# MITIGATION OF RESIDUAL AFLATOXINS IN EDIBLE TISSUES OF BROILER CHICKENS

**[*†]Adeogun, J. B., [†]Omidiwura, B. R. O., [#]Mosobalaje, M. A., [†]Agboola, F. A., [+]Ewuola, E. O. and [†]Abu, O. A.**

[†]Agricultural Biochemistry and Nutrition Unit, Department of Animal Science, University of Ibadan [+]Animal Physiology and Bioclimatology Unit, Department of Animal Science, University of Ibadan, Ibadan, Nigeria. [#]Department of Animal Production and Health, Oyo State College of Agriculture and Technology, Igboora, Nigeria. [*]Correspondence: Adeogun, J. B. E-mail: adeogunjbayo@gmail.com

## ABSTRACT

Aflatoxins result in retarded growth or stunting in children. Strategies to reduce retention of inevitably absorbed aflatoxins in broiler chickens are less reported. Therefore, effect of mitigants made of yeast beta-glucans, supplemental dietary antioxidants (DA) and vitamin K (VK) in reducing residual aflatoxins in broiler chickens was investigated. Unsexed 180-day-old Arbor Acres chicks were randomly allotted into six dietary treatments for 49 days. Treatment (T) 1 was negative control (NC), T2 was positive control (PC), with 270±16µg.kg$^{-1}$ total aflatoxins ($AFB_1$ and $AFB_2$). T3 to T6 had 270µg.kg$^{-1}$ aflatoxins, DA and VK. T3 and T4 had 250mg.kg$^{-1}$ of beta-glucans inclusion, while T5 and T6 had 375mg.kg$^{-1}$. Inclusion rates of DA were: vitamin E (VE) 200mg.kg$^{-1}$; vitamin C (VC) 250mg.kg$^{-1}$; selenium 0.3mg.kg$^{-1}$ and VK 3mg.kg$^{-1}$. Only T4 and T6 had selenium. The experiment was completely randomised in a 2 + (2x2) augmented factorial arrangement. Aflatoxins retention and their residual in liver and breast meat (BM) were measured. Data were analysed using Anova at $\alpha_{0.05}$. Aflatoxins retention was reduced ($P < 0.05$) from 55.97% in PC birds to 12.00% in birds on T6. Residual aflatoxins in BM (2.56µg.kg$^{-1}$) and liver (3.46µg.kg$^{-1}$) in PC were reduced ($P < 0.05$) to 0.23µg.kg$^{-1}$ in BM and 0.35µg.kg$^{-1}$ in liver of birds on T5 and T6, respectively. Birds on T3 to T6 had reduction ($P < 0.05$) in aflatoxin retention and residual aflatoxins in BM and liver below those on PC. In conclusion, addition of beta-glucans, supplemental dietary antioxidants and vitamin K was effective in reducing residual aflatoxins in breast meat and liver of broiler chickens.

**Keywords**: Aflatoxins retention, residual aflatoxins, retarded growth, breast meat, liver

## INTRODUCTION

Apart from milk and milk derived products, aflatoxins can also be deposited in other edible animal products such as meat and eggs. Field strategies aimed at reducing pre-harvest aflatoxins contamination such as aflasafe® application can reduce contamination by 80-90 percent and Bühler LumoVision® optical sorting machine is up to 85-90 percent efficient in removing aflatoxins from contaminated grains. These further underscores the unavoidable presence of aflatoxins in maize/ feed materials, as efficiency is less than 100%. Since mycotoxin binder cannot not completely preclude mycotoxins from being absorbed in the digestive system of animals, Kolawole *et al*. (2019), there is the need to reduce retention of inevitably absorbed aflatoxins in broiler chickens' meat.

There are evidences that aflatoxins cause or aggravate retarded growth in children, in a condition referred to as stunting or kwashiorkor (Lamplugh and Hendrickse, 1982). Another reason which further underscores the need to reduce residual aflatoxins in broilers' meat is the strong synergy between aflatoxins and chronic hepatitis B virus (HBV), which present a greater risk in the induction of liver cancer in people positive to HBV (Wu and Guclu, 2012).

