Detoxification of Poultry Feed Using *Candida tropicalis* Isolated from Palm Wine

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Abstract: Aflatoxin contamination in poultry feed poses a significant threat to both poultry health and food safety. This study aimed to explore the potential of using *Candida tropicalis*, isolated from palm wine, to detoxify aflatoxins in poultry feed ingredients. In this study, *Candida tropicalis* was isolated from palm wine samples collected within Akure metropolis, Ondo State, Nigeria. The isolated yeast strain was identified morphologically, through sugar fermentation tests and molecularly. Concurrently, toxigenic *Aspergillus flavus* strains was isolated from contaminated poultry feed ingredients and was confirmed for aflatoxin production through quantification using thin layer chromatography (TLC). The ability of the isolated *Candida tropicalis* to degrade aflatoxins was assessed by inoculating toxigenic *A. flavus* into formulated poultry feed, followed by treatment with a suspension of *C. tropicalis*. The detoxification efficacy was evaluated by comparing the aflatoxin levels in treated and untreated feed samples. The *in vivo* effects of detoxified feed on broiler chickens, dividing them into five dietary groups and their haematological and liver enzyme parameters were monitored using standard techniques. Broilers fed with *Candida tropicalis*-treated feed showed improved hematological profiles, including higher white blood cell counts, compared to those fed with aflatoxin-contaminated feed. Additionally, liver enzyme activity was closer to normal in broilers consuming detoxified feed, demonstrating the protective effects of *Candida tropicalis.* Findings of the research demonstrates the potential of *Candida tropicalis*, as an effective biological agent for mitigating the effects of aflatoxins in poultry feeds.
Key word: Aflatoxins, *Candida tropicalis***,** detoxification, haematological parameters

INTRODUCTION

Poultry meat is becoming more and more popular as the world's population grows at a rapid pace. This is mostly because it is readily available and has a magnificent reputation as a protein source (Bilal *et al*., 2023). The growing number of people consuming chicken products means that concerns about microbial contamination must be addressed (Danbappa *et al*., 2018). Poultry products are at high risk of contamination from their feed, which can affect both animal health and consumers (Suleman *et al.,* 2022). Despite being dry, poultry feed provides a favourable environment for microbial growth, especially fungi and bacteria, due to its nutrient content, environmental factors like moisture and temperature further promote contamination (Gicheha *et al.,* 2021).

Mycotoxins which are harmful compounds produced by moulds, are a common contaminant in feeds. These toxins, including aflatoxins from *Aspergillus*, deoxynivalenol and fumonisin from *Fusarium*, and ochratoxin from *Penicillium*, are dangerous to both poultry and humans (Ochieng *et al.,* 2021). Among these, aflatoxins are particularly concerning due to their lower tolerance levels in poultry feed, causing symptoms like reduced growth, lower egg production, and increased mortality rates (Okasha *et al.,* 2024). Aflatoxins refer to a collective term for a group of chemically similar compounds with similar toxicity levels, including aflatoxin B1, B2, G1, and G2. Among these, AFB1 is usually the most commonly found in feedstuffs. In terms of toxicity, the order is typically Aflatoxin B1 > aflatoxin B2 > aflatoxin G1 > aflatoxin G2 (Nazhand *et al.,* 2020). Many studies have been conducted to determine the teratogenic, carcinogenic, mutagenic, and growth-inhibiting effects of aflatoxins in poultry in order to assess its toxicity. The detrimental consequences of aflatoxins on the body's hematological and histological systems have also been well documented (Oguz, 2011). Preventing the growth of moulds and contamination by aflatoxins in feed and feedstuffs is paramount.

However, in cases where contamination occurs despite preventive measures, it is crucial to decontaminate aflatoxins before

using these materials. To combat aflatoxins, biological detoxification using yeasts has emerged as an effective method. Yeasts can break down aflatoxins and also preserve feed quality. It also promote growth in broilers and enhances immunity by raising macrophages and antibodies. Yeasts cell wall derivatives are known to bind aflatoxins, reducing their harmful effects. Additionally, yeast produces enzymes that degrade aflatoxins by breaking down their chemical structure (Bilal *et al.,* 2023).

Palm wine, a fermented product of various palm trees, serves as an excellent and accessible source of yeast (Sarma *et al.,* 2022). Common yeast species from palm wine include *Saccharomyces* and *Candida*, which have shown promise in detoxifying poultry feeds (Djeni *et al.,* 2020). This study aims to assess the effectiveness of yeast isolated from palm wine in detoxifying poultry feed, while also evaluating the impact of detoxified feed on the hematological and histological health parameters of broilers.

