IMPACT OF *JUSTICIA CARNEA* LEAF AQUEOUS EXTRACT ON HAEMATOLOGICAL INDICES OF AFLATOXIN B1 EXPOSED BROILER CHICKENS.

Ayodele, O.A.[1]*, Oloruntola , O.D.[1], Ayodele, S.O.[2], Falowo, A.B.[1], Adeyeye, S.A.[3], and Gbore, F.A.[1]
[1]Department of Animal Science, Adekunle Ajasin University, Akungba-Akoko, Nigeria.
[2]Department of Agricultural Technology, The Federal Polytechnic, Ado Ekiti, Nigeria.
[3]Department of Animal Health and Production Technology, Federal College of Agriculture, Akure, Nigeria.
*Corresponding author: ayodeleojurereoluwa@gmail.com

ABSTRACT

Aflatoxicosis negatively impacts haematological indices in broiler chickens, leading to a reduction in red blood cells and a weakened immune response. However, phytobiotic compounds demonstrate protective and mitigating effects against the harmful impacts of aflatoxin B1. This study assessed the effects of Justicia carnea leaf aqueous extract (JCLE) on haematological indices of broiler chickens exposed to aflatoxin B1 (AFB1). Two hundred and forty day-old broiler chicks were randomly assigned to four treatment groups: CNTL (no AFB1 or leaf extract), AFT (0.3 mg/kg AFB1), AFJE1 (0.3 mg/kg AFB1 + 1g J. carnea leaf powder/L drinking water), and AFJE2 (0.3 mg/kg AFB1 + 2g J. carnea leaf powder/L drinking water), with six replicates of 10 chicks each. The birds in AFB1-treated group (AFT) demonstrated a low (P<0.05) packed cell volume (PCV), haemoglobin concentration (HbC) levels, red blood cell (RBC) count and white blood cell (WBC) count, including granulocytes, lymphocytes, and monocytes compared to the birds in the control group (CNTL), thus, indicating the occurrence of AFB1-induced anaemia. Supplementation with JCLE at 1g/L and 2g/L of drinking water in birds of AFJE1 and AFJE2 groups respectively, improved these haematological parameters. However, mean cell haemoglobin concentration (MCHC), mean cell volume (MCV), and mean cell haemoglobin (MCH) showed no significant changes. In conclusion, JCLE mitigated AFB1-induced haematotoxic effects in broiler chickens, exhibiting dose-dependent restorative properties and highlighting its potential as a natural remedy for managing aflatoxicosis.

Keywords: Aflatoxins, Haematological indices, *Justicia carnea,* Aqueous extract, Amelioration

INTRODUCTION

Broiler chicken production is a critical sub-sector of livestock farming globally, playing a key role in food security, economic growth, and public health. As a primary source of affordable, high-quality protein, broilers address malnutrition and meet the growing global demand for animal protein. Their efficient feed-to-meat conversion makes them a sustainable protein source for diverse populations (Onunkwo et al., 2021). Safeguarding the health of broiler chickens for optimal productivity is essential to ensuring the supply of safe poultry products, reducing zoonotic disease risks, and maintaining consumer trust (LaPelusa & Kaushik, 2022).

Aflatoxin B1 (AFB1), a potent mycotoxin commonly contaminating poultry feed, poses significant challenges to the poultry industry. Its detrimental effects include impaired growth performance, reduced feed efficiency, immunosuppression, anaemia, increased disease susceptibility, and mortality, resulting in substantial economic losses. The impact of AFB1 on haematological indices highlights the urgency of exploring effective mitigation strategies.

