekly. The exposure of the rats to aflatoxin-B1 lasted for 28 days. Upon completion of the exposure to aflatoxin-B1, the animals were sacrificed for

Table 1: The food ingredients and their respective percentages in the rat food and composition of the nutrients supplied

Ingredients	**Percentages in the diet**
Wheat	35.50
Maize	25.00
Wheat bran	13.00
Fishmeal	9.00
Soybean meal	6.00
Groundnut cake	5.00
Soybean oil	2.50
Brewer's dried yeast	1.00
Dicalcium phosphate	1.50
Ground limestone	0.50
Salt	0.50
Vitamin-mineral premix	0.25
Choline chloride	0.13
L-lysine	0.10
L-methionine	0.10
	100.08
Nutrient composition of the diet	
Protein (%)	19.75
Crude Fibre (%)	3.63
Ether Extract (%)	4.91
Ash (%)	3.57
Carbohydrate (%)	46.67
Total sugar (%)	3.62
Gross energy (kCal/g)	4.31

The vitamins and minerals premix supplied vitamin A 24300000 IU, vitamin D 4,200,000 IU, vitamin K 22.0 g, vitamin E 16.5g, Biotin 132.0 mg, Folic acid 1.1 g, Niacin 22.0 g, Pantothenic acid 27.5 g, Pyridoxine 2.2 g, Riboflavin supplement 5.5 g, Thiamine 71.5 g, Vitamin B12 supplement 15,400.0 µg, Cobalt 440.0 mg, Copper 4.4 g, Iron 66.0 g, Magnesium 440.0 g, Manganese 110.0 g, Zinc 11.0 g, and Iodine 1.7 g.

internal organs observation and collection of blood used in downstream haematology and serum biochemical analyses. The aflatoxin-B1 (AFB1) exposures are as indicated below:

i. Control – Unexposed rats
ii. T1 – rats orally exposed to 150 µg AFB1 per kg body weight daily
iii. T2 – rats orally exposed to 150 µg AFB1 per kg body weight every two days
iv. T3 – rats orally exposed to 150 µg AFB1 per kg body weight twice in a week

Procedures of Animal Sacrifice and Collection of Blood

On the last day of the animal experimentation, rats in each group were placed under chloroform anaesthesia and euthanised by cervical dislocation while the blood was collected through cardiac puncturing of each rat (Everitt & Gross, 2006). The blood was collected in two separate bottles (anticoagulants and non-anticoagulants bottles), the samples collected in the anticoagulant bottle were used for haematological analysis, while the samples collected in the non-anticoagulant bottles were used for serum biochemical profiling (Sikiru *et al*., 2021).

Assessment of Heamatological, Liver and Kidney Function Parameters of the Rats

The haematology analysis was carried out using Sysmex XN Haematology Analyzer (Sysmex Corporation, Japan). The instrument performs automatic reflex testing of blood using a novel white blood cell differential technology and enumeration of nucleated red blood cells (Briggs *et al*., 2012). The serum analysis was performed using ChemWell® - T 4600 Chemistry Analyzer (Awareness Technology, USA). The haematological analysis implemented was full blood count parameters determination from which the heterophil-lymphocyte ratio values were computed using the values obtained from the full blood counts. The serum parameters determined include sodium, potassium, bicarbonate, chloride, urea, creatinine, total bilirubin, alkaline phosphatase, aspartate aminotransferase, and alanine transferase concentrations in each of the samples.

Statistical Data Analysis

All data obtained were subjected to one-way analysis of variance (ANOVA) using SPSS version 20.0. The exposure to aflatoxin-B1 was used as the factor upon which the data were analysed. The results were presented as Mean±SEM with significant differences in the means determined at the probability of $p < 0.05$, while homogenous means were separated by subjecting the means to Duncan's Multiple Range Test.

Results

Body Weight Changes of the Rats in Response to the Aflatoxin-B1 Exposures

The average body weight of each rat was 102.92±5.69 g. There was no difference in the body weight changes of the rats within the first 20 days of the experiment ($p > 0.05$) but, there was a significant difference observed in the body weight changes of the rats from 21 days of the exposure to aflatoxin-B1 till the end of the experiment ($p = 0.05$). The changes in body weights showed that the unexposed rats (control group) progressively gained more weight from day 0 of the experiment till the end. Similarly, the rats exposed every two days, and twice weekly also gained weight, which was lower compared with the control. The rats exposed to aflatoxin-B1 on a daily basis lost more body weight compared with the other rats in the control, T1, T2, and T3, respectively (Table 2).

Average daily food intake, weekly and total food intakes of the rats

There was a significant difference observed in the average daily food intake, weekly, and total food intake of the rats ($p < 0.05$). All the feeding parameters including daily food intake, weekly and total food intake were significantly higher in the control group compared with the exposed groups. However, except for the food intake in the first week of the experiment (days 1-7) when there was no significant difference in the quantity of food consumed, the weekly food intake was significantly higher in the control group compared with other groups throughout the experiment. The mean average daily food intake was 18.16±0.62 g, which shows that the rats in the control group consumed more food daily than the exposed rats ($p = 0.001$). The mean total food intake was 508.70±17.39 g, respectively (Table 3).

