THE IMPACT OF *VERNONIA AMYGDALINA* AQUEOUS LEAF EXTRACTS ON THE PERFORMANCE OF BROILER CHICKENS EXPOSED TO AFLATOXIN B_1

Oladebeye, F.S.[α,*], Oloruntola , O.D. [α], Adeyeye, S.A.[β], Ayodele, S.O.[γ], Falowo, A.B. [α], Olarotimi, O.J. [α], Gbore, F.A. [α], Ayodele, O.A.[α], Adeniji, O.E.[α] and Adu O.A. [Ł]

α Department of Animal Science, Adekunle Ajasin University, Akungba-Akoko, Nigeria.

β Department of Animal Health and Production Technology, Federal College of Agriculture, Akure, Nigeria.

γ Department of Agricultural Technology, The Federal Polytechnic, Ado Ekiti, Nigeria.

Ł Department of Animal Production and Health. The Federal University of Technology, Akure, Nigeria.

**Corresponding author: Oladebeye F.S., Email:* fehintoladebeye@gmail.com

ABSTRACT

The aim of this study was to assess the impact of Vernonia amygdalina aqueous leaf powder extract (VLAE) on the performance of broiler chickens exposed to aflatoxin B1. A total of 200-day-old broilers were divided into four groups, designated as treatments 1, 2, 3, and 4 (with 50 birds per treatment and 10 birds per replicate). Treatment assignments were as follows: Treatment 1 (Control), Treatment 2 (0.5 mg/kg aflatoxin B1 contamination), Treatment 3 (0.5 mg/kg aflatoxin B1 contamination + 1g VLAE per liter of water), and Treatment 4 (0.5 mg/kg aflatoxin B1 contamination + 2 g VLAE per liter of water). The body weight gain of broiler chickens in Treatments 3 and 4 was statistically similar ($P > 0.05$) but significantly ($P < 0.05$) higher than in the remaining diets. Conversely, the body weight gain of birds in Treatment 2 was lower ($P < 0.05$) compared to the other treatments. The feed conversion ratio was the lowest in Treatment 3, while the feed conversion ratio in Treatment 4 was comparable ($P > 0.05$) to Treatments 2 and 1. In conclusion, Vernonia amygdalina aqueous leaf powder extract (VLAE) demonstrates the potential to alleviate the adverse effects of aflatoxin B1 (AFB1) on the performance characteristics of broiler chickens exposed to 0.5 mg/kg aflatoxin B1.

Key words: Performance, Aflatoxin B_1, *Vernonia amygdalina*, Body weight gain, Feed conversion ratio.

INTRODUCTION

Mycotoxins represent a significant contributor to the health and disease challenges faced in chicken production, as highlighted by Nakavuma *et al*. (2020). These toxic compounds are secondary metabolites produced by various fungal species such as Aspergillus, Penicillium, Fusarium, and Trichoderma, posing a substantial threat to the well-being of poultry through their presence in food and feed (Tola and Kebede, 2016). According to Gizachew *et al*. (2016), mycotoxins can be found as harmful substances in staples like cereals and oilseeds. The detrimental effects of mycotoxins encompass a range of health hazards, including carcinogenic, cytotoxic, teratogenic, immunosuppressive, and neurotoxic impacts (Benkerroum, 2016). Mycotoxins exist in various classes, with aflatoxin being a prominent example. Aflatoxins are classified into different types, namely aflatoxin B_1, G_1, B_2, and G_2, each posing distinct risks to the health of poultry and other animals.

Aflatoxin B_1 stands out as a significant concern for chicken farmers, exerting a substantial impact on the health and productivity of poultry, particularly since it can be present in corn, a primary source of energy for these birds (Abbasi *et al*., 2018). The findings of Olarotimi *et al*. (2023) underscore the negative repercussions when chickens consume feed contaminated with aflatoxin B_1, including reduced growth rates, smaller body size, and lower weight. Furthermore, AFB1 toxicity is closely linked to its potent induction of oxidative stress, defined as an imbalance between the intracellular production of reactive oxygen species (ROS) and the antioxidant system (Halliwell *et al*., 2004). This imbalance results from the excessive generation of free radicals beyond the cellular antioxidant capacity, leading to the overproduction of ROS in macrophages, a critical element in the toxic mechanism (Wang *et al*., 2017).