Growing restrictions on the use of antibiotics in broiler production, plus concerns over resistance and residues in meat, have intensified the search for alternatives. Plant-based bioactive compounds also known as phytobiotics, have emerged as a natural solution to improve poultry health without the drawbacks of antibiotics. *Justicia carnea*, commonly known as the blood leaf, is a medicinal plant valued for its therapeutic properties, including antidiabetic and antioxidant effects. Rich in phytonutrients like proteins, vitamins, and minerals (Usunomena and Okolie, 2016), it also contains beneficial phytochemicals such as tannins, flavonoids, saponins, and alkaloids (Olasupo et al., 2017). These qualities make it a promising natural feed additive for enhancing broiler health and productivity (Oleforuh-Okoleh *et al.*, 2015). This study evaluates the potential of *Justicia carnea* leaf (JCL) in ameliorating AFB1-induced haematological alterations in broiler chickens, contributing to sustainable and productive poultry farming practices (Dönmez *et al*, 2012).

MATERIALS AND METHODS

Justicia carnea leaf collection and processing

Fresh *Justicia canea* leaves were collected from the Teaching and Research Farm of Adekunle Ajasin University (AAUA). The leaves were washed, chopped, and dried under shade before being ground into a fine powder (JCLP) and stored in an airtight container.

Aflatoxin B1 production

Culture of *Aspergillus flavus* were inoculated on HiMedia potato dextrose agar plate, incubated at 28^0C for seven days afterwards, inoculated on maize grit for the production of aflatoxin B1 (AFB1) following the method described by Sundaram *et al*, (2001). Thereafter, the AFB1 cultured maize was ground to powder. The concentration of AFB1 in the maize grit was measured using the method described by Mgbeahuruike *et al*. (2018).

Experimental Diets

Two basal diets were prepared for the starter phase (1–21 days) and finisher phase (22–42 days) to meet the nutrient requirements of Cobb500 broilers (Table 1). The basal diets were divided into four portions: 1, 2, 3, and 4. Portion 1 is the CNTL (control, no AFB1 contamination, no leaf extract administration); portions 2 (AFT), 3 (AFJE1), and 4 (AFJE2) were contaminated at rate of 0.3 mg AFB1/kg feed following the procedure outlined by Oloruntola *et al.* (2024). Furthermore, the birds on AFJE1 and AFJE2 were offered 1g *Justicia carnea* leaf powder/L of water and 2g *Justicia carnea* leaf powder/L of water, respectively.

The summary of the experimental treatments is as follows:

CNTL: No AFB1 or leaf extract,

AFT: 0.3 mg/kg AFB1,

AFJE1: 0.3 mg/kg AFB1 + 1g *Justicia. carnea* leaf powder/L of water and

AFJE2: 0.3 mg/kg AFB1 + 2g *Justicia. carnea* leaf powder/L of water

Table 1: Composition of the basal diets

Ingredients (%)	Broilers' starter phase (1-3 weeks)	Broilers' finisher Phase (4-6 weeks)
Maize	50.36	58.36
Maize bran	3.00	0.00
Rice bran	0.00	3.02
Fish meal	3.00	3.00
Soybean meal	38.00	30.00
Bone meal	3.00	3.00
Premix	0.31	0.31
Limestone	0.49	0.47
Salt	0.31	0.31
Lysine	0.24	0.24
Methionine	0.29	0.29
Soy oil	1.00	1.00

Study Area, Experimental Birds and Design

This study was carried out at the Teaching and Research Farm of Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria. A total of 240-day-old broiler chicks were procured, weighed and assigned to the four dietary treatments in a completely randomized design (6 replicates/treatment;10 birds/replicate). Birds in all treatments had unrestricted access to feed and water throughout the experiment.

Data Collection and Analysis

Blood samples were aseptically collected from the branchial vein of six selected birds per treatment group (one bird per replicate) using a syringe and needle. The samples were transferred into bottles containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant for subsequent haematological analysis. Parameters analysed included haemoglobin concentration (HbC), packed cell volume (PCV), total erythrocyte and its differential counts, total leukocyte count, and leukocyte differential count. Statistical analysis of the data was conducted using analysis of variance and Duncan's Multiple Range Test was applied to identify significant differences, utilizing SPSS software (version 20).