Organ Weights (G) and Organ-Body Weight Ratios of the Rats

There was a significant difference observed in the kidney, liver, lungs, and testes weights of the rats ($p < 0.05$), while no significant difference was observed in the heart weights of the rats ($p = 0.387$). There was also a significant difference observed in the percentages of the liver to body weight ($p = 0.001$), kidney to body weight ($p = 0.001$), heart to

Table 2: Body weight changes of the rats measured in grams (Mean±SEM) weekly in response to oral exposure to aflatoxin-B1 through food

Parameters	Control	T1	T2	T3	Mean	p-values
Day 0	101.76±14.20	100.12±9.10	102.80±12.68	107.12±15.62	102.92±5.69	0.984
Day 7	125.00±14.24	120.00±6.73	120.55±8.01	116.10±15.87	120.42±4.08	0.925
Day 14	138.89±11.48	109.99±17.40	131.66±8.55	128.30±30.96	127.22±5.78	0.373
Day 21	142.22±9.80^{a}	98.33±15.12^{b}	97.22±16.56^{b}	134.40±42.42^{ab}	118.05±8.10	0.05
Day 28	151.99±12.73^{a}	60.30±8.44^{c}	108.50±10.84^{b}	130.80±11.66^{ab}	112.90±11.01	0.001

Means with different superscripts on the same row are different ($p < 0.05$) for the parameter measured per group. Control – rats in the unexposed group, T1 – rats exposed to 150 µg aflatoxin-B1 per kg body weight on a daily basis, T2 – rats exposed to 150 µg aflatoxin-B1 per kg body weight every two days, T3 – rats exposed to 150 µg aflatoxin-B1 per kg body weight twice weekly.

Table 3: Food consumption (g) characteristics of the rats showing average daily, weekly, and total food intakes

Parameters	Control	T1	T2	T3	Mean	p-values
Average daily food intake	21.53±0.21^{a}	16.60±0.05^{b}	16.69±0.66^{b}	17.54±0.50^{b}	18.16±0.62	0.001
Days 1 - 7	140.85±6.49	128.18±7.83	128.53±11.28	129.40±10.18	131.74±5.25	0.170
Days 8 – 14	150.22±0.22^{a}	115.99±4.04^{b}	115.24±7.65^{b}	116.93±6.28^{b}	124.00±5.01	0.004
Days 15 – 21	158.11±1.78^{a}	114.44±3.38^{c}	122.00±2.60^{b}	129.44±0.29^{b}	131.00±5.08	0.001
Days 22 – 28	153.76±1.22^{a}	114.68±3.80^{c}	111.69±4.12^{c}	135.32±1.94^{b}	128.86±5.29	0.001
Total food intake	602.94±5.98^{a}	463.30±1.45^{b}	467.47±18.64^{b}	491.10±14.04^{b}	508.70±17.39	0.001

Means with different superscripts on the same row are different ($p < 0.05$) for the parameter measured per group. Control – rats in the unexposed group, T1 – rats exposed to 150 µg aflatoxin-B1 per kg body weight daily, T2 – rats exposed to 150 µg aflatoxin-B1 per kg body weight every two days, T3 – rats exposed to 150 µg aflatoxin-B1 per kg body weight twice weekly.

body weight ($p = 0.001$), and testes to body weight ($p = 0.002$), of the rats, respectively. The rats exposed to a continuous daily dose of aflatoxin-B1 had a higher internal organ weight and percentage of body weight compared with the unexposed rats (Tables 4a and 4b).

Haematology and Serum Biochemical Profile of the Exposed and Unexposed Rats

There was a significant increase in haemoglobin concentration ($p = 0.001$) in the exposed rats compared with the unexposed rats. Similarly, there was a significant difference in the red blood cells, white blood cells, PCV, MCHC, and the heterophil-lymphocytes ratio of the rats ($p=0.001$). The mean red blood cell count was 6.82±0.22 ($x10^{12}$ cpL), and the mean heterophil-lymphocytes ratio was 0.16±0.01, respectively (Table 5). There was a significant difference observed in sodium concentration ($p = 0.008$), bicarbonate concentration ($p = 0.014$), creatinine ($p = 0.002$), total bilirubin ($p = 0.001$), aspartate

Table 4a: Internal organs weight (in grams) of the rats exposed to aflatoxin-B1 on daily, every two days, and weekly basis compared with the internal organs of the unexposed rats

Parameters	Control	T1	T2	T3	Mean	p-values
Liver	5.25±0.08[a]	6.12±0.08[b]	5.42±0.28[a]	5.40±0.11[a]	5.55±0.12	0.023
Kidney (left)	1.08±0.01[a]	1.66±0.12[b]	1.53±0.16[b]	1.66±0.18[b]	1.48±0.10	0.050
Kidney (right)	1.09±0.01[a]	1.68±0.12[b]	1.55±0.16[b]	1.68±0.18[b]	1.50±0.10	0.047
Heart	0.56±0.07	0.61±0.02	0.57±0.03	0.49±0.04	0.56±0.02	0.387
Lungs	1.04±0.01[a]	1.05±0.05[a]	1.08±0.15[a]	1.62±0.12[b]	1.19±0.08	0.009
Testes (left)	1.39±0.05[ab]	1.30±0.02[b]	1.33±0.03[ab]	1.43±0.04[a]	1.36±0.02	0.050
Testes (right)	1.43±0.02[a]	1.32±0.01[b]	1.37±0.02[ab]	1.42±0.01[a]	1.38±0.02	0.014

Means with different superscript on the same row are different ($p < 0.05$) for the parameter measured per group. Control – rats in the unexposed group, T1 – rats exposed to 150 µg aflatoxin-B1 per kg body weight on daily basis, T2 – rats exposed to 150 µg aflatoxin-B1 per kg body weight every two days, T3 – rats exposed to 150 µg aflatoxin-B1 per kg body weight twice weekly.