MATERIALS AND METHODS

Isolation and identification of yeast from palm wine samples: Freshly tapped palm wine samples were obtained in sterile containers from various locations within Akure metropolis, Ondo State, Nigeria, including Ipinsa, Ibule, Ilara, and Oba-ile and transported to the laboratory for microbial analysis. Yeast isolation was carried out by serially diluting 1 ml of each sample in 9 ml of sterile distilled water. From the diluted suspensions, 0.1 ml was aseptically transferred onto sterile yeast extract agar (YEA) plates. The plates were incubated at 28±2°C for 48 hours, after which yeast colonies were enumerated. Pure cultures of the yeast isolates were obtained through repeated streaking on YEA. The yeast isolates were identified based on morphological characteristics, sugar fermentation profiles and molecular techniques (Olaniyi *et al*., 2019).

Isolation and identification of toxigenic fungus from the poultry feed samples: The suspected contaminated feed ingredient sample was homogenized to increase the surface area for fungal growth. The homogenized sample was plated on potato dextrose agar (PDA). The plate was incubated at room temperature (usually around 30-37°C) for five (5) days to allow fungal colonies to grow. To get pure fungal cultures, individual colonies were sub-cultured onto sterile PDA plates and observed for growth (Olaniyi *et al.,* 2019).

Quantification of aflatoxin in toxigenic fungi using thin layer chromatography: The method described by Leszczynska *et al*. (2001) was used in which, 50 g of the ground sample was weighed, filtered, and concentrated. Silica gel G was spread on a 20x20 cm plate and spotted with different volumes of the sample extract and standard aflatoxins. The plate was developed in a solvent system and the disintegrated sample was extracted with a methanol-water mixture to separate aflatoxin. The sample was homogenized, centrifuged, and diluted with phosphate buffer at pH=7.2. The absorbance of the solution was measured at 450 nm using a UV/Visible spectrophotometer, and the aflatoxin content was calculated using a prepared standard curve

Identification of yeast and toxigenic fungi isolate using molecular method: Genomic DNA of yeast and *Aspergillus* was extracted from the fungal samples with the Zymo Research Quick-DNA fungal/bacterial kit. The Internal Transcribed Spacer (ITS) region was amplified using OneTaq Quick-Load 2X Master Mix and specific primers. The amplified PCR products were enzymatically cleaned using the EXOSAP method. Sequencing was carried out in both directions with the Brilliant Dye Terminator Cycle Sequencing Kit, and the DNA fragments were purified using the ZR-96 DNA Sequencing Clean-up Kit. The purified sequences were analyzed on an ABI 3500xl Genetic Analyzer, and the resulting .ab1 files were processed using DNASTAR software. Sequence identification was conducted via BLAST search in the

GenBank database of the National Centre for Biotechnology Information (NCBI) (Stephen *et al.*, 1997).

Screening of yeast isolate for aflatoxin degrading enzymes: Laccase production was assessed by culturing each fungal strain on potato dextrose agar (PDA) plates supplemented with 0.02% guaiacol and 0.5% tannic acid, which were sterilized separately and incorporated into the medium before solidification. Additionally, 0.01% Remazol Brilliant Blue R (RBBR) was used as an indicator. The plates were incubated at 30°C for 7 days (Senthivelan *et al*., 2019). Chitinase activity was evaluated using the dinitrosalicylic acid (DNS) method, with colloidal chitin serving as the substrate. A mixture of 1.0 ml enzyme solution, 1.0 ml of 0.5% colloidal chitin, and 1.0 ml of 0.1 m citrate buffer (pH 7.0) was incubated at 37°C in a Wincom shaker water bath (Model-WBS-C2) at 280rpm for 30 minutes. The reaction was terminated by adding 2 ml of DNS reagent, followed by heating the mixture in a boiling water bath for 10 minutes. After cooling, the mixture was centrifuged at 10,000 rpm for 10 minutes at room temperature, and the absorbance of the supernatant was measured at 540 nm (Gonfa *et al*., 2023).