Unfortunately, there is no regulatory limit for aflatoxins in animal products except for milk and milk derived products. The US Food and Drug Administration (FDA) set the Maximum Residue Limit (MRL) for aflatoxin $M_1$ ($AFM_1$) in milk at 0.5 µg/kg (FDA, 2019) while the European Union (EU) set MRL for $AFM_1$ at 0.05 µg/kg (EU Commission, 2020). In Nigeria, there is no regulatory limit for aflatoxins in animal products (Miklós *et al*., 2020). Using the EU MRL for $AFM_1$ as the standard, it was reported that nearly 100% of samples of milk and milk products analysed in Nigeria and Sudan had exceptionally high levels of $AFM_1$ compared to the EU standard (Susan *et al*., 2012).

There is inadequate documentation on the retention of aflatoxins within the animal, from the fraction of the ingested toxin that could not be adsorbed by toxin binders, which ends up being absorbed into the animal's body. Therefore, an investigation was conducted on the effects of baker's yeast (*Saccharomyces cerevisiae*) derived beta-glucans, supplemental dietary antioxidants (vitamins C and E) and vitamin K in reducing residual aflatoxins in broiler chickens' meat below or within the permissible regulatory limit.

## MATERIALS AND METHODS

### Experimental Site

The study was carried out at the Teaching and Research Farm of the University of Ibadan, Ibadan, Nigeria. This experiment was reviewed and approved by the Department of Animal Science, in accordance to ARRIVE guidelines 2.0.

### Experimental Materials

Whole and clean maize grains were inoculated with toxigenic strain of *Aspergillus flavus*- isolate 3228, to generate total aflatoxins, using the method of Atehnkeng *et al*. (2008). The grain culturing lasted for 10 days and the contaminated grains were oven dried at about 60 – 70 ºC and finely ground. The grains were quantified (AOAC, 19090), and added to other Aflasafe® treated maize grains to produce a basal diet containing 270±16 ppb total aflatoxins (aflatoxins $B_1$ - $AFB_1$ and $B_2$ - $AFB_2$).

### Experimental Animals, Management and Diets

One-day-old Arbor Acres broiler chicks of mixed sexes (n = 180) were randomly allotted into six dietary treatments. Each treatment had 30 chicks, replicated three times with 10 chicks per replication. Treatment 1 was negative control- NC (uncontaminated diet), while Treatment 2 was basal diet (BD) or positive control- PC (diet with aflatoxins but without beta-glucans, antioxidants and vitamin K). Treatments 3 to 6 were contaminated diets having beta-glucans, dietary antioxidants and vitamin K. However, Treatments 3 and 5 do not contain selenium. The experiment lasted for 49 days.

### Dietary Treatments Layout

Treatment 1 (T1) = Negative Control (NC)
T2 = BD (Positive Control)
T3 = BD + 250 ppm beta-glucans + [(200 mg VE + 250 mg VC) + 3 mg VK]
T4 = BD + 250 ppm beta-glucans + [(200 mg VE + 250 mg VC) + 3 mg VK + 0.3 mg Se]
T5 = BD + 375 ppm beta-glucans + [(200 mg VE + 250 mg VC) + 3 mg VK]
T6 = BD + 375 ppm beta-glucans + [(200 mg VE + 250 mg VC) + 3 mg VK + 0.3 mg Se]
Where: VE- Vitamin E, VC- Vitamin C, VK- Vitamin K, Se- Selenium

### Parameters Measured

Average daily feed intake, daily aflatoxins intake and output, aflatoxin retention from the absorbed fraction and residual aflatoxins in blood, liver and breast meat were determined.