Table 4b: The percentages (%) of internal organs by body weights of the unexposed rats compared with the exposed rats to aflatoxin-B1

Parameters	Control	T1	T2	T3	Mean	p-values
Liver to body weight	3.53±0.27[a]	10.53±1.30[b]	5.17±0.36[a]	4.13±0.10[a]	5.83±0.90	0.001
Kidney to body weight	1.45±0.13[a]	5.77±0.86[b]	2.88±0.30[a]	2.55±0.28[a]	3.16±0.52	0.001
Heart to body weight	0.38±0.10	1.05±0.12	0.53±0.02	0.37±0.03	0.58±0.10	0.001
Testes to body weight	1.89±0.16[a]	4.50±0.56[b]	2.55±0.25[a]	2.18±0.02[a]	2.78±0.34	0.002

Means with different superscript on the same row are different ($p < 0.05$) for the parameter measured per group. Control – rats in the unexposed group, T1 – rats exposed to 150 µg aflatoxin-B1 per kg body weight on daily basis, T2 – rats exposed to 150 µg aflatoxin-B1 per kg body weight every two days, T3 – rats exposed to 150 µg aflatoxin-B1 per kg body weight twice weekly

amino transaminase ($p = 0.001$), and alanine amino transaminase ($p = 0.050$), respectively (Table 6).

Discussion

Findings from this study suggest that dietary exposure of rats to AFB1 could cause a reduction in body weight, and compromise hepatological and serum biochemical profiles including reduced concentrations of sodium, bicarbonate, and total bilirubin, and increased creatinine, and aspartate amino. transaminase, and alanine amino transaminase concentrations in rats. Also, observations showed that there are relationships between the frequency of the rat's exposure to AFB1 and the body weight change because there

Table 5: Haematological parameters of the rats exposed to aflatoxin-B1 orally through food contaminations

Parameters	Control	T1	T2	T3	Mean	p-values
Haemoglobin (g/dL)	10.48±0.62[a]	15.00±0.35[b]	14.30±0.10[b]	15.23±0.33[b]	13.75±0.48	0.001
PCV (%)	30.11±2.08[a]	40.50±0.88[b]	38.71±0.13[b]	42.10±1.00[b]	37.05±1.20	0.001
White Blood Cell (x10^9 cpL)	7.86±0.94[a]	5.82±0.54[b]	5.42±0.25[bc]	3.81±0.20[c]	5.73±0.42	0.001
Red Blood Cell (x10^{12} cpL)	5.45±0.44[a]	7.30±1.00[b]	7.02±0.07[b]	7.52±0.21[b]	6.82±0.22	0.001
MCV (fL/L)	56.40±0.96	55.73±0.44	55.20±0.43	54.46±0.95	55.45±0.38	0.344
MCHC (gHb/100mL)	35.26±0.76[a]	36.50±0.28[ab]	36.96±0.10[b]	35.90±0.16[ab]	36.15±0.24	0.05
MCH (fL/L)	19.90±0.71	20.36±0.25	20.43±0.11	20.30±0.14	20.25±0.18	0.77
Neutrophils (10^9 cells/L)	5.03±0.28[a]	15.16±1.36[b]	14.83±1.71[b]	16.55±0.76[b]	12.89±1.81	0.001
Lymphocytes (10^3 cells/µL)	82.20±0.33[a]	79.43±1.14[b]	73.61±1.55[b]	75.48±1.09[b]	77.68±1.02	0.001
Heterophil/Lymphocytes ratio	0.01±0.01[a]	0.20±0.02[b]	0.18±0.02[b]	0.22±0.01[b]	0.16±0.01	0.001

Means with different superscript on the same row are different ($p < 0.05$) for the parameter measured per group. Control – rats in the unexposed group, T1 – rats exposed to 150 µg aflatoxin-B1 per kg body weight on daily basis, T2 – rats exposed to 150 µg aflatoxin-B1 per kg body weight every two days, T3 – rats exposed to 150 µg aflatoxin-B1 per kg body weight twice weekly.

Table 6: Analysis of serum biochemical parameters in rats subjected to oral aflatoxin-b1 exposure via food contamination