Given this understanding, numerous studies have explored botanical approaches to mitigate the effects of AFB1, involving the utilization of natural substances such as essential oils, spices, herbs, and crude extracts (Iram *et al*., 2016). These botanical interventions exhibit the potential to enhance performance and maintain physiological well-being by improving immunity and antioxidant status in birds (Oloruntola *et al*., 2018). Importantly, this method is considered environmentally friendly, cost-effective, and a safer alternative source of bioagents for reducing the impact of aflatoxin B_1 in animals (Alberts *et al*., 2019).

One of the extensively studied plants for application in animal nutrition today is bitter leaf, scientifically known as *Vernonia amygdalina* (VA). Commonly referred to as bitter leaf due to its notable bitter component (Ekpo *et al*., 2018), this plant is recognized for its antidiabetic and antioxidant properties (Erukainure *et al*., 2019). According to Usunomena and Ngozi (2016), VA is a rich source of phytonutrients, encompassing proteins, carbohydrates, minerals, and vitamins. Additionally, it contains various phytochemicals such as tannins, flavonoids, saponins, and alkaloids (Olasupo *et al*., 2017). As a result, bitter leaf stands out as a natural feed additive with significant implications for both productivity and health in the broiler industry (Oleforuh-Okoleh *et al*., 2015).

MATERIALS AND METHODS

Study area

This study was performed in the Poultry unit of the Teaching and Research Farm, Adekunle Ajasin University, Akungba-Akoko (AAUA), Ondo State, Nigeria.

***Vernonia amygdalina*, experimental birds, diets and design.**

Fresh *Vernonia amygdalina* leaves (VL) were harvested from the Adekunle Ajasin University, Akungba Akoko, Teaching and Research Farm. Subsequently, the leaves were washed, drained, and finely chopped before being spread for air-drying over a span of 7 days. The dried leaves were then ground to produce *Vernonia amygdalina* leaf powder (VLP), which was stored in an airtight container until use. AFB_1 was inoculated in the AAUA microbiology laboratory. Maize was ground into grits and cultured with a toxigenic strain of *Aspergillus flavus*, and AFB_1 levels were quantified following the method outlined by Mgbeahuruike *et al*. (2018).

Two basal diets, formulated for the starter phase (0 to 21 days of age) and the grower phase (22 to 42 days of age), were created to meet the recommended nutrient levels for Cobb500 broiler chickens (refer to Table 1). The basal diets underwent proximate analysis (AOAC 2002) and were subsequently divided into four portions, named treatments 1, 2, 3, and 4. Treatments 2, 3, and 4 were contaminated with aflatoxin B_1 by incorporating AFB1-cultured maize to achieve a concentration of 0.5mg AFB1/kg feed, following the procedure described by Olarotimi *et al*. (2023).

Two hundred day-old Cobb 500 broiler chicks were randomly allocated to the four dietary treatments, with 50 birds per treatment and 10 birds per replicate. The feeding trial was conducted using a completely randomized design (CRD). For treatment 3, 1g of VLP was added to 1 liter of warm water, stirred, and sieved with a muslin cloth. In treatment 4, 2g of VLP was added to 1 liter of warm water, stirred, and sieved with a muslin cloth. These VLP solutions were then provided to the chickens in treatments 3 and 4, respectively.

Maintaining a temperature of 31ºC±2.0 for the initial 7 days, the temperature of the experimental pen was reduced by 2ºC after each consecutive 7 days until reaching 26ºC±2.0. Throughout the experimental period, feeds were provided *ad libitum*.

Data collection

Chickens were randomly weighed at the beginning of the experiment and thereafter their body weight (BW) and feed intake (FI) were measured and recorded every weekly. The average body weight gain (BWG) was calculated by subtracting the initial and final weights of the birds, and the feed conversion ratio (FCR) was calculated by dividing the feed consumed by the weight gained (g feed/ g gain).

RESULT AND DISCUSSION

The result of the growth performance of broiler chickens exposed to aflatoxin B_1 and *Vernonia amygdalina* aqueous leaf powder extract VLAE is presented in Table 1.

