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Effect of Mycotoxins Produced by *Fusarium moniliforme* and *Aspergillus flavus* Associated with Wheat Grains Cultivated in the Al-Muthanna Desert and Used as Poultry Feed

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ABSTRACT

Background: Fungi such as *Fusarium moniliform* and *Aspergillus flavus* are known to cause plant diseases through a variety of mechanisms, such as competing with plants for food or blocking the plant's vessels, preventing water and nutrients from reaching the plant and secreting a number of toxins that negatively affect animals that feed on the infected plants.

Methods: This study was conducted in the poultry farms of the College of Agriculture, University of Al-Muthanna, during the 2024-2025 season to evaluate the effect of mycotoxin- contaminated diet on some immune and productive symptoms of Ross 308 broiler chickens at 20 days of age. Birds were divided into four groups, which included 10 birds in each group. Birds were divided as follows: (T1) A natural, food-free diet; (T2) a diet contaminated with *Fusarium moniliform*; (T3) a diet contaminated with *A. flavus* and (T4) a diet contaminated by both *F. moniliform* and *A. flaves*.

Result: The results demonstrated that mycotoxin affected the immune parameters significantly, causing reducing their resistance to infectious bronchitis (IB), Newcastle disease virus (NDV) and heterofil-to-lymphocyte (H/L). The most pronounced effect was seen in treatment (T3) and (T4) (2103.80 and 1253.30) and (4557.20 and 790.90), respectively. In addition, a decline in concentrations of alpha, beta and gamma globulin, as well as minor changes in the weight of internal organs such as the heart, liver, gizzard and abdominal fat.

Key words: *Aspergillus flavus*, Broiler immunity, *Fusarium moniliforme*, Mycotoxins.

INTRODUCTION

Cereals and their derivatives represent essential food resources for the global population (Deibel and Swanson, 2001). Studies worldwide indicate that approximately 25-40% of cereal grains are annually contaminated with varying levels of mycotoxins (EL Khoury and Atoui, 2010), with nearly 50% of the daily intake of these grains and their products by humans and animals being affected. This contamination can occur either in the field or during storage, particularly in regions with hot climatic conditions (Batool *et al.,* 2012; Sofia *et al.,* 2023; Aruna *et al.,* 2025).

Several studies have highlighted that fungal genera such as *Aspergillus, Penicillium, Fusarium* and *Rhizopus* are among the most prevalent species found on wheat grains post-harvest (Jubair *et al.,* 2024). These fungi are well-known storagemolds capable of producing mycotoxins during grain storage (Joshaghani *et al.,* 2013). Mycotoxin contamination of feed is an often underestimated stress factor for broiler chickens. Mycotoxins are known to reduce growth performance and feed efficiency, increase mortality rates and impair the immune system of chickens (Smith *et al.,* 2012). Fumonisins are secondary metabolites of the fungus *F. verticillioides* and fumonisin B1 (FB) is the predominant form among fumonisin isoforms (referred to as A1, A2, B1, B2, B3 and B4) (Voss *et al.,* 2007). Feed contaminated with phospholipid bacteria has been reported to reduce villus height, villus-to-cell ratio (Rauber *et al.,* 2013) and body weight gain (Antonissen *et al.,* 2015) and increase several clinical symptoms associated with phospholipid bacteria, including liver necrosis, diarrhea and rickets in poultry. Since phospholipid bacteria may affect the intestinal epithelium (Ashraf and Shah, 2014), they are known to be a predisposing factor for necrotic enteritis in broiler chickens (Antonissen *et al.,* 2015). Nutritional strategies (*e.g.*, the use of toxin inhibitors, TB) are commonly used to form stable complexes with mycotoxins or to convert mycotoxins into non-toxic metabolites to reduce toxicity in chickens (Pappas *et al.,* 2014; Jubair *et al.,* 2020; Neamah *et al.,* 2020). The present study was conducted to determine the

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effects of fungal toxins secreted from contaminated grains on poultry birds fed with fungus contaminated diets, *F. moniliforme* and *A. flavus*. This study monitored some biochemical and immunological parameters and examined the internal organs of broiler chickens to determine the degree of susceptibility to these toxins.