	Control	T1	T2	T3	Mean	p-values
Sodium (mmol/L)	139.00±0.46[a]	137.37±0.70[b]	138.00±0.66[b]	140.37±0.48[a]	138.68±0.36	0.008
Potassium (mmol/L)	4.35±0.19	3.86±0.17	4.13±0.10	4.10±0.26	4.11±0.10	0.217
Bicarbonate (mmol/L)	26.3±70.11[a]	25.12±0.50[b]	26.12±0.33[ab]	27.00±0.31[a]	26.10±0.24	0.014
Chloride (mmol/L)	109.37±0.11[a]	107.75±0.64[b]	108.50±0.50[ab]	110.00±0.98[a]	108.90±0.35	0.106
Urea (mg/dL)	7.33±1.48	6.23±0.35	6.75±0.27	7.78±0.46	7.12±0.40	0.461
Creatinine (µmol/L)	135.25±12.40[ab]	175.00±4.42[c]	145.50±9.70[b]	141.75±10.66[a]	149.36±6.95	0.002
Total Bilirubin (µmol/L)	22.26±2.56[a]	8.73±1.83[b]	9.82±1.19[b]	5.67±0.28[b]	11.62±1.60	0.001
Alkaline Phosphatase (U/L)	40.25±3.46	40.50±2.99	47.37±6.04	34.87±2.89	40.75±2.12	0.23
Aspartate Aminotransaminase (U/L)	24.25±1.34[a]	85.00±1.73[b]	129.87±21.53[b]	130.62±19.33[b]	92.43±11.92	0.001
Alanine Aminotransaminase (U/L)	113.00±15.30[a]	155.00±1.07[b]	141.12±6.89[ab]	139.50±10.06[a]	137.18±5.70	0.05

Means with different superscripts on the same row are different ($p < 0.05$) for the parameter measured per group. Control – rats in the unexposed group, T1 – rats exposed to 150 µg aflatoxin-B1 per kg body weight daily, T2 – rats exposed to 150 µg aflatoxin-B1 per kg body weight every two days, T3 – rats exposed to 150 µg aflatoxin-B1 per kg body weight twice weekly.

was higher weight loss observed in the group continuously exposed to the mycotoxin daily compared with the other groups. Aflatoxin-B1 and other mycotoxins could be accumulated in grains consumed as food by humans and used to produce animal feeds. Upon consumption, because of the highly liposoluble nature of the aflatoxins, they could be easily solubilized and get into the blood circulation and eventually get distributed to different tissues, and liver (Mogopodi *et al*., 2022). The distribution in the gastrointestinal tract could cause alteration in the absorptive capacities of the intestinal tissue and suppression of immune function through modulation of cytokine gene expression (Grenier & Applegate, 2013; Thompson-Chagoyán *et al*., 2005). These complex processes could result in poor nutrient absorption and utilization of the derived nutrients which could both lead to poor body weight gain, and the poor body weight cannot be unconnected with the reduction in body weight of the exposed rats in this study. In agreement with this, some common symptoms of aflatoxin-induced food poisoning in humans reported include impaired food conversion ratio and slower growth rates (Fink-Grernmels, 1999; Godfrey *et al*., 2013). Further to these, the rate of exposure could also aggravate the loss of body weight because of the higher body weight loss recorded in the rats exposed every day compared with the others. This also agrees with the observations of Zain (2011), and Yu et al. (2020) who reported that the effect of aflatoxins in infants is dose-dependent and suggested that the higher dietary dosage could lead to a higher rate of body weight loss.

This study also suggests that continuous dietary exposure of rats to AFB1 could first be tolerated, but lead to accumulation of the toxin before the onset of body weight loss. This is because the significant decrease in body weight loss started only after 21 days of exposure to the mycotoxin in this present study. The implication of this is that irrespective of the exposure frequency, accumulation of aflatoxin-B1 over time can lead to a reduction in body weight at the exposure dosage of 150 µg per kg body weight used in this present study. While the reduction in body weight became noticeable 21 days after exposure, the reduction in food intake becomes noticeable by 14 days after; this means that the reduction in food intake commenced before the weight loss. Compared with the control group where the food intake continued to increase throughout the study period, the food intake of the exposed rats reduced from day 14 till the end of the study, culminating in an overall lower total food intake of the exposed rats compared with the control. It could be inferred from this that aflatoxin-B1 exposure complications on body weight changes commenced with reduced food intake which manifested later as body weight loss. This implied that the rat could be progressively losing the capacity for food digestion and nutrient utilization before actual weight loss; this agrees with the reports of Kennedy & Short (1986), and Early, (1995). This could be a food insecurity risk in sub-Saharan Africa because, the accumulation of aflatoxins in some food crops is possible right from the field which means that human and animal exposure, even at low doses on a continuous rate, could cause poor food intake and nutrient utilization before weight loss becomes evident (Mahuku *et al*., 2019; Noreddine, 2020; Tirado *et al*., 2010). Hence, there is a need to promote the elimination of aflatoxins right from the cultivation stage across the entire food chain to avoid its negative consequences such as malnutrition and children's stunted growth, and other complex dysfunctions such as infertility and reproductive ill-health, liver cirrhosis, hepatitis, liver cancer, infant and maternal mortality (Patial *et al*., 2018; Stehbens, 2003; Whitmee *et al*., 2015).

This study also showed that exposure of the rats to aflatoxin-B1 could negatively affect internal organs; this is because, while the weights of the liver and kidney of the exposed rats were higher than the unexposed group, the testes of the group exposed daily, and every two days were smaller compared with the control. These observations could be a result of inflammation of the liver and poor cellular activities in the testes which could pose negative critical dysfunctions on the metabolic and reproductive performances of the rats (Abdel-Wahhab *et al*., 2018; Alsayyah *et al*., 2019; Fan *et al*., 2021). Also, the percentages of liver, kidney, and testes to body weight of the rats in the group exposed continuously every day was significantly

higher compared with the other groups; this is an indication that the rate of exposure is a factor determining the percentages of these organs to the body weight. The liver and kidney damage by aflatoxin-B1 could cause a reduction in total protein circulation which is a prominent toxic effect occasioned by failure in the synthesis of protein due to its possible adducts formation with nucleic acids, gene expressions inhibition, and degranulation of the endoplasmic reticulum which were all reported consequences of aflatoxin-B1 according to the reports of Mallmann & Dilkin (2011), Sharma *et al.* (2011) and Wangikar *et a*l. (2005). These could also not be unconnected with the recorded elevated concentrations of aspartate aminotransferase, alanine aminotransferase, and creatinine found in the serum of rats exposed to aflatoxin-B1 in this study.