Inoculation of toxigenic Aspergillus flavus into the formulated poultry feed and detoxification with Candida tropicalis: Eighty kilograms (80 kg) of formulated poultry feeds were divided into four portions. The first portion contained feed inoculated with 500 ml of spore suspension of toxigenic *A. flavus*, while the second portion contained feed inoculated with 500 ml of spores suspension of toxigenic *A. flavus* plus 500 ml cell wall suspension of *Candida tropicalis*. The third portion contained feed inoculated with 500 ml cells wall suspension of *Candida tropicalis*, while the fourth portion contained feed that was not inoculated with either fungal spores or *Candida tropicalis* (control) (Ibitoye *et al.,* 2021). These detoxified feed samples were used for *in vitro* assessment and then administered to broiler chickens in accordance with International Standards of Animal Welfare. Fifteen four-week-old broilers were sourced from a commercial hatchery and divided into five groups, each containing three broilers. The groups were assigned as follows: Group 1 was fed with a basal diet (uninoculated feed), Group 2 was fed with *A. flavus*-contaminated feed, Group 3 was fed with *A. flavus*-contaminated feed treated with *Candida tropicalis*, Group 4 was fed with *Candida tropicalis*-inoculated feed, and Group 5 was fed with commercially purchased feed.

Quantitative analysis of aflatoxins in the formulated poultry feed samples: Disintegrated sample was extracted with 10 ml of methanol–water mixture (7:3) to separate aflatoxin. To that end, the rest was homogenized for 10 min at room temperature and the resultant deposit was centrifuged. An aliquot (100 µl) of the supernatant was diluted with 600 µl of phosphate buffer at pH = 7.2. The samples were incubated for 30 min at room temperature in the darkness. Then, 50 µl of tetramethylbenzidine and 50 µl of urea peroxide were added and incubated again for 30 min in darkness. The reaction was terminated by adding 100 µl of the stop reagent. The absorbance of solution was measured at a wavelength of 450 nm, using Longmed UV/Visible spectrophotometer (Model-SHZ82). The content of aflatoxins was calculated using prepared standard curve.

Haematology of the blood and serum biochemistry: At the end of the study, blood samples were collected directly from the axillary veins of the wing, obtaining 3 cc of blood into a sterilized glass tube containing Ethylene diamine tetra acetic acid (EDTA) and another glass tube without anti-coagulant for haematological and serum biochemistry (liver enzymes) respectively. Blood samples were separated, centrifuged, and frozen at 100℃ for the serum analysis (Hidayat *et al*., 2020).

Statistical analysis of data obtained: All generated data were subjected to one way analysis of variance (ANOVA) using

Statistical package for social sciences (SPSS) version 23.0. Treatment means were compared using Duncan's new multiple range test and differences were considered significant at P>0.05.

RESULTS

Total yeast counts and characteristics of yeast isolates

Figure 1 displays the total yeast counts from palm wine samples collected from various locations. The sample from Ibule exhibited the highest yeast count of 2.46×10^2 cfu/ml, while the sample from Oba-Ile had the lowest count of 1.50×10^2 cfu/ml. Table 1 presents the cultural and morphological characteristics of yeast isolated from different locations. Table 2 details the sugar fermentation profiles of the yeast isolates using carbon substrates such as galactose, glucose, sucrose, maltose, and raffinose. The colour change from red to yellow after 48 hours of fermentation suggests acid production.

Molecular identification of the yeast and fungi isolates

The yeast isolate was confirmed to be *Candida tropicalis* and the fungus as *Aspergillus flavus* using molecular methods. The sequences were matched with the GenBank database using the Basic Local Alignment Search Tool (BLAST) of the National Centre for Biotechnology Information (NCBI) as shown in Table 3.

Quantity of degrading enzymes present in *Candida tropicalis* isolates

Table 4 depicts the quantities of the degrading enzymes namely laccase and chitinase present in *Candida tropicalis.* The relatively high quantity of laccase (4.851 mg/ml/min) and the presence of chitinase at 1.211 mg/ml/min in *Candida tropicalis* could lead to both direct degradation of aflatoxins and inhibition of aflatoxin-producing fungus, contributing to safer feed and improved poultry health.

Concentration of aflatoxin in the toxigenic *Aspergillus flavus* isolated from the feed ingredients

The aflatoxin B1 (AFB1) content of the fungal biomass was quantified and determined to be 1.563 µg/mg using thin layer chromatography while AFB2 was not detected.

Quantitative values of aflatoxins in the formulated feed samples

The quantitative values of aflatoxins in Table 5 indicates high aflatoxin B1 production in formulated feed with *A. flavus*, low production in yeast and *A. flavus*, and no aflatoxin B1 production in the control and yeast-only samples.