MATERIALS AND METHODS

Pathogenic agents

Wheat grain suspected of being infected with *F. moniliforme* and *A. flavus* was collected from a grain storage facility that had recently received wheat grains from infected farms in the Al-Muthanna desert. The field samples were brought to the laboratory, they were investigated for the pathogens and after that, they were diagnosed to confirm that they were infected by the target agents studied. The fungi were isolated and purified individually on Petri dishes using Potato Dextrose Agar (PDA) medium. They were allowed to grow for seven days to complete their development and were then stored in a refrigerator at 4°C until used in subsequent experiments.

Contamination of wheat grains with pathogenic fungi

Samples of wheat seeds, free from infection and with an acceptable moisture content for use as poultry feed, were collected. The seeds were thoroughly washed with water to remove any adhering dust and then sterilized using a 1% sodium hypochlorite solution for two minutes. They were subsequently rinsed well with water to eliminate any traces of the sterilizing agent and dried thoroughly. The seeds were divided into four portions, each weighing 5 kg. The first portion was contaminated with *F. moniliforme* by adding several Petri dishes containing the pathogenic fungus at seven days of growth. The second portion was contaminated with *A. flavus* in the same manner, using Petri dishes containing the seven-day-old fungus. The third portion was co-contaminated with both *F. moniliforme* and *A. flavus*. The fourth portion was left uncontaminated as a control treatment. The seeds were incubated at 25°C in an incubator, with the seed samples stirred every two days to ensure even distribution of fungal spores across the wheat grains. This process was carried out over a period of seven days.

Broiler chicken rearing

A total of 40 broiler chickens of the Ross 308 strain, aged 20 days and fed on a starter diet (Table 1), were brought and vaccinated against Gumboro disease. The birds were divided into four groups, with 10 birds in each group, to align with the prepared treatments as follows: T1: A natural diet, free from any pathogenic fungus; T2: A diet contaminated with *F. moniliforme* only; T3: A diet contaminated with *A. flavus* only and T4: A diet contaminated with both *F. moniliforme* and *A. flavus.*

The composition of the feed is detailed in Table (1) and the feeding continued until the birds reached 35 days of age. The Central Research Laboratory for Grain Products performed the mycotoxin analysis using the ELISA technique. The results showed final mycotoxin levels were; Aflatoxin B1 (0.001 mg/kg), DON (1.24 mg/kg), Zearalenone (0.068 mg/kg), Ochratoxin (0.005 mg/kg) and T-2 Toxin (0.09 mg/kg). Blood samples were taken at the end of all experimental groups to investigate the birds immune response against IBV, NDV and AIV. The blood samples were placed in EDTA-containing tubes to prevent clotting. Subsequently, biochemical traits were measured after centrifugation to obtain plasma, following the method described by Shen (1983). Ready-to-use diagnostic kits from Randox were utilized for the tests and each parameter was calculated according to the manufacturer's instructions. The immune response in blood serum against *Avian Influenza*, *Newcastle Disease* and Infectious Bronchitis was also measured using commercial kits from Affini Tech. Additionally, five birds from each group were randomly selected to examine liver and kidney changes across all treatments.

A completely randomized design (CRD) was used to analyze the effects of different treatments on biochemical and immune parameters. Significant differences between mean values were determined using the SPSS software, with statistical significance set at $P<0.05$.

RESULTS AND DISCUSSION

The results indicate a highly significant differences between treatments T3 and T4 compared to the control (T1) (Table 2). The control group (T1) demonstrated a significantly higher resistance to Infectious Bronchitis Virus (IBV), with the highest recorded value of 3423.30, whereas treatments T3 and T4 showed significantly lower resistance levels, recorded at 2103.80 and 1253.30, respectively. Likewise, in terms of Newcastle Disease Virus (NDV) resistance value, T1 was 6350.11 higher than the other treatments, while T3 and T4 was significantly lower (4557.20 and 790.90, respectively). The lowest significant value for heterophil-to-lymphocyte (H/L) ratio (0.46) was obtained by the T1 treatment who are therefore expected to be less physiologically stressed, while the treatment T4 exhibited the highest ratio (0.89) indicating

Table 1: Composition of the diets used for feeding broiler chickens during the starter and finisher phases.