While some of the observations highlighted above agree with some previous studies on aflatoxin-B1 exposures, this present study suggests bilirubin and heterophil-lymphocyte ratio as possible haematological biomarkers of stress related to performance dysfunction of body weight changes and food intakes in the rats. This is because there was a significant reduction in total bilirubin in the exposed rats which could be linked with lowered total antioxidant capacities. The reduction in bilirubin could also be linked with a possible response of the rats to stress induction due to aflatoxin-B1 and the use of the bilirubin for counterbalancing of the prooxidants generated because of oxidative stress induction by the mycotoxin (Adin *et al*., 2005; Clark *et al*., 2000; Stocker, 2004). Apart from the reduced bilirubin concentration identified as a possible haematological marker of stress associated with the aflatoxin-B1 exposure in this study, elevated heterophil-lymphocyte count ratio could also be recognized as another haematological biomarker of stress concerning the AFB1 exposure. This is because, compared with rats in the control group, the exposed rats have a significantly higher heterophil-lymphocytes (H/L) ratio. Meanwhile, as a biomarker of stress, a decreased H/L ratio has been reported as a signal of strong antioxidant defence status which corresponds with reduced malondialdehyde and higher body weight gain in broilers (Aengwanich & Suttagit, 2010; Hosseini-Vashan & Raei-Moghadam, 2019; Özkan *et al*., 2007). Hence, elevated H/L recorded in the exposed rats could be because of possible stress induction by the aflatoxin in the rats, because the rats lost body weight and consumed less food compared with the unexposed group and these cannot be unconnected with the possible induction of oxidative stress damages in the rats.

Mycotoxins, including aflatoxin-B1, are toxic principles in mammalian species including humans, birds, swine, fish, and rodents; while oxidative stress is an underlying promoter of performance dysfunction associated with the toxin principle (Lahiani *et al*., 2017). Exposure of humans and animals to AFB1 has been reported to be linked with increased malondialdehyde concentration, lipid peroxides, and protein conjugates in the liver, kidney, brain, and cutaneous tissues (Lee *et al*., 2005; Madhusudhanan *et al*., 2004; Ravinayagam *et al*., 2012). In addition, it has also been reported to cause structural and functional deformations to DNA as well as oxidation of amino acid residue side chains, formation of protein-protein cross-linkages, and oxidation of the protein backbone resulting in protein fragmentation, and the modified forms of proteins accumulation in organisms resulting in changed physiological functions (Hatziagapiou & Lambrou, 2020; Marin & Taranu, 2012). However, despite this evidence, there is a scarce approach to assessment of oxidative stress complexities in aflatoxin poisoning due to variations in the measurement of oxidative stress biomarkers.

Therefore, there is a need for a simple and rapid assessment of AFB1-induced oxidative stress-related physiological functional compromise to contribute to the prevention of aflatoxicosis in humans and animals. Hence, linking of higher heterophil-lymphocyte count ratio, and reduced bilirubin concentration to aflatoxin-B1 toxicity as demonstrated in this study could suggest the suitability of these haematology biomarkers as suitable options for measuring oxidative stress-induced performance dysfunctions in mammalian species such as rats. Stress induction could be regarded because of toxicity associated with depression and compromised welfare leading to

loss of body weight in the exposed rats. The induction of stress in the rats, like in other animals, usually caused an increase in corticosterone due to an increase in the secretion of adrenocorticotrophic hormone (ACTH), and corticotropin-releasing hormone.

These changes could be both environmentally or biologically induced, but could also be triggered by dietary means such as fasting, feed restriction, nutrient deficiency, and toxicity as shown in the present study. However, under any of these conditions, there could be an elevation of free corticosterone and production compromises such as decreased growth, increased liver, intestinal, and adipose tissue weights, and depressed immune function which are all associated with elevated heterophil-lymphocyte ratio (Roushdy *et al*., 2020). Meanwhile, measuring these parameters using endocrine biomarkers could be complex when compared with the simplicity of the H/L count ratio which can be easily computed from full blood counts. Hence, since there are relationships among H/L count ratio, stress induction, and performances as observed in this study, the H/L count ratio could serve as a convenient means of determining aflatoxin-B1-induced stress complications in rats and similar mammalian species.

Conclusion

It could be concluded from the data obtained in this study that, aflatoxin-B1 is a mycotoxin capable of inducing stress-related performance compromise in Sprague Dawley rats. Also, blood heterophil-lymphocyte (H/L) count ratio and serum bilirubin concentration are worthy potential biomarkers suitable for measuring performance compromise associated with AFB1 toxicity in rats. Therefore, further study on H/L count ratio, bilirubin concentration, performances, oxidative stress status, and their molecular regulations in rats exposed to AFB1 is warranted for determining and establishing the validity of H/L and bilirubin concentration changes as suitable biomarkers for assessing aflatoxin-B1 stress-related performance compromises in rats and other mammalian species.