### Aflatoxins Retention and Residual Aflatoxins Determinations

On day 39, two birds per replicate were selected and housed in the metabolic cage for 10 days. Total faecal outputs were collected daily for five days, weighed and oven-dried at 60 ºC to a constant weight and thereafter air-dried for 24 hours. Aflatoxins content in feed and faecal samples was extracted using AOAC (1990) method and quantified with High Performance Thin Layer Chromatography (HPTLC) with a scanning densitometer.

The birds were later sacrificed for residual aflatoxins concentration in blood, liver and breast meat. Residual aflatoxins were assayed with enzyme-linked immunosorbent assays (ELISA) method, using Romer Labs AgraQuant® Total Aflatoxin Assay 4/40 kit and ELISA reader.

Aflatoxins retention was estimated by adapting the method of Kolawole *et al*. (2019), similar to nutrients balance trial procedure, i.e., $\left(\frac{\text{Intake}-\text{Output}}{\text{Intake}}\right) \times 100\%$.

Below are the calculations used in determining percentage aflatoxins retention:

$$\text{Average daily aflatoxins intake (ADAI)} = \text{ADFI} \times \text{FAC}, \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad \text{Eq. 1}$$

where ADFI = Average daily feed intake; FAC = Feed aflatoxins concentration

$$\text{Average daily faecal aflatoxins (ADFA)} = \text{ADFO} \times \text{FSAC}, \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad \text{Eq. 2}$$

where ADFO = Average daily faecal output; FSAC = Faecal samples aflatoxins concentration

$$\text{Aflatoxins retention (\%)} = \left(\frac{\text{ADAI} - \text{ADFA}}{\text{ADAI}}\right) \times 100\% \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{Eq. 3}$$

**Experimental Design and Statistical Analysis**

Experimental layout was a 2 + (2 x 2) augmented factorial arrangement in completely randomised design. Data collected were analysed with analysis of variance (ANOVA), significant differences in treatment means were separated with Duncan Multiple Range Test at 0.05% level of probability.

**RESULTS**

Table 1. Effect of varied levels of beta-glucans and supplemental selenium on aflatoxins retention, aflatoxin concentrations in blood, breast meat and liver of broiler chickens offered aflatoxin-contaminated poultry feed.

| Treatments | Aflatoxins (ppb) | Beta-glucans (ppm) | Selenium (mg/kg) | Aflatoxins concentrations | | | Aflatoxins Retention (%) |
|---|---|---|---|---|---|---|---|
| | | | | Blood (ng/mL) | Breast meat (µg/kg) | Liver (µg/kg) | |
| T1 (NC) | 0 | 0 | 0.00 | 0.03±0.01$^{d}$ | 0.00±0.00$^{c}$ | 0.00±0.00$^{d}$ | 0.00$^{e}$ |
| T2 (BD) | 270 | 0 | 0.00 | 9.17±0.60$^{a}$ | 2.56±0.34$^{a}$ | 3.46±0.43$^{a}$ | 55.97$^{a}$ |
| T3 | 270 | 250 | 0.00 | 3.85±0.26$^{b}$ | 0.52±0.27$^{b}$ | 0.78±0.13$^{b}$ | 29.27$^{b}$ |
| T4 | 270 | 250 | 0.30 | 3.91±0.71$^{b}$ | 0.29±0.10$^{bc}$ | 0.52±0.19$^{bc}$ | 18.45$^{cd}$ |
| T5 | 270 | 375 | 0.00 | 2.89±0.21$^{c}$ | 0.23±0.02$^{bc}$ | 0.46±0.13$^{bc}$ | 19.77$^{c}$ |
| T6 | 270 | 375 | 0.30 | 2.77±0.35$^{c}$ | 0.27±0.05$^{bc}$ | 0.35±0.11$^{c}$ | 12.00$^{d}$ |
| ±SEM | | | | 0.24 | 0.11 | 0.12 | 2.13 |
| P-value | | | | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |

[ab] Means of treatments along the same column with different superscript differed significantly (P < 0.05). NC- Negative control, SEM- standard error of means, P-value-probability, T- Treatment, T1- NC (0ppb aflatoxins), T2- Basal diet (BD) - 270±16 ppb total aflatoxins), T3- BD + 250 mg/kg beta-glucans + (E+C+K), T4- BD + 250 mg/kg beta-glucans + Vitamins (E+C+K)+Se, T5- BD + 375 mg/kg beta-glucans + Vitamins (E+C+K), T6- BD + 375 mg/kg beta-glucans + Vitamins (E+C+K)+Se, Se- Selenium.