Haematological parameters and liver enzyme test of broilers fed with different diets

Table 6 and 7 shows the haematological parameters and liver enzyme test of broilers from the different diet groups. The hematological parameters suggest that broilers fed with feed containing toxigenic *A. flavus* (A.F) exhibit signs of immunosuppression and potential anemia. The introduction of *Candida tropicalis* (C.T) as a treatment appears to ameliorate some of these adverse effects, as evidenced by improved red and white blood cell counts and platelet parameters. The findings statistically had no significant differences ($p < 0.05$).

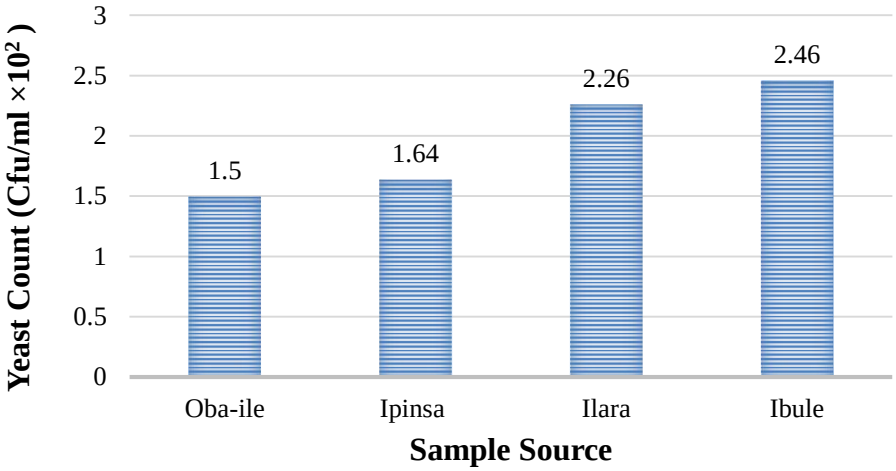

Figure 1: Total yeast counts from palm wine obtained from different locations

Table 1: Morphological and microscopic characteristics of yeast isolates

Isolate code	Microscopic structure	Shape	Color	Elevation	Margin	Opacity	Surface
IP	Long chain	Oval	Cream	Slightly raised	Regular	Opaque	Smooth, glossy
IB	Short chain	Oval	Cream	Slightly raised	Regular	Opaque	Smooth, glossy
IL	Short chain	Oval	Cream	Slightly raised	Regular	Opaque	Smooth, glossy
OB	Short chain	Oval	Cream	Slightly raised	Regular	Opaque	Smooth, glossy

Key: Ip = Ipinsa ; Ib = Ibule ; Il = Ilara and Ob = Oba-Ile

Table 2: Sugar fermentation activity of the yeast isolates

Isolate code	Lactose	Glucose	Arabinose	Xylose	Sucrose	Raffinose	Galactose	Maltose
IP	-	+	-	-	+	+	+	+
IB	-	+	-	-	+	+	+	+
IL	-	+	-	-	+	+	+	+
OB	-	+	-	-	+	+	+	+

Key: Ip = Ipinsa ; Ib = Ibule ; Il = Ilara ; Ob = Oba-Ile ; + = fermentable and - = non-fermentable

Table 3: Molecular identification of the isolated fungi and yeast (Blast prediction)

S/N	Sample ID	Organism	Sequence length (bp)	% Identity	Accession no of BLAST hit	E-value	Alignment score	Highest query coverage (%)
1	F	*Aspergillus flavus*	595	99.83%	MT645322.1	0.0	≥200	100%
2	Yeast	*Candida tropicalis*	523	99.24%	MZ363119.1	0.0	≥200	99%

Key: F- *Aspergillus flavus*

Table 4: Quantity of aflatoxin degrading enzymes in *Candida tropicalis*

Enzyme in *Candida tropicalis*	mg/ml/min
Laccase	4.851
Chitinase	1.211

Table 5: Quantitative values of aflatoxins in the formulated feed sample

Sample	Aflatoxin content µg/mg
A. flavus	1.50 ± 0.00^{b}
Candida tropicalis only	0.00 ± 0.00^{a}
Candida tropicalis and A.flavus	0.12 ± 0.00^{a}
Control (formulated)	0.00 ± 0.00^{a}

Data are represented as mean ± standard error where n=2. Mean of groups with the same superscript across the column are not statistically significant (P> 0.05)

Table 6: Haematological parameters of broilers fed with different formulated diets