Feed ingredients	Starter phase (1-22 days)	Finisher phase (22-35 days)
Maize (%)	56.5	62.0
Wheat (%)	4.00	3.50
Soybean meal 44% (%)	31.0	25.00
Protein concentrate (%)	4.00	4.00
Oil (%)	2.00	3.00
Limestone (%)	1.50	1.50
Dicalcium phosphate (%)	1.00	1.00
Total	100%	100%

elevated levels of physiological stress in this treatment group.

The results indicate the effects of mycotoxicosis on the specific immunoproteins; alpha globulin, beta globulin and gamma globulin respectively (Table 3). The data reveal significant differences among the dietary treatments (T1, T2, T3 and T4), it indicates that the presence of mycotoxins has negative effects on the immune system of birds.

For alpha globulin , the control treatment (T1) showed the highest concentration at 12.85, while the lowest value was observed in T4 (6.79). This progressive decline in alpha globulin levels from T1 to T4 suggests that mycotoxins, particularly in combination (as seen in T4), suppress the production of this protein, which plays a role in immune defense and transport functions.

Similarly, beta globulin showed the highest mean concentration in T1 (11.21), significantly decreasing in T3 (6.58) and T4 (6.80). Impaired immune function is reflected by a reduction in beta globulin which is involved in pathway complementing and defence on the pathogens. It is also apparent from the high values for T3 and T4 that the contaminants on their own or together, are equally effective at affecting the synthesis of beta globulin.

For gamma globulin, which is critical for antibody production and adaptive immunity, T1 again exhibited the highest concentration (21.50), while T4 had the lowest (16.42). The decline in gamma globulin levels across treatments highlights the negative impact of mycotoxins on humoral immunity, with combined contamination (T4) causing the most pronounced suppression.

In conclusion, broiler chickens fed mycotoxin-contaminated diet had significantly decreased concentrations of important immunoproteins and the mycotoxin-contaminated diet impaired the immune response of broiler chickens. The strongest reductions in alpha, beta and gamma globulins were observed with the combination of *F. moniliforme* and *A. flavus* (T4). A holistic approach to poultry nutritional management and mycotoxin prevention is essential to improving or even restoring proper immune response and health in poultry to the benefit of animal production and the wider population.

The results presented in the (Table 4) highlight the effects of dietary treatments (T1, T2, T3 and T4) on various internal organ weights and carcass characteristics of broiler chickens. These findings provide insights into how mycotoxin contamination in feed influences physiological traits and organ development.

Table 2: Effect of using diets containing mycotoxins on some immunological traits of broiler chickens.

Studied traits	Diet treatments			
	T1	T2	T3	T4
Resistance to IB	3423.30	3153.20	2103.80	1253.30
Resistance to NDV	6350.11	6125.30	4557.20	790.90
H/L ratio	0.46	0.50	0.70	0.89
LSD 0.05	Diet treatments	Immunological traits	Interaction	
	24.78	28.62	49.56	

Table 3: Effect of using diets containing mycotoxins on some immunoproteins of broiler chickens.

Type of immune proteins	Diet treatments			
	T1	T2	T3	T4
Alpha globulin	12.85	11.80	9.00	6.79
Beta globulin	11.21	10.23	6.58	6.80
Gamma globulin	21.50	20.32	18.57	16.42
LSD 0.05	Diet treatments	Type of immuno	Interaction	
	0.67	proteins 0.58	1.160	

Table 4: Effect of using diets containing mycotoxins on the relative weight percentages of some internal organs in broiler chickens.

Internal organs	Diet treatments			
	T1	T2	T3	T4
Cold carcass weight (g)	1970.12	1852.32	1790.15	1680.33
Gizzard weight (%)	2.44	2.14	2.05	1.88
Liver weight (%)	3.11	3.0	2.81	2.68
Heart weight (%)	0.75	0.63	0.59	0.58
Spleen weight (%)	0.3	0.28	0.28	0.27
Intestine weight (%)	4.72	3.51	3.44	3.3
Abdominal fat weight (%)	2.32	1.98	1.68	1.45
LSD 0.05	Diet treatments	Mean organ weight	Interaction	
	1.212	1.604	2.208	

The cold carcass weight (in grams) decreased progressively from T1 (1970.12 g) to T4 (1680.33 g). This decline suggests that mycotoxin contamination, particularly in T4 where both *F. moniliforme* and *A. flavus* were present, negatively impacted growth performance and muscle development. The reduction in carcass weight is likely due to impaired nutrient absorption, metabolic disturbances, or reduced feed intake caused by mycotoxins.