The body weight gain (BWG) of broiler chickens in treatments 3 and 4 exhibited no significant difference (P>0.05), yet both were significantly (P<0.05) higher than the other diets. Conversely, the body weight gain of birds in treatment 2 was significantly lower (P<0.05) compared to the remaining treatments. Treatment 3 demonstrated the lowest feed conversion ratio (FCR), while treatment 4 exhibited a FCR similar (P>0.05) to those in treatments 2 and 1. The reduced body weight gain observed in treatment 2 can be attributed to the ingestion of aflatoxin B_1, causing a decline in growth rate, resulting in smaller body size and lower weight. This finding aligns with the support provided by Olarotimi *et al*. (2023). Zou *et al*. (2023) further emphasized that aflatoxin B_1 disrupts the efficiency of the gastrointestinal tract, impacting the conversion and absorption of food components, ultimately leading to reduced nutrient absorption and conversion in birds. In contrast, the presence of *Vernonia amygdalina* leaf powder (VLP) in treatments 3 and 4, as reported by Daramola *et al*. (2018), enhanced gastrointestinal enzymes, thereby improving digestion and nutrient assimilation. The increase in feed conversion ratio in birds fed with treatment 4 suggests that the inclusion of 1 g VLP/liter of water is within the tolerable level for broiler chickens (Gemede and Ratta, 2014).

Table 1. Performance of broiler chickens exposed to Aflatoxin B_1 and administered *Vernonia amygdalina* aqueous leaf powder extract

Parameters	Treatment 1	Treatment 2	Treatment 3	Treatment 4	SEM	P value
Initial weight (g/bird)	40.22	40.55	40.26	40.36	0.18	0.94
Final weight (g/bird)	2538.47^{b}	2349.30^{c}	2813.34^{a}	2805.72^{a}	62.75	0.01
Weight gain (g/bird)	2498.25^{b}	2308.74^{c}	2773.07^{a}	2765.35^{a}	62.79	0.01
Feed consumption (g/bird)	3448.34	3221.53	3330.56	3500.17	43.69	0.07
Feed conversion ratio	1.38^{ab}	1.39^{a}	1.20^{c}	1.26^{bc}	0.02	0.02

Means within a row with different letters are significantly different (P<0.05); VLP: Vernonia amygdalina leaf powder; Treatment 1: No aflatoxin B1 contamination; no aqueous extract administration; Treatment 2: 0.5 mg/kg aflatoxin B1 contamination; Treatment 3: 0.5mg/kg aflatoxin B1 contamination + 1 g VLP/litre of water; Treatment 4: 0.5 mg/kg aflatoxin B1 contamination + 2 g VLP/litre of water; SEM: Standard error of the mean.

CONCLUSION

The VLAE at 1 g VLP/litre of water and 2 g VLP/litre of water mitigates the effect of AFB_1 on broiler chicken performance.

REFERENCES

Abbasi, F., Liu J., Zhang H., Shen X., & Luo X. (2018). Effects of feeding corn naturally contaminated with aflatoxin on growth performance, apparent ileal digestibility, serum hormones levels and gene expression of Na+, K+-ATPase in ducklings. Asian-Australasian Journal of Animal Science, 31(1), 91-97. https://doi.org/10.5713/ajas.17.0383

Alberts J., Rheeder J., Gelderblom W., Shephard G., & Burger H. (2019). Rural subsistence maize farming in South Africa: risk assessment and intervention models for reduction of exposure to Fumonisin mycotoxins. Toxins, 11:334. https://doi:10.3390/toxins11060334

AOAC (2002). Association of Analytical Chemist. Official Method of Analysis. 18th edition. Washington DC., USA.

Benkerroum, N. (2016). Mycotoxins in dairy products: A review. International Dairy Journal, 62, 63-75. https://doi.org/10.1016/j.idairyj.2016.07.002

Daramola, O.T., Oloruntola, O.D., & Ayodele, S.O. (2018). Nutritional Evaluation of Bitter Leaf meal (Vernonia amygdalina): Effects on Performance, Carcass and Serum Metabolites of Broiler Chickens. Nigerian Journal of Animal Science, 20(2): 211-221.

Ekpo, A., Eseyin, O.A., Ikpeme, A.O., & Edoho, E.J. (2007). Studies on some biochemical effects of Vernonia amygdalina in rats. Asian Journal of Biochemistry, 2, 193-197. https://doi.org/10.3923/ajb.2007.193.197

Erukainure, O.L., Chukwuma, C.I., Sanni, O., Matsabisa, M.G., & Islam, M.S. (2019). Histochemistry, phenolic content, antioxidant, and anti-diabetic activities of Vernonia amygdalina leaf extract. Journal of Food Biochemistry, 43(2), 12737. https://doi.org/10.5897/JMPR2020.6931