LAB	WBC (10^9/L)	RBC	PLT	P-LCR (%)	MPV (fL)	NEU (%)	EOS (%)	BAS (%)	HCB (g/L)
C1	3.08 ± 0.03^{b}	2.48 ± 0.55^{a}	164.5 ± 99.5^{a}	26.85 ± 3.25^{a}	8.3 ± 0.00^{ab}	51 ± 1.00^{a}	1.00 ± 0.00^{a}	0.50 ± 0.50^{a}	7.25 ± 0.15^{a}
C2	3.42 ± 0.02^{b}	2.33 ± 0.01^{a}	188.5 ± 100.5^{a}	26.85 ± 3.25^{a}	8.5 ± 0.70^{ab}	50.5 ± 2.50^{a}	1.00 ± 0.00^{a}	1.00 ± 0.00^{a}	8.80 ± 0.10^{ab}
A.F	2.46 ± 0.24^{a}	2.53 ± 0.08^{a}	72.5 ± 0.50^{a}	14.6 ± 5.90^{a}	7.7 ± 0.10^{b}	56.50 ± 1.50^{a}	0.50 ± 0.50^{a}	0.00 ± 0.00^{a}	9.35 ± 0.05^{b}
A.F-C.T	2.45 ± 0.25^{a}	2.53 ± 0.08^{a}	72.5 ± 0.50^{a}	19.9 ± 4.60^{a}	7.0 ± 0.10^{b}	56.50 ± 1.50^{a}	0.50 ± 0.50^{a}	0.00 ± 0.00^{a}	9.35 ± 0.05^{b}
C.T	2.90 ± 0.30^{a}	2.50 ± 0.57^{a}	185 ± 102.0^{a}	24.6 ± 0.10^{a}	8.5 ± 0.40^{a}	50.50 ± 2.50^{a}	0.50 ± 0.50^{a}	0.50 ± 0.50^{a}	10.25 ± 1.05^{b}

Data are represented as mean ± standard error where n=2. Mean of groups with the same superscript across columns are not statistically significant (P> 0.05). Keys: Basophil-Bas(%); Eosinophil-Eos(%); Hemoglobin Concentration-HCG(G/L); Mean Platelet Volume-MPV(Fl); Platelet-PLT(10^9/L); Plateletcrit-Large Cell Ratio- P-Lcr(%); Red Blood Cell Or Erythrocyte-RBC(10^{12} /L); White Blood Cell-WBC(10^9 /L); Neutrophil-NEU(%); Broilers Fed With *C.Tropicalis*- C.T; Broilers Fed With Commercially Purchased Feed- C2; Broilers Fed With The Basal Diet (Bd) (Uninoculated Sample)-C1; Broilers Fed With Toxigenic *A. Flavus*-A.F; Broilers Fed With Toxigenic *A. Flavus* & Treated With *C.Tropicalis*- A.F&Ct

Table 7: Liver enzyme markers

LAB	ALP (U/L)	AST (U/L)	ALT (U/L)	LDH (U/L)
C1	58.20 ± 0.00^{ab}	142 ± 33.01^{a}	390.19 ± 7.48^{a}	36.05 ± 0.53^{a}
C2	58.35 ± 40.94^{ab}	154.6 ± 53.503^{a}	396.78 ± 23.52^{a}	29.98 ± 8.05^{a}
A.F	101.85 ± 20.58^{ab}	184.6 ± 11.08^{a}	406.35 ± 6.64^{a}	48.57 ± 5.72^{a}
A.F & C.T	58.20 ± 0.00^{b}	177.90 ± 17.22^{a}	401.11 ± 1.07^{a}	42.50 ± 14.31^{a}
C.T	29.15 ± 0.07^{a}	145.88 ± 43.357^{a}	399.75 ± 4.20^{a}	40.54 ± 5.63^{a}

Data are represented as mean ± standard error where n=3. Mean of groups with the same superscript across the columns are not statistically significant (P> 0.05). Keys: Alkaline phosphatase (ALP), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Lactate dehydrogenase (LDH), Broilers fed with toxigenic *A. flavus* and treated with *C.tropicali*s (A.F & C.T), Broilers fed with toxigenic *A. flavus* (A.F), Broilers fed with *C.tropicalis* (C.T), Broilers fed with the basal diet (BD) (uninoculated sample) (C1) and Broilers fed with commercially purchased feed (C2)

DISCUSSION

In commercial poultry farming, aflatoxins present serious financial difficulties. There is proof that aflatoxin contamination in animal diets has a detrimental effect on animal productivity (Mgbeahuruike *et al*., 2016). Given that between 65 and 75% of the costs associated with producing chickens are related to feed, changes in feed quality would inevitably impact the productivity of poultry (Hassan *et a*l., 2021). The significant presence of fungi can be linked to the low water activity in animal feed and the characteristics of the contaminating fungal

genera. These fungal species might have originated from carry-over of fungi that were already present in the field. Additionally, handling and other post-harvest processes may also be key contributors to the contamination (Danbappa *et al.,* 2018).

