

## Research Article



# Effects of Administering Fungi Isolated from Poultry Feed on Some Physiological and Productive Parameters in Rose Chickens in Several Regions of the Karbala Governorate

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**Abstract** | Research have shown that supplementation with probiotic fungi improves digestive health and reduces populations of harmful bacteria in birds. This study aimed to evaluate the impact of fungi isolated from poultry feed collected from various regions of Karbala Governorate, Iraq, on selected physiological and productive traits in broilers. A total of 180 broiler chicks were used, divided into three equal groups. Each group was further divided into six replicates of 10 chicks each. The second group received a dose of 1 ppm *Aspergillus flavus*, while the third group received 1 ppm *Aspergillus niger*. The first group, which was not exposed to fungal contamination, served as the control. Both fungal species, *Aspergillus flavus* and *Aspergillus niger*, were isolated from poultry feed. The study measured productive parameters including body weight, weight gain, feed conversion ratio (FCR), and feed intake, along with physiological parameters such as blood cell counts, lipid profile, blood proteins, and liver and kidney function indicators. The results showed that the third group (*A. niger*) had significantly higher average weekly weight compared to the other groups ( $P < 0.05$ ). In the second group (*A. flavus*), there was a significant increase in white blood cells, red blood cells, hemoglobin, and lymphocyte percentage compared to the control group ( $P < 0.05$ ). Both treatment groups exhibited a reduction in total cholesterol compared to the control, while high-density lipoprotein (HDL) levels were significantly higher in the third group than in the others ( $P < 0.05$ ). Regarding blood proteins, the third group showed significantly higher levels than both the second group and the control ( $P < 0.05$ ). However, liver enzymes and creatinine were elevated in the second group, indicating potential hepatic and renal stress in birds exposed to *A. flavus*. In conclusion, inclusion of *A. niger* in the diet of broilers exhibited some beneficial effects as compared to *A. flavus*.

**Keywords** | Fungi, Poultry feed, Physiological, Productive, Rose type broiler

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## INTRODUCTION

The economic cost of feed significantly influences the growth of the livestock sector and constrains animal production. Therefore, rationing feed while maintaining production quality and quantity is crucial. Numerous researchers have explored the use of low-cost feed additives in minimal ratios to enhance the quality and quantity of

meat, eggs, and other products by improving feed quality with additives not suitable for human consumption (Ayoola *et al.*, 2024; Classen and Cope, 1998). Agricultural animals, including broilers, can convert low-quality feed unsuitable for humans into high-quality animal protein, with feed accounting for about 75% of total poultry farming costs (Atteh, 2004).

Poultry can be exposed to various pathogens, including fungi and their toxins, through the consumption and digestion of contaminated feed especially yellow corn (Canas and Aranda, 1996; Al-Tememe *et al.*, 2025). Fungal contamination of poultry feed is a global issue, leading to altered blood parameters, impaired liver function, reduced growth and development, and significant economic losses for poultry meat and egg producers (Che *et al.*, 2011). Additionally, fungi can also be found in broiler drinking water and airborne dust (Ibrahim *et al.*, 2021). *Aspergillus* species can affect birds of any age and in any environment (Abd El-Ghany, 2021).

The high concentration of fungi or their toxins in the feed leads to an increase in the percentage of halogens, a decrease in red blood cells, egg production, deterioration of the nutritional conversion rate, inhibition of immune system function, decreased response to vaccines, and pathological changes in the liver and other parts of the bird's body (Kamalavenkatesh *et al.*, 2005). Studies have pointed to a scientific fact that the higher the concentration of mycotoxins, the activity the immune system is inhibited, the effectiveness of phagocytic cells decreases, and lymphocytes decrease (Fung and Clark, 2004). *Aspergillus flavus* produces mycotoxins specifically aflatoxins B1, B2, G1, and G2 which commonly contaminate cereals such as wheat, yellow corn, and soybeans at various stages of production (Cole and Richard, 1993). Among these, aflatoxin B1 is considered the most harmful to poultry, leading to symptoms like reduced appetite, poor weight gain, decreased feed intake, impaired feed conversion ratio, and even mortality (Girish and Devegowda, 2006). The liver is the primary target organ affected by mycotoxins (Miazzo *et al.*, 2000), and mycotoxin contamination has long been a challenge in the poultry industry (Kubena *et al.*, 1993). In contrast, *Aspergillus niger* exhibits biostimulant properties and is commonly found in nature and easily cultured in laboratories (Piva *et al.*, 1995). It is a valuable source of bioactive compounds such as citric, gluconic, and itaconic acids, and it produces beneficial enzymes like protease, cellulase, xylanase, and phytase, which support bird health and can enhance the economic efficiency of poultry farming (Nadumane *et al.*, 2016).