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Animal Ethics and Use Clearance

The clearance for implementation of the research was given after several reviews of the protocol by the Institutional Animal Ethics Committee (IAEC) of the Federal University of Technology, Minna, Nigeria. The assigned number was 0000013EAU, approved on 9th November 2020.

References

Abdel-Wahhab, M. A., El-Nekeety, A. A., Hassan, N. S., Gibriel, A. A. Y., & Abdel-Wahhab, K. G. (2018). Encapsulation of cinnamon essential oil in whey protein enhances the protective effect against single or combined sub-chronic toxicity of fumonisin B1 and/or aflatoxin B1 in rats. *Environmental Science and Pollution Research*, *25*(29), 29144–29161. https://doi.org/10.1007/s11356-018-2921-2

Adin, C. A., Croker, B. P., & Agarwal, A. (2005). Protective effects of exogenous bilirubin on ischemia-reperfusion injury in the isolated, perfused rat kidney. *American Journal of Physiology-Renal Physiology*, *288*(4), F778 - F784. https://doi.org/10.1152/ajprenal.00215.2004

Aengwanich, W., & Suttagit, M. (2010). Effect of polyphenols extracted from Tamarind (Tamarindus indica L.) seed coat on physiological changes, heterophil/lymphocyte ratio, oxidative stress, and body weight of broilers (Gallus domesticus) under chronic heat stress. *Animal Science Journal*, *81*, 264–270.

Alahlah, N., El Maadoudi, M., Bouchriti, N.,

Triqui, R., & Bougtaib, H. (2020). Aflatoxin M1 in UHT and powder milk marketed in the northern area of Morocco. *Food Control*, *114*, 107262.https://doi.org/10.1016/j.foodcont.2020.107262

Alsayyah, A., ElMazoudy, R., Al-Namshan, M., Al-Jafary, M., & Alaqeel, N. (2019). Chronic neurodegeneration by aflatoxin B1 depends on alterations of brain enzyme activity and immunoexpression of astrocyte in male rats. *Ecotoxicology and Environmental Safety*, *182*, 109407. https://doi.org/10.1016/j.ecoenv.2019.109407

Awapak, D., Petchkongkaew, A., Sulyok, M., & Krska, R. (2021). Co-occurrence and toxicological relevance of secondary metabolites in dairy cow feed from Thailand. *Food Additives & Contaminants: Part A*, *38*(6), 1013–1027. https://doi.org/10.1080/19440049.2021.1905186

Benevenga, N. J., Calvert, C., Eckhert, C. D., Fahey, G. C., Greger, J. L., Keen, C. L., Knapka, J. J., Magalhaes, H., Oftedal, O. T., Reeves, P. G., Shaw, H. A., Smith, J. E., & Steele, R. D. (1995). *Nutrient requirements of laboratory animals* (J. Overton, Ed.; Fourth). National Academy Press.

Briggs, C., Longair, I., Kumar, P., Singh, D., & Machin, S. J. (2012). Performance evaluation of the Sysmex haematology XN modular system. *Journal of Clinical Pathology*, *65*(11), 1024–1030. https://doi.org/10.1136/jclinpath-2012-200930

Clark, J. E., Foresti, R., Sarathchandra, P., Kaur, H., Green, C. J., & Motterlini, R. (2000). Heme oxygenase-1-derived bilirubin ameliorates postischemic myocardial dysfunction. *American Journal of Physiology-Heart and Circulatory Physiology*, *278*(2), H643–H651. https://doi.org/10.1152/ajpheart.2000.278.2.H643

Early, D. A. (1995). *Determination of exposure of humans to selected mycotoxins with particular reference to aflatoxins*. University of Natal.

El-Sayed, R. A., Jebur, A. B., Kang, W., El-Esawi, M. A., & El-Demerdash, F. M. (2022). An overview on the major mycotoxins in food products: characteristics, toxicity, and analysis. *Journal of Future Foods*, *2*(2), 91–102. https://doi.org/10.1016/j.jfutfo.2022.03.002

Everitt, J. I., & Gross, E. A. (2006). Euthanasia and Necropsy. In *The Laboratory Rat* (pp. 665–678). Elsevier. https://doi.org/10.1016/B978-012074903-4/50023-6

Fan, T., Xie, Y., & Ma, W. (2021). Research progress on the protection and detoxification of phytochemicals against aflatoxin B1-Induced liver toxicity. *Toxicon*, *195*, 58–68. https://doi.org/10.1016/j.toxicon.2021.03.007

Fink-Grernmels, J. (1999). Mycotoxins: Their implications for human and animal health. *Veterinary Quarterly*, *21*(4), 115–120. https://doi.org/10.1080/01652176.1999.9695005

Gallo, M., Ferrara, L., Calogero, A., Montesano, D., & Naviglio, D. (2020). Relationships between food and diseases: What to know to ensure food safety. *Food Research International*, *137*, 109414. https://doi.org/10.1016/j.foodres.2020.109414

Godfrey, S., Kitya, D., Lubega, A., Ogwal-Okeng, J., W., W., & B., D. (2013). Review of the Biological and Health Effects of Aflatoxins on Body Organs and Body Systems. In *Aflatoxins - Recent Advances and Future Prospects*. InTech. https://doi.org/10.5772/51201