Aflatoxins, particularly aflatoxin B1 (AFB1), have been shown to adversely affect poultry health. Studies indicate that AFB1 can lead to hepatotoxicity, resulting in liver damage and impaired growth rates in broilers (Bilal *et al.,* 2023). Reducing the detrimental effects of aflatoxins in animal nutrition may be accomplished through the use of yeast to detoxify feeds contaminated with the toxin (Oluwole *et al.*, 2023).

Palm wine is a great medium for microorganisms like yeasts and bacteria to develop and proliferate with its high nutrient content. The isolation of diverse yeast populations from palm wine were previously published by Boboye *et al.* (2008) and Oluwole *et al.* (2023). *Candida tropicalis* is a common microorganism in various fermented foods. Its ability to bind aflatoxin indicates its potential use in removing toxins from cereals during fermentation. Magnoli *et al*. (2016) identified *Candida tropicalis* as one of the most effective non-Saccharomyces yeasts for aflatoxin binding, along with other strains like *Clavispora lusitaniae* and *Pichia anomala. Candida tropicalis* possesses enzymes that play a crucial role in the degradation of aflatoxins, particularly through its metabolic pathways. In this study, laccase and chitinase enzymes were found present and quantified in the isolated *Candida tropicalis*. Numerous studies have focused on the measurement of these enzymes which are involved in the decomposition of aflatoxin. These studies have also examined the enzymes' effectiveness in combating aflatoxin B1 (AFB1). When attempting to target aflatoxin breakdown, Dellafiora *et al*. (2017) stressed how crucial it is to comprehend the distinct actions of laccase isoforms. The authors showed that *Trametes versicolor* laccase (enzymes) had the ability to break down toxic substances like aflatoxins, which is similar with findings in this study. During which a decrease in the aflatoxin B1 level in the *Candida tropicalis* + *A.flavus* feed sample was observed, compared to the aflatoxin B1 level in the *A. flavus* feed sample. The formulated feed sample treated solely with *C. tropicalis* also had undetectable levels of aflatoxin B1. In another study conducted by Xiong *et al*. (2022), a new laccase (enzyme) produced from *Bacillus amyloliquefaciens* B10 was also shown to be capable of digesting aflatoxin. The authors identified key active site residues and successfully cloned and expressed the laccase gene in *E. coli*. This provided a strong foundation for further development and optimization of the enzyme for practical applications. Notably, the laccase exhibited high degradation activity, suggesting its effective utilization under a range of environmental conditions commonly found in food and feed storage.

The physiological state of an animal is reflected in its haematological constituents in relation to both its internal and external surroundings (Mulatu *et al.,* 2019). Reactive oxygen species (ROS), which are created by AFB1, have the potential to harm cells, including those found in the bone marrow where red blood cells are synthesized. The generation of white blood cells (WBC) and red blood cells (RBC) may be hampered by this injury. Findings in this study indicates that aflatoxin contamination leads to a significant reduction in WBC counts, highlighting its immunosuppressive effects. The treatment with *Candida tropicalis* shows some improvement in WBC counts, suggesting a partial protective effect, though not enough to fully counteract the suppression caused by aflatoxins. This is consistent with the findings of Riahi *et al*. (2021) where the mycotoxin binder was shown to stabilize certain haematological parameters but, did not completely negate the effects of mycotoxins. Another study conducted by Basmacioglu *et al*. (2005) found that broilers' hemoglobin levels and lymphocyte counts decreased when fed with an aflatoxin-contaminated diet. In this recent

study, exposure to toxigenic *Aspergillus flavus* resulted in a higher RBC count but, significantly lower hemoglobin levels, indicating anemia and stress from aflatoxin toxicity. Treatment with *Candida tropicalis* only had a lower RBC count showing partial recovery towards normal hemoglobin levels, although not completely restoring RBC health. Yeast supplementation has been shown to positively influence hematological parameters in poultry affected by aflatoxicosis. Specifically, the inclusion of yeast in the diet can lead to improvements in red blood cell counts, hemoglobin levels, and overall blood health, which are often negatively impacted by aflatoxin exposure (Oguz, 2011).