Gemede, H.F., & Ratta, N. (2014) Antinutritional factors in plant foods: potential health benefits and adverse effects. International Journal of Nutrition and Food Science, 3(4), 284–289. https://doi.org/10.11648/j.ijnfs.20140304.18

Gizachew, D., Szonyi, B., Tegegne, A., Hanson, J., & Grace, D. (2016). Aflatoxin contamination of milk and dairy feeds in the Greater Addis Ababa milk shed, Ethiopia. Food Control, 59, 773-779. https://doi.org/10.1016/j.foodcont.2015.06.060

Halliwell B., & Whiteman M. (2004). Measuring reactive species and oxidative damage in vivo and in cell culture: How should you do it and what do the results mean? British Journal of Pharmacology, 142(2), 231–255. https://doi.org/10.1038/sj.bjp.0705776

Iram, W., Anjum, T., Iqbal, M., Ghaffar, A., & Abbas M. (2016). Structural elucidation and toxicity assessment of degraded products of aflatoxin B1 and B2 by aqueous extracts of Trachyspermum ammi. Frontier in Microbiology, 7, 346. https://doi.org/10.3389/fmicb.2016.00346

Mgbeahuruike, A.C., Ejioffor, T.E., Christian, O.C., Shoyinka, V.C., Karlsson, M., & Nordkvist, E. (2018). Detoxification of aflatoxin-contaminated poultry feeds by 3 adsorbents, bentonite, activated charcoal, and Fuller's earth. Journal of Applied Poultry Research, 27(4), 461–71. https://doi.org/10.3382/japr/pfy054.

Nakavuma, J.L., Kirabo, A., Bogere, P., Nabulime, M.M., Kaaya, A.N., & Gnonlonfin, B. (2020).
Awareness of mycotoxins and occurrence of aflatoxins in poultry feeds and feed ingredients in selected regions of Uganda. International Journal of Food Contamination, 7 (1), 1-10. https://doi.org/10.12691/ajphr-10-4-1

Olarotimi, O.J., Gbore, F.A., Adu, O.A., Oloruntola, O.D., & Jimoh, O.A. (2023). Ameliorative effects of Sida acuta and vitamin C on serum DNA damage, pro- inflammatory and anti-inflammatory cytokines in roosters fed aflatoxin B1 contaminated diets. Toxicon, 236(2), 107330. https://doi.org/10.1016/j.toxicon.2023.10.107330/rs.3.rs-3293152/v1

Olasupo, A., Olagoke, O., & Aborisade. A. (2017). Qualitative phytochemical screening of bologi (Senecio biafrae) and bitter leaf (Vernonia amygdalina) leaves. Chemical Science International Journal, 20(3), 1-6. https://doi.org/10.9734/CSJI/35753

Oleforuh-Okoleh, V.U., Olorunleke, S.O., & Nte, J.I. (2015). Comparative Response of Bitter Leaf (Vernonia amygdalina) Infusion Administration on Performance, Haematology and Serum Biochemistry of Broiler Chicks. Asian Journal of Animal Sciences, 9 (5), 217-224. https://doi.org/10.3923/ajas.2015.217.224

Oloruntola, O.D., Ayodele, S.O., Agbede, J.O and Oloruntola D.A. (2018). Neem, pawpaw and bamboo leaf powder in broiler chickens. Effect on performance and health. Journal of Food Biochemistry, 42(2): e12723. https://doi.org/10.1111/jfbc.12723

Tola, M., & Kebede, B. (2016). Occurrence, importance and control of mycotoxins: A review. Cogent Food and Agriculture 2, 1191103. https://doi.org/10.1080/23311932.2016.1191103

Usunomena, U., & Ngozi, O.P. (2016). Phytochemical analysis and proximate composition of Vernonia amygdalina. International Journal of Scientific World, 4(1), 11-14. https://doi.org/10.14419/ijsw.v4i1.5845

Wang W.J., Xu Z.L., Yu C., & Xu X.H. (2017). Effects of aflatoxin B1 on mitochondrial respiration, ROS generation and apoptosis in broiler cardiomyocytes. Animal Science Journal, 88: 1561–1568. https://doi.org/10.1111/asj.12796

Zou, Y., Liu, S.B., Zhang, Q., & Tan, H. (2023). Effects of Aflatoxin B1 on growth performance, carcass traits, organ index, blood biochemistry and oxidative status in Chinese yellow chickens. Journal of Veterinary Medical Science, 85(9), 1015–1022. https://doi: 10.1292/jvms.23-0130