Grenier, B., & Applegate, T. (2013). Modulation of Intestinal Functions Following Mycotoxin Ingestion: Meta-Analysis of Published Experiments in Animals. *Toxins*, *5*(2), 396–430. https://doi.org/10.3390/toxins5020396

Guerre, P. (2020). Mycotoxin and Gut Microbiota Interactions. *Toxins*, *12*(12), 769. https://doi.org/10.3390/toxins12120769

Hatziagapiou, K., & Lambrou, G. I. (2020). Antitoxicant Properties of Saffron and Relevance to Protection from Toxins and Drugs. *Current Bioactive Compounds*, *16*(3), 265–283. https://doi.org/10.2174/1573407214666181003123707

Hosseini-Vashan, S. J., & Raei-Moghadam, M. S. (2019). Antioxidant and immune system status, plasma lipid, abdominal fat, and growth performance of broilers exposed to heat stress and fed diets supplemented with pomegranate pulp (Punica granatum L.). *Journal of Applied Animal Research*, *47*(1), 521–531. https://doi.org/10.1080/09712119.2019.1676756

IARC. (1993). Aflatoxins: naturally occurring aflatoxins (Group 1), aflatoxins M1 (Group 2B). *Int Agency Res Cancer*, *56*, 245.

Kennedy, G. L., & Short, R. D. (1986). Biological Effects of Acetamide, Formamide, and Their Monomethyl and Dimethyl Derivatives. *CRC Critical Reviews in Toxicology*, *17*(2), 129–182. https://doi.org/10.3109/10408448609023768

Khan, F. A. (2000). *Physiological responses of acclimated and non-acclimated broilers exposed to heat stress and continuous delivery of ACTH*. Mississippi State University.

Kumari, S., Sharma, S., Advani, D., Khosla, A., Kumar, P., & Ambasta, R. K. (2021). Unboxing the molecular modalities of mutagens in cancer. *Environmental Science and Pollution Research*. https://doi.org/10.1007/s11356-021-16726-w

Lahiani, A., Yavin, E., & Lazarovici, P. (2017). The Molecular Basis of Toxins' Interactions with Intracellular Signaling via Discrete Portals. *Toxins*, *9*(3), 107. https://doi.org/10.3390/toxins9030107

Lee, J. K., Choi, E. H., Lee, K. G., & Chun, H. S. (2005). Alleviation of aflatoxin B1-induced oxidative stress in HepG2 cells by volatile extract from Allii Fistulosi Bulbus. *Life Sciences*, *77*, 2896–2910.

Madhusudhanan, N., Kavithalakshmi, S. N., Shanmugasundaram, R. K., & Shanmugasundaram, E. R. (2004). Oxidative damage to lipids and proteins induced by aflatoxin B(1) in fish (Labeo rohita)-protective role of Amrita Bindu. *Environmental Toxicology and Pharmacology*, *17*, 73–77.

Mahato, D. K., Lee, K. E., Kamle, M., Devi, S., Dewangan, K. N., Kumar, P., & Kang, S. G. (2019). Aflatoxins in Food and Feed: An Overview on Prevalence, Detection and Control Strategies. *Frontiers in Microbiology*, *10*. https://doi.org/10.3389/fmicb.2019.02266

Mahuku, G., Nzioki, H. S., Mutegi, C., Kanampiu, F., Narrod, C., & Makumbi, D. (2019). Pre-harvest management is a critical practice for minimizing aflatoxin contamination of maize. *Food Control*, *96*, 219–226. https://doi.org/10.1016/j.foodcont.2018.08.032

Mallmann, C. A., & Dilkin, P. (2011). *Mycotoxins and mycotoxicosis in swine* (G. Zaviezo & D. Zaviezo, Eds.; Special Nu).

Marchese, S., Polo, A., Ariano, A., Velotto, S., Costantini, S., & Severino, L. (2018). Aflatoxin B1 and M1: Biological properties and their involvement in cancer development. In *Toxins* (Vol. 10, Issue 6). MDPI AG. https://doi.org/10.3390/toxins10060214

Marin, D. E., Bulgaru, C. V., Anghel, C. A., Pistol, G. C., Dore, M. I., Palade, M. L., & Taranu, I. (2020). Grape Seed Waste Counteracts Aflatoxin B1 Toxicity in Piglet Mesenteric Lymph Nodes. *Toxins*, *12*(12), 800. https://doi.org/10.3390/toxins12120800

Marin, D. E., & Taranu, I. (2012). Overview on aflatoxins and oxidative stress. *Toxin Reviews*, *31*(3–4), 32–43. https://doi.org/10.3109/15569543.2012.730092

Mogopodi, D., Mbisana, M., Raditloko, S., Chibua, I., & Paphane, B. (2022). Toward Safe Food Systems: Analyses of Mycotoxin Contaminants in Food and Preventive Strategies Thereof for Their Formation and Toxicity. In *Food Systems Resilience [Working Title]*. IntechOpen. https://doi.org/10.5772/intechopen.101461

Navale, V., Vamkudoth, K. R., Ajmera, S., & Dhuri, V. (2021). Aspergillus derived mycotoxins in food and the environment: Prevalence, detection, and toxicity. *Toxicology Reports*, *8*, 1008–1030. https://doi.org/10.1016/j.toxrep.2021.04.013

Noreddine, B. (2020). Aflatoxins: Producing Molds, Structure, Health Issues and Incidence in Southeast Asian and Sub-Saharan African