Yeast supplementation has been linked to lower levels of liver enzymes such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT), which are indicators of liver health. A reduction in these enzymes suggests less hepatic stress and better overall liver function (Hassan, 2021). Broilers exposed to toxigenic *Aspergillus flavus* (A.F) in this recent study showed higher levels of liver enzymes, suggesting liver damage or stress due to aflatoxins. This is similar to a study done by Bhatti *et al*. (2016), which showed that broilers fed dietary contamination with 0.1, 0.2, and 0.6 mg/kg aflatoxin B1 had an elevated blood concentration of ALT compared to broilers given a non-contaminated food. In another study by Rashidi *et al*. (2020), a substantial increase in the blood levels of ALT was observed when feeding broilers an aflatoxin B1-contaminated food at a rate of 0.5 mg/kg in comparison to the control group. The group treated with both *A.flavus* and *Candida tropicalis* showed similar ALP levels but, still elevated AST, ALT, and LDH levels. *Candida tropicalis* treatment helped normalize ALP levels but, did not completely mitigate liver damage caused by aflatoxins. The findings of this study align with that of Riahi *et al*. (2021), where the presence of mycotoxins (OTA and T-2) also resulted in altered liver enzyme activities, suggesting liver stress. The authors further reported the inclusion of the Multicomponent mycotoxin detoxifying agent (MMDA) improved some liver enzyme activities but did not fully reverse the negative effects of the mycotoxins on liver function. The group treated solely with *Candida tropicali*s had the lowest liver enzyme levels, suggesting *Candida tropicalis* treatment alone did not stress the liver and may have a protective effect. This study is in contrast to a study carried out by Hashmi *et al.* (2006) where yeast sludge did significantly improve the levels of serum total protein, serum albumin, and alanine transaminase (ALT) in the broilers. Notably, while *Candida tropicalis* may not completely eliminate the effects of aflatoxins in the diet, it plays a significant role in reducing their impact.

CONCLUSION

This study underscores the significant threat posed by aflatoxins in poultry feed, which can severely impair broiler health, growth performance, and liver function thus affecting poultry business. The findings align with previous research, highlighting the widespread occurrence of aflatoxin B1 in contaminated feed and its adverse effects on broiler chickens. The study confirms that dietary contamination with toxigenic *Aspergillus flavus* thereby altered hematological parameters and elevated liver enzyme activities. Importantly, the research demonstrates the potential of *Candida tropicalis*, a yeast strain isolated from palm wine, as an effective biological agent for mitigating the effects of aflatoxins in poultry feed. The yeast treatment significantly reduced aflatoxin B1 levels in formulated feed, reduced some of the adverse hematological and liver enzyme changes induced by aflatoxins. Although, *Candida tropicalis* did not completely reverse the effects of aflatoxin exposure, it provided a protective effect, particularly in reducing the severity of liver damage.

REFERENCES

Bilal, R.N., Tahir, M.A., Shahzad, A., Farag, M.R., Siddiq, A., El-Shall, N.A., Dhama, K., Elnesr, S.S and Alagawany, M. (2023). Yeast and derived products; Their uses in preventing mycotoxins in poultry feeds. *World's Poultry Science Journal,* 2: 45-83.

Danbappa, A.A.R., Alhassan, K.A. and Shah, M.M. (2018). Isolation and identification of microbial contaminants associated with commercial poultry feeds. *Journal of Applied and Advanced Research,* 3(5): 142−147.

Dellafiora, L., Galaverna, G., Reverberi, M. and Dall'Asta, C. (2017). Degradation of aflatoxins by means of laccases from trametes versicolor: An in silico insight. *Toxins,* 9(1): 17.

Djeni, T. N., Kouame, K. H., Ake, F. D., Amoikon, L. S., Dje, M. K. and Jeyaram, K. (2020). Microbial diversity and metabolite profiles of palm wine produced from three different palm tree species in Côte d'Ivoire. *Scientific Reports,* 10(1): 15-17.

Gicheha, M. G. (2021). The effects of heat stress on production, reproduction, health in chicken and its dietary amelioration. *Advances in Poultry Nutrition Research,* 1: 212-214.

Gonfa, T. G., Negessa, A. K. and Bulto, A. O. (2023). Isolation, screening, and identification of chitinase-producing bacterial strains from riverbank soils at Ambo, Western Ethiopia. *Heliyon,* 9(11): 32-44.

Hassan, S. S. (2021). Evaluation of Nigella sativa seeds on broiler chicks hematological, blood biochemical parameters and antioxidant enzymes. *Egyptian Poultry Science Journal*, 41(3):439-459.