As a bio-enhancer, probiotics (when alive) and prebiotics (when inactivated) support the growth of beneficial gut bacteria in poultry and help birds utilize the physiologically active secondary metabolites produced by fungi (Hong *et al.*, 2004). Previous studies have shown that supplementation with *Aspergillus niger* improves digestive health and reduces populations of *E. coli* and *Salmonella* in birds (Saleh *et al.*, 2017).

The aim of the study was to make a comparison between the effect of two types of fungi that were isolated from

a poultry feed in Karbala on some productive and physiological parameters in broiler.

## MATERIALS AND METHODS

This study was conducted at the Animal Field, Faculty of Agriculture, Karbala University, using 180 broiler chickens of the Rose breed. The experimental birds were reared and handled in line with all ethical standards and study protocols were approved by the University of Kerbala. The birds were randomly assigned to three equal groups, with each group further divided into six replicates of 10 chicks each. All birds were maintained on a standard balanced diet. The second group received a 1 ppm solution of *Aspergillus flavus*, while the third group was dosed with a 1 ppm solution of *Aspergillus niger*. The first group, which received no fungal treatment, served as the control.

### ISOLATION AND DIAGNOSIS OF FUNGI USED IN THE STUDY

*A. flavus* and *A. niger* fungi were isolated from poultry feed pellets in Karbala, Iraq, using a modified method described by Ali *et al.* (1991) and identified according to the procedure outlined by Kayode and Sani (2008).

### PRODUCTIVE PARAMETERS

Body weight and feed efficiency were measured weekly, starting from the seventh day of the experiment. Each chick was individually weighed on the first day, and this procedure was repeated every weekend throughout the trial. Live body weight and weight gain were calculated using the method described by Al-Musodi *et al.* (2024), by subtracting the bird's weight at the start of the week from its weight at the end of the week.

Feed consumption was determined based on the actual feed intake of each group, following the method of Tarrage and Puchal (1977). It was calculated by subtracting the amount of feed remaining at the end of the week from the amount provided at the beginning.

The feed conversion ratio (FCR), which indicates feed efficiency, was calculated using the following formula:

$$FCR = \text{Feed consumed (g)} / \text{Average weight gain (g)}$$

This value represents the grams of feed required to produce one gram of body weight gain (Al-Musodi *et al.*, 2024).

### BLOOD COLLECTION

At the end of the rearing period, blood samples were collected from the birds' jugular veins. A total of 5 mL of blood was drawn from each bird and divided into two tubes. One tube contained an anticoagulant for the

analysis of white blood cells, hemoglobin, packed cell volume (PCV), and leukocyte counts. The second tube, left without anticoagulant, was used to collect serum for the measurement of lipid profile, liver enzyme activity, and blood protein levels.

### HEMATOLOGICAL ANALYSIS

Bird blood cells were diluted using Natt and Herrick solution and analyzed using the hemocytometer counting method, following the procedure described by Campbell (1988). For red blood cell (RBC) counting, 0.5 mL of blood was drawn and diluted with the solution to the 101 mark, resulting in a 1:200 dilution. For white blood cell (WBC) counting, the same volume of blood was diluted to the 11 mark, yielding a 1:20 dilution. The cells were then counted using standard hemocytometer procedures.

Differential leucocyte counts were performed by preparing a blood smear. A drop of blood was placed on one end of a clean glass slide, and another slide held at a 45° angle was used to spread the blood into a thin film. After air-drying, the smear was stained with Wright's stain, gently rinsed, and dried again. The slide was examined under an oil immersion lens, and 100 leukocytes were counted. The different types of white blood cells were identified based on morphological characteristics, as per the method of Burton and Harrison (1969).

Packed Cell Volume (PCV) was measured by collecting blood in heparinized capillary tubes after puncturing the wing vein. One end of the tube was sealed with artificial clay, and the tube was centrifuged at 12,000 rpm for 5 minutes using a hematocrit centrifuge. PCV was then determined using a hematocrit reader, following Campbell's (1988) method.