RESULTS AND DISCUSSION

The effects of *Justicia carnea* aqueous leaf extract administration on the haematological indices of AFB1 exposed broiler chickens are shown in Table 2. PCV was significantly reduced in birds of AFT compared to the CNRL, thus indicating anaemia induced by aflatoxin toxicity. Supplementation with JCL aqueous extract improved PCV values in AFJE1 (30.00%) and restored it to near-normal levels in birds of AFJE2 (35.33%). These results conform to the outcome of Rathod *et al*, (2017) showcasing the extract's ability to mitigate AFB1-induced haematotoxicity.

Birds on AFB1 exposure in the AFT had a significantly lowered ($P<0.05$) haemoglobin levels while those of the extract-treated groups had higher haemoglobin concentrations, with birds in AFJE2 group almost reaching control

values. Donmez *et al*, (2012) in their work on the effects of aflatoxin on some haematological parameters, agrees with this outcome. This in essence implies that JCL, a phytobiotic source has restorative effect on erythropoiesis.

RBC count was significantly lower (P<0.05) in birds of AFT compared to those of CNRL. On treatment with JCL aqueous extract, birds in AFJE1 and AFJE2 showed improved RBC levels, demonstrating the protective effect of JCL aqueous extract's phyto-contents against AFB1-induced erythrocyte damage.

WBC count, granulocytes, lymphocytes, and monocytes were all significantly lowered (P<0.05) in birds of the AFT compared to birds in the CNRL, thereby reflecting an impaired immune function and immunosuppression. This is in accordance with the results of Yohannes *et al,* (2013) who observed leucocytopenia in broilers at relatively lower level of aflatoxicosis (100 ppb and 150 ppb respectively). JCL aqueous supplementation at 1g/L drinking water partially restored these parameters in birds of AFJE1 and the birds in AFJE2 nearing normalcy as in birds of the CNRL. Treatment with JCLA significantly improved these parameters in both AFJE1 and AFJE2 groups, with AFJE2 values closely matching those of the control. This indicates JCLA's capacity to counteract AFB1-induced leukocyte depletion and enhance immune competence.

No significant differences were observed among the groups for mean cell Haemoglobin concentration (MCHC), mean cell volume (MCV), and mean cell Haemoglobin (MCH). This indicates that the size and Haemoglobin content per cell remained unaffected.

Table 2: The haematological indices of broiler chickens exposed to Aflatoxin B1 and administered aqueous *Justicia carnea* leaf extract via daily drinking water.

Parameters	CNRL	AFB	AFJE1	AFJE2	SEM	P value
Packed cell volume (%)	35.67^{a}	26.00^{c}	30.00^{b}	35.33^{a}	1.30	0.01
Haemoglobin conc. (g/dl)	11.85^{a}	8.64^{c}	9.96^{b}	11.73^{a}	0.43	0.01
Red blood cells ($x10^{12}$/l)	3.43^{a}	2.54^{b}	2.88^{ab}	3.34^{a}	0.13	0.03
Mean cell haemoglobin conc. (g/dl)	33.26	33.26	33.29	33.26	0.02	0.78
Mean cell volume (fl)	103.88	102.54	106.24	106.17	2.99	0.97
Mean cell haemoglobin (pg)	34.51	34.07	35.29	35.27	0.99	0.98
White blood cells ($x10^{9}$/l)	10.94^{a}	7.75^{b}	9.44^{ab}	10.63^{a}	0.45	0.02
Granulocytes ($x10^{9}$/l)	3.72^{a}	2.63^{b}	3.20^{ab}	3.61^{a}	0.16	0.02
Lymphocytes ($x10^{9}$/l)	5.66^{a}	3.54^{c}	4.60^{b}	5.42^{ab}	0.27	0.01
Monocytes ($x10^{9}$/l)	1.54^{a}	1.09^{b}	1.32^{ab}	1.49^{a}	0.06	0.02

Means within a row with different letters are significantly different (P<0.05); CNRL: No aflatoxin B1 contamination; no supplementation; AFB1: 0.3 mg/kg aflatoxin B1; AFJE1: 0.3mg/kg aflatoxin B_1 + 1 g/l aqueous *Justicia carnea* leaf extract; AFJE2: 0.3mg/kg aflatoxin B_1 + 2 g/l aqueous *Justicia carnea* leaf extract; SEM Standard error of the mean.