Table 1 shows relative aflatoxins retention, aflatoxins concentration in blood, breast meat and liver of broiler chickens fed contaminated diets mitigated with varied inclusion levels of beta-glucans, supplemental antioxidants (with or without selenium) and vitamin K. The least aflatoxins retention (12.00%) within the body was recorded in birds on T6, significantly reduced (P < 0.05) below those birds on other treatments but similar (P > 0.05) to aflatoxins retention in birds on T4 (18.45%). However, higher (P < 0.05) aflatoxins retention (55.97%) was obtained in birds on PC diet, above those of other dietary treatments. Birds on PC diet had higher (P < 0.05) blood aflatoxins concentration, than the other dietary treatments. Blood aflatoxins level in birds on T3 to T6 reflected the level of beta-glucans inclusion. Birds on T3 and T4 had similar (P > 0.05) blood aflatoxin level, while birds on T5 and T6 also had similar (P > 0.05) blood aflatoxin concentration, that were significantly below (P < 0.05) those of T3 and T4. Aflatoxins concentrations in breast meat were similar (P > 0.05) in T4, T5 and T6, and also similar (P > 0.05) in liver, but lower (P < 0.05) significantly to those birds on PC diet. Birds fed PC diet had approximately 10 times higher aflatoxins concentration in breast meat (2.56 µg/kg) and liver (3.46 µg/kg) compared to those of birds on the mitigated test diets which ranged from 0.23 – 0.52 µg/kg in breast meat and 0.32 – 0.78 µg/kg in liver. Breast meat residual aflatoxin concentration in birds on T4 (0.29 µg/kg); T5 (0.23 µg/kg) and T6 (0.27 µg/kg) were similar (P > 0.05) to that of birds on NC diet, which had zero aflatoxins concentration.

**Discussion**

Addition of beta-glucans facilitated the reduction of aflatoxins absorption (Adeogun *et al*., 2021) from the gastrointestinal tract. The addition of beta-glucans, supplemental dietary antioxidants with or without selenium and vitamin K enhanced further reduction in aflatoxins' concentration in edible tissues (breast meat and liver). Earlier report by Adeogun *et al*. (2021) showed that 375 mg/kg of beta-glucans addition had higher gastrointestinal tract (GIT) aflatoxins adsorption than diet with 250 mg/kg inclusion. However, percentage aflatoxin retention in birds on T4 (with 250 mg/kg beta-glucans and supplemental selenium), which was similar to birds on 375 mg/kg beta-glucans, indicated that the addition of supplemental selenium was important in facilitating the excretion of aflatoxins metabolites away from the body. Enhanced disposal of aflatoxin metabolites may be the reason for the reduction in aflatoxins retention in all birds on mitigated test diets, compared to those on unmitigated diet. Results from the current study showed that birds fed PC diets retained more that 50 percent of the ingested aflatoxins. Significant reduction in aflatoxin retention in birds on T4 and T6, may be due to supplemental selenium. Currently, no regulatory limit exists for aflatoxins in edible animal products except for milk and milk derived products. Of all the different regulatory limits for aflatoxins available, only aflatoxin $M_1$ has a limit of 0.05µg/kg in milk and 0.025µg/kg in infant formulae (EU Commission, 2010), while 0.5µg/kg was permitted in milk by FDA (FDA, 2019). If these two regulatory limits were assumed for broiler chicken edible products, it will take the use of uncontaminated feed as seen in negative control diet, to achieve EU's aflatoxin maximum residual limit of 0.05µg/kg in broiler chickens' breast meat and liver. However, the mitigation strategies enumerated in the current study towards reducing residual aflatoxins in breast meat and liver of broiler chickens, gave breast meat residual aflatoxin concentration range of 0.23 to 0.29 µg/kg while in liver it ranged from 0.35 to 0.52 µg/kg. These two sampled tissues had residual aflatoxins levels that were within the 0.5 µg/kg of US FDA permissible aflatoxin $B_1$ level in milk (FDA, 2019).