Hemoglobin concentration was estimated using Drabkin's solution, which converts hemoglobin to cyanmethemoglobin. A volume of 20 µL of blood was mixed with 5 mL of Drabkin's solution and allowed to react for 5 minutes. The mixture was then centrifuged at 2,500 rpm for 5 minutes, and absorbance was read at 540 nm using a spectrophotometer. The spectrophotometer was zeroed with Drabkin's solution, in accordance with Varley and Bell (1980).

### DETERMINATION OF THE TOTAL PROTEIN LEVEL IN THE BLOOD

The Biuret Method, described by Wotton (1964), was applied to measure the total protein concentration in the blood. This method is based on the interaction between copper ions in the biuret reagent and protein peptides in an alkaline medium, forming a violet-colored complex. The Rodkey (1965) method was employed to measure

the albumin concentration in the blood. To determine the globulin concentration, the total protein level was subtracted by the albumin concentration, as described by Ghanim *et al.* (2016).

### DETERMINATION OF LIPID PROFILE

Serum total cholesterol concentration (mg/dL) was measured using a commercial kit (Spinreact, Spain) following the procedure outlined by Allian *et al.* (1974). Triglyceride levels were also determined with a commercial kit (Spinreact, Spain), following the method described by Fossati and Prencipe (1982).

High-Density Lipoprotein (HDL-C) concentration (mg/dL) was measured using a commercial kit (Spinreact, Spain), as per the procedure outlined by Tietz (1995). Similarly, Low-Density Lipoprotein (LDL-C) concentration (mg/dL) was calculated using the method of Buritus and Ashwood (1999) with the following equation:

$$LDL-C \text{ concentration (mg/dL)} = \text{Total cholesterol} - (HDL-C + vLDL-C)$$

Very Low-Density Lipoprotein (vLDL-C) concentration (mg/dL) was measured according to Buritus and Ashwood (1999), using the following formula based on triglyceride levels:

$$vLDL-C \text{ concentration (mg/dL)} = \text{Triglycerides}/5$$

### LIVER FUNCTION ENZYMES

Alanine Aminotransferase (ALT) activity (U/L) was measured using the method described by Wroblewski and Ladue (1956). Aspartate Aminotransferase (AST) activity was quantified according to the procedure outlined by Jain (1986).

### RENAL FUNCTION

The concentration of urea in the blood of meat chicks was determined using the method described by Chawla (1999), while the blood creatinine level was measured according to the method of Naseem *et al.* (2018).

### STATISTICAL ANALYSIS

Statistical analyses in this study were performed using SPSS 15.0. ANOVA was used to assess the significance of the main effects and interactions in the data. The Duncan Multiple Range Test was employed to compare the means. The significance threshold was set at  $P < 0.05$  (SAS, 2010).

## RESULTS AND DISCUSSION

### LIVE BODY WEIGHT

It is noted from the Table 1 that the weights of the chicks

in the third group were significantly higher ( $p<0.05$ ) than those of the control group and the second group in the fourth week of life. Additionally, the weights of the control group were significantly higher ( $p<0.05$ ) than those of the second group in the same week. However, the chicks in the first and third groups showed similar weights.

### BODY WEIGHT GAIN

The chicks of the third group were significantly superior ( $p<0.05$ ) in terms of weekly weight gain over the chicks of the second group in the last week of the study (Table 2).

### FEED INTAKE

It is noted that there were no significant differences ( $p>0.05$ ) in the amount of feed intake between the chicks of the study groups, as shown in Table 3.

### FEED CONVERSION RATIO (G/ WEEK)

The addition of *A. niger* to the broiler chicks resulted in a

significant ( $p<0.05$ ) improvement in the feed conversion rate compared to the control and second groups in the fourth week of the study, as shown in Table 4.

### BLOOD CELL PARAMETERS

The third group significantly ( $p<0.05$ ) outperformed the second group in the number of red and white blood cells, hemoglobin levels, and the percentage of packed cell volume (PCV), while the control group surpassed the second group in the number of white blood cells, as shown in Table 5.