CONCLUSION

The administration of *Justicia carnea* leaf extract at concentrations of 1g and 2g per liter of drinking water effectively mitigated the haematotoxic effects of AFB1 exposure in broiler chickens. The restorative effects demonstrated a dose-dependent response, with the 2g/L dosage (AFJE2) yielding the most significant recovery. These results highlight the potential of *Justicia carnea* leaf extract as a natural and effective intervention for managing aflatoxicosis in poultry.

REFERENCES

Dönmez N., Dönmez H. H., Keskin E. and Kısadere İ. (2012) Effects of aflatoxin on some haematological parameters and protective effectiveness of esterified glucomannan in Merino rams. Scientific World Journal. 342468. doi: 10.1100/2012/342468.

LaPelusa, A., and Kaushik, R. (2022). Physiology, Proteins. In StatPearls. StatPearls Publishing. Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK555990/

Mgbeahuruike A. C (2016) Aflatoxin contamination of Poultry Feeds in Nigerian Feed Mills and the Effect on the Performance of Abor Acre Broilers. Animal Research International (2016) 13(2): 2436 – 2445.

Olasupo, A.D., Olagoke, O. and Aborisade, A.B. (2017). Qualitative phytochemical screening o biologi (*Senecio biafrae*) and bitter leaf (*Verninia amygdalina*) leaves. Chemical Science International Journal 20(3), 1-6. http://doi.org/10.9734/CSJI/2017/35753

Oleforuh-Okoleh, V. U., Ndofor-Foleng, H. M., Olorunleke, S. O., and Uguru, J. O. (2015). Evaluation of growth performance, haematological and serum biochemical response of broiler chickens to aqueous extract of ginger and garlic. *Journal of Agricultural Science, 7*(4), 167.

Oloruntola, O.D,Ayodele,S.O, Oloruntola, D.A Olarotimi,O.J,Falowo,A.B,Akinduro,V.O, Gbore, F. A, Adu, O. A and Agbede, A.O. (2024) Dietary supplementation of *Capsicum* powder affects the growth immunoglobulins, pro-inflammatory cytokines, adipokines, meat, and liver histology of aflatoxin B1 exposed broiler chickens Toxin, 240, 107640. https://doi.org/10.1016/j.toxicon.2024.107640

Onunkwo, D. N., Udokwu, I. U., Ezea, J. and Ekundayo, E. O. (2021). Haematological indices of broiler chickens administered water containing medicinal plant leaf methanol extract. *Nigeria Agricultural Journal*, 52(2), 297-300. ISSN: 0300-368X.

Rathod, P., Gangadhar, K., Gangane, G., and Bhojane, N. (2017). Effect of aflatoxin on haematological and biochemical alteration in broilers. *International Journal of Science,* Environment and Technology, 6(1), 824–831. ISSN 2278-3687 (O).

Sundaram, T.K., A. Natrajan, D. Chandrasekaran and K. Mani. (2001) Feed analytical tech. *Manual of Animal feed Analytical and Quality Control Laboratory, Veterinary College and Research Institute, Namakkal.*

Usunomena, U., Okolie, N. P. (2016). Phytochemical analysis and proximate composition of *Vernonia amygdalina*. *International Journal of Scientific World,* 4(1), 11. https://doi.org/10.14419/ijsw.v4i1.5845

Yohannes, T., Sharma, A.K., Singh, S.D., and Sumi, V., (2013) Experimental haematobiochemical alterations in broiler chickens fed with t-2 toxin and co-infected with IBV. Open Journal of Veterinary Medicine, http://dx.doi.org/10.4236/ojvm.2013.35040. 3, 252-258.