## CONCLUSION AND RECOMMENDATIONS

It was noticed that only birds on uncontaminated diet gave edible products that was free of aflatoxins and safe for human consumption by any available regulation. However, addition of beta-glucans, supplemental dietary antioxidants and vitamin K to aflatoxin-contaminated poultry feed ensured the production of broiler chickens having breast meat and liver with safe level of aflatoxins within the permissible limit for human consumption. Therefore, where possible, aflatoxin-contaminated poultry feed/or feed materials should be avoided in practical broiler production, to ensure the production of safe edible products. This can be achieved by an act of law, not only to regulate aflatoxins level in feed and feed materials, but also backed up with effective monitoring and enforcement to ensure compliance.

## REFERENCES

Adeogun, J. B., Abu, O. A. and Ewuola, E. O. (2021). Effect of yeast beta-glucans on aflatoxin absorption in the gut of broiler chickens. *Nigerian Journal of Animal Production*, 48(6): 198-208. Available on: https://doi.org/10.5179/njap.v48i6.3292

AOAC, (1990). Association of Official Analytical Chemists. *Official Method of Analysis*, 15th edition, Arlington, VA, Section 968.22.

Atehnkeng, J., Ojiambo, P. S., Donner, M., Ikotun, T., Sikora, R. A., Cotty, P. J. and Bandyopadhyay, R. (2008). Distribution and toxigenicity of Aspergillus species isolated from maize kernels from three agro-ecological zones in Nigeria. *International Journal of Food Microbiology*, 122: 74–84.

European Commission- EC (2010). Commission Regulation (EU) No. 165/2010 of 26 February 2010, amending regulation (EC) No. 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards aflatoxin. *Official Journal of the European Union*, L 50: 8–12.

FDA (2019). Sec. 683.100 Action Levels for Aflatoxins in Animal Food, Compliance Policy Guide, *Guidance for FDA Staff, Food and Drug Administration Office of Regulatory Affairs and Centre for Veterinary Medicine*, pp. 1-4. Available at: https://www.fda.gov/ICECI/ComplianceManuals/CompliancePolicyGuidanceManual/default.htm.

Kolawole, O., Meneely, J., Greer, B., Chevallier, O., Jones, D. S., Connolly, L. and Elliott, C. (2019). Comparative *In Vitro* Assessment of a Range of Commercial Feed Additives with Multiple Mycotoxin Binding Claims. *Toxins*, 11, 659; https://doi.org/10.3390/toxins11110659

Lamplugh, S. M. and Hendrickse, R. G. (1982). Aflatoxins in the livers of children with kwashiorkor. *Annals of Tropical Paediatrics*, vol. 2, no. 3, pp. 101-104.

Miklós, G., Angeli, C., Ambrus, A., Nagy, A., Kardos, V., Zentai, A., Kerekes, K., Farkas, Z., Józ´wiak, A. and Bartók, T. (2020). Detection of Aflatoxins in Different Matrices and Food-Chain Positions. *Frontiers in Microbiology*, Volume 11, Article 1916. Available at: www.frontiersin.org; https://doi.org/10.3389/fmicb.2020.01916

Susan, O. K., Obansa, A. I., Anthony, M. H. and Chidawa, M. S. (2012). A preliminary survey of AFM1 in dairy cattle products in Bida, Niger State, Nigeria. *African Journal of Food Science and Technology*.,3:273–6.

Wu, F. and Guclu, H. (2012). Aflatoxin Regulations in a Network of Global Maize Trade. *PLoS ONE*, 7(9): e45151. https://doi.org/10.1371/journal.pone.0045151