Hidayat, M.N., Malaka, R., Agustina, L. and Pakiding, W. (2020). Effect of probiotic *Lactobacillus paracasei* on hematology and relative weight of lymphoid organs of broiler. *IOP Conference Series: Earth and Environmental Science,* 492: 1-7.

Ibitoye, O. A., Olaniyi, O. O., Ogidi, C. O. and Akinyele, B. J. (2021). Lactic acid bacteria bio-detoxified aflatoxins contaminated cereals, ameliorate toxicological effects and improve haemato-histological parameters in albino rats. *Toxin Previews,* 40(4): 985-996.

Leszczyńska, J., Masłowska, J., Owczarek, A. and Kucharska, U. (2001). Determination of aflatoxins in food products by the ELISA method. *Czech Journal of Food Science,* 19(1): 8-12.

Magnoli, A. P., Rodriguez, M. C., Poloni, V. L., Rojo, M. C., Combina, M., Chiacchiera, S. M. and Cavaglieri, L. R. (2016). Novel yeast isolated from broilers' feedstuff, gut and faeces as aflatoxin B1 adsorbents. *Journal of Applied Microbiology*, 121(6): 1766-1776.

Mulatu, K., Ameha, N. and Girma, M. (2019). Effects of feeding different levels of baker's yeast on performance and hematological parameters in broiler chickens. *Journal of World's Poultry Research*, 9(2): 38-49.

Nazhand, A., Durazzo, A., Lucarini, M., Souto, E. B., and Santini, A. (2020). Characteristics, occurrence, detection and detoxification of aflatoxins in foods and feeds. *Foods,* 9(5): 644.

Ochieng, P.E., Scippo, M.L., Kemboi, D.C., Croubels, S., Okoth, S., Kang'ethe, E.K., Doupovec, B., Gathumbi, J.K., Lindahl, J.F. and Antonissen, G. (2021). Mycotoxins in poultry feed and feed ingredients from Sub-saharan Africa and their impact on the production of broiler and layer chickens: A review. *Toxins,* 13(9):633.

Oguz, H. (2011). A review from experimental trials on detoxification of aflatoxin in poultry feed. *Eurasian*

Journal of Veterinary Sciences, 27(1): 1-12.

Okasha, H., Song, B. and Song, Z. (2024). Hidden Hazards revealed: mycotoxins and their masked forms in poultry. *Toxins*, 16(3): 137.

Olaniyi, O.O., Akinlami, O.R., Adeleke, B.S., Alabi, G.O. and Akinyele, B.J. (2019). Isolation and characterization of alcohol-tolerant *Saccharomyces cerevisiae* from palm wine (raffiapalm). *Annals. Food Science and Technology*, 20(4): 701- 709.

Olaniyi, O.O. and Akinyele B.J. (2019). Isolation of toxigenic *Aspergillus flavus* and evaluation of aflatoxins in "Burukutu", sorghum fermented beverage sold in Akure, Nigeria. *Journal of Food Safety and Hygiene*, 5*(*1): 30-38.

Riahi, I., Ramos, A. J., Raj, J., Jakovčević, Z., Farkaš, H., Vasiljević, M. and Pérez-Vendrell, A. M. (2021). Effect of a mycotoxin binder (MMDA) on the growth performance, blood and carcass characteristics of broilers fed ochratoxin a and t-2 mycotoxin contaminated diets. *Animals*, 11(11): 32-65.

Sarma, C., Mummaleti, G., Sivanandham, V., Kalakandan, S., Rawson, A. and Anandharaj, A. (2022). Anthology of palm sap: The global status, nutritional composition, health benefits & value-added products. *Trends in Food Science and Technology*, 119**:** 530-549.

Senthivelan, T., Kanagaraj, J., Panda, R. C. and Narayani, T. (2019). Screening and production of a potential extracellular fungal laccase from *Penicillium chrysogenum*: Media optimization by response surface methodology (RSM) and central composite rotatable design (CCRD). *Biotechnology Reports*, 23: 344-368.

Stephen F. A, Thomas L. M, Alejandro A. S, Jinghui, Z., Zheng, Z, Webb, M, and David J. L (1997), Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", *Nucleic Acids Research*, 25:3389-3402.

Suleman, S., Qureshi, J. A., Rasheed, M., Farooq, W., and Yasmin, F. (2022). Poultry feed contamination and its potential hazards on human health. *Biomedical Letters*, 8(1):70-81.