Countries. *International Journal of Environmental Research and Public Health*, *17*(4), 1215. https://doi.org/10.3390/ijerph17041215

Özkan, S., Malayoğlu, H. B., Yalçin, S., Karadaş, F., Koçtürk, S., Çabuk, M., Oktay, G., Özdemir, S., Özdemir, E., & Ergül, M. (2007). Dietary vitamin E (α-tocopherol acetate) and selenium supplementation from different sources: performance, ascites-related variables, and antioxidant status in broilers reared at low and optimum temperatures. *British Poultry Science*, *48*(5), 580–593. https://doi.org/10.1080/00071660701593951

Patial, V., Asrani, R. K., & Thakur, M. (2018). Food-Borne Mycotoxicoses: Pathologies and Public Health Impact. In *Foodborne Diseases* (pp. 239–274). Elsevier. https://doi.org/10.1016/B978-0-12-811444-5.00009-9

Price, P. T. (2016). *Food Safety and Heat Stress Response of Broiler Chickens Fed a Saccharomyces Cerevisiae Fermentation Product*. Texas A & M University.

Ravinayagam, V., Jaganathan, R., Panchanadham, S., & Palanivelu, S. (2012). Potential Antioxidant Role of Tridham in Managing Oxidative Stress against Aflatoxin-B(1)-Induced Experimental Hepatocellular Carcinoma. *International Journal Hepatology*, 428–373.

Roushdy, E. M., Zaglool, A. W., & Hassan, F. A. M. (2020). Thermal stress consequences on growth performance, immunological response, antioxidant status, and profitability of finishing broilers: transcriptomic profile change of stress-related genes. *Tropical Animal Health and Production*, *52*(6), 3685–3696. https://doi.org/10.1007/s11250-020-02405-4

Shabeer, S., Asad, S., Jamal, A., & Ali, A. (2022). Aflatoxin Contamination, Its Impact and Management Strategies: An Updated Review. *Toxins*, *14*(5), 307. https://doi.org/10.3390/toxins14050307

Sharma, V., Sharma, C., Paliwal, R., Pracheta, S. S., & Sharma, S. (2011). Ameliorative effects of Curcuma longa and curcumin on aflatoxin B1 induced serological and biochemical changes in kidney of male mice. *Asian Journal of Biochemical and Pharmaceutical Research*, *1*(2), 338–351.

Sikiru, A. B., Arangasamy, A., Alemede, I., Egena, S., Ippala, J., & Bhatta, R. (2021). Effects of dietary supplementation of Chlorella vulgaris on oxidative stress attenuation and serum biochemical profile of pregnant New Zealand White rabbits. *Indian Journal of Animal Science*, *90*(9), 88–91.

Stehbens, William. E. (2003). Oxidative stress, toxic hepatitis, and antioxidants with particular emphasis on zinc. *Experimental and Molecular Pathology*, *75*(3), 265–276. https://doi.org/10.1016/S0014-4800(03)00097-2

Stocker, R. (2004). Antioxidant Activities of Bile Pigments. *Antioxidants & Redox Signaling*, *6*(5), 841–849. https://doi.org/10.1089/ars.2004.6.841

Thompson-Chagoyán, O. C., Maldonado, J., & Gil, A. (2005). Aetiology of inflammatory bowel disease (IBD): Role of intestinal microbiota and gut-associated lymphoid tissue immune response. *Clinical Nutrition*, *24*(3), 339–352. https://doi.org/10.1016/j.clnu.2005.02.009

Tirado, M. C., Clarke, R., Jaykus, L. A., McQuatters-Gollop, A., & Frank, J. M. (2010). Climate change and food safety: A review. *Food Research International*, *43*(7), 1745–1765. https://doi.org/10.1016/j.foodres.2010.07.003

Wangikar, P. B., Dwivedi, P., Sinha, N., Sharma, A. K., & Telang, A. G. (2005). Teratogenic effects in rabbits of simultaneous exposure to ochratoxin A and aflatoxin B1 with special reference to microscopic effects. *Toxicology*, *215*(1–2), 37–47. https://doi.org/10.1016/j.tox.2005.06.022

Whitmee, S., Haines, A., Beyrer, C., Boltz, F., Capon, A. G., de Souza Dias, B. F., Ezeh, A., Frumkin, H., Gong, P., Head, P., Horton, R., Mace, G. M., Marten, R., Myers, S. S., Nishtar, S., Osofsky, S. A., Pattanayak, S. K., Pongsiri, M. J., Romanelli, C., … Yach, D. (2015). Safeguarding human health in the Anthropocene epoch: report of The Rockefeller Foundation–Lancet Commission

on planetary health. *The Lancet*, *386*(10007), 1973–2028. https://doi.org/10.1016/S0140-6736(15)60901-1

Yu, S., Jia, B., Liu, N., Yu, D., & Wu, A. (2020). Evaluation of the Individual and Combined Toxicity of Fumonisin Mycotoxins in Human Gastric Epithelial Cells. *International Journal of Molecular Sciences*, *21*(16), 5917. https://doi.org/10.3390/ijms21165917

Zain, M. E. (2011). Impact of mycotoxins on humans and animals. *Journal of Saudi Chemical Society*, *15*(2), 129–144. https://doi.org/10.1016/j.jscs.2010.06.006