Overall, the results demonstrate that mycotoxin-contaminated diets adversely affect the growth and physiological health of broiler chickens. The most pronounced impacts were observed in T4, where combined contamination with *F. moniliforme* and *A. flavus* led to significant reductions in carcass weight, increases in liver and intestinal weights and subtle changes in other organs. These findings emphasize the critical need to prevent mycotoxin contamination in poultry feed to ensure optimal growth, health and productivity in broiler chickens.

The incidence rate for poultry diseases varies, as shown in Table 2. This variation can be attributed to the immune response which is very sensitive to toxins produced by pathogenic fungi. These can suppress the immune system in poultry, making them more prone to diseases, causing vaccination failure or low immunity to responsiveness. This is caused by the effect on primary lymphoid cells, causing atrophy, in addition to degeneration of secondary lymphoid organs such as spleen (Okoli *et al.,* 2007; Mahmood and AL-Abedy, 2021).

Heterophil-to-lymphocyte (H/L) ratio has increased sharply treatments consisting of both pathogenic fungi (T4). This growth can be attributed to increased stress due to high secretions of mycotoxin from pathogenic fungi, which increases the physical stress that birds experience. An increase in this ratio leads to the suppression of level of corticosterone hormone in the plasma (Ghareeb *et al.,* 2012; Muteab and AL-Abedy, 2025), which is quite corrected with the H/L ratio. These conclusions match the results reported by Bankole and Kpodo (2005).

Reduction in concentrations of alpha, beta and gamma globulin in birds can increase with a high level of mycotoxin in a diet feeding from pathogenic fungi (Lillehoj and Li, 2004). These are protein lipoproteins that play an important role in the lymphatic immune system and increase the immune response to birds. The decline in these proteins can be attributed to the decline in the number of lymphocytes responsible for their production, which in turn reflects a weak immune response in birds (Phillips *et al.,* 2002).

The weight loss of internal organs, including gizzard, heart, liver and abdominal fat, was also seen. This deficiency can be attributed to the presence of mycotoxin in the diet used to feed (Antonissen *et al.,* 2015). These toxins increase the absorption of the bird's body, causing them to move directly through the blood circulation to the gut and other organs, increasing their harmful effects on the bird. These conclusions are in line with those reported by Al-Jubouri (2002) and Ezz El-Din and Al-Naimi (2008).

In summary, it was observed that the T3 and T4 treatments had the greatest impact on the susceptibility of poultry to IBV and reduced resistance to Newcastle disease virus (NDV). We also observed a reduction in the activity of immune proteins, which weakened the immune system of poultry and had a negative impact on growth indicators of broilers, such as organ weights and carcass characteristics. Therefore, we recommend that several studies be conducted to reduce the toxicity of poultry feeds, such as exposing the feed to some kind of radiation, such as mutagenic rays (UV), to investigate whether radiation affects the *via*bility of the fungi and their toxin production, or using liquid nitrogen as a cooling method for feed storage and observing whether the activity of toxigenic fungi has been suppressed.

CONCLUSION

This study demonstrates that mycotoxin-contaminated diets, particularly those involving *F. moniliforme* and *A. flavus* , significantly impair the immune response, reduce growth performance and alter physiological traits in broiler chickens. Combined contamination (T4) caused the most severe effects, including low resistance to diseases (IB and NDV), reduction in immunooprotein levels and unfavorable changes in organ weight and carcass characteristics. These findings highlight the importance of preventing mycotoxin pollution in poultry feed to ensure optimal health and productivity. Effective storage practices and monitoring are important to reduce these risks.

Conflict of interest

All authors declare that they have no conflict of interest.

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