### DIFFERENTIAL LEUCOCYTE COUNT

The results of the differential white blood cell count showed a significant ( $p<0.05$ ) higher percentage of heterophils in the control group compared to the second group. In contrast, the second group significantly outperformed the control group in the percentage of lymphocytes and eosinophils, as shown in Table 6.

**Table 1:** The effect of treatment on weekly body weight (g/week) of broilers.

| Groups/ week                 | 1 <sup>st</sup> week | 2 <sup>nd</sup> week | 3 <sup>rd</sup> week | 4 <sup>th</sup> week       | 5 <sup>th</sup> week       |
|------------------------------|----------------------|----------------------|----------------------|----------------------------|----------------------------|
| Control                      | 185.57±5.91          | 518.07±36.21         | 882.77±34.61         | 1482.08±31.23 <sup>b</sup> | 2150.4±60.1 <sup>a</sup>   |
| Group 2 ( <i>A. flavus</i> ) | 174.81±20.11         | 489.01±30.13         | 810.11±13.54         | 1314.12±30.17 <sup>c</sup> | 1892.15±58.07 <sup>b</sup> |
| Group 3 ( <i>A. niger</i> )  | 178.84±8.73          | 532.24±31.02         | 982.14±13.15         | 1565.9±9.11 <sup>a</sup>   | 2254.9±71.02 <sup>a</sup>  |
| LSD                          | 45.04                | 126.11               | 94.12                | 82.91                      | 201.4                      |
| P value                      | N.S                  | N.S                  | N.S                  | <0.05                      | <0.05                      |

**Table 2:** The effect of treatment on weekly weight gain (g/week) of broilers.

| Groups/ week                 | 1 <sup>st</sup> week | 2 <sup>nd</sup> week | 3 <sup>rd</sup> week | 4 <sup>th</sup> week       |
|------------------------------|----------------------|----------------------|----------------------|----------------------------|
| Control                      | 332±50               | 364.70±25.40         | 599.30±37.41         | 668.40±24.64 <sup>ab</sup> |
| Group 2 ( <i>A. flavus</i> ) | 314.2±30.71          | 321.10±32.18         | 504±61.60            | 578.04±99.34 <sup>b</sup>  |
| Group 3 ( <i>A. niger</i> )  | 353.40±20.14         | 449.90±40.01         | 583.74±17.90         | 689.05±29.11 <sup>a</sup>  |
| LSD                          | 90.06                | 111.91               | 154.12               | 104.1                      |
| P value                      | N.S                  | N.S                  | N.S                  | <0.05                      |

**Table 3:** The effect of treatment on feed intake (g/Week).

| Groups/ week                 | 1 <sup>st</sup> week | 2 <sup>nd</sup> week | 3 <sup>rd</sup> week | 4 <sup>th</sup> week |
|------------------------------|----------------------|----------------------|----------------------|----------------------|
| Control                      | 509.70±40.10         | 657.40±23.41         | 679.80±24.10         | 936.50±37.39         |
| Group 2 ( <i>A. flavus</i> ) | 440.81±70.10         | 627.97±25.01         | 677.80±29.60         | 805.17±36.06         |
| Group 3 ( <i>A. niger</i> )  | 478.40±21.17         | 624.40±27.51         | 807.10±27.31         | 808.19±61.40         |
| LSD                          | 97.11                | 88.40                | 590.10               | 163.90               |
| P value                      | N.S                  | N.S                  | N.S                  | N.S                  |

**Table 4:** The effect of treatment on feed conversion ratio (g/week).

| Groups/ week                 | 1 <sup>st</sup> week | 2 <sup>nd</sup> week | 3 <sup>rd</sup> week | 4 <sup>th</sup> week    |
|------------------------------|----------------------|----------------------|----------------------|-------------------------|
| Control                      | 1.53±0.4             | 1.80±1.24            | 1.13±0.10            | 1.40±0.32 <sup>a</sup>  |
| Group 2 ( <i>A. flavus</i> ) | 1.40±0.44            | 1.95±2.30            | 1.34 ±0.17           | 1.39±1.03 <sup>ab</sup> |
| Group 3 ( <i>A. niger</i> )  | 1.35±0.90            | 1.38±1.13            | 1.38±3.21            | 1.17±0.33 <sup>b</sup>  |
| LSD                          | 0.713                | 0.631                | 0.591                | 0.291                   |
| P value                      | N.S                  | N.S                  | N.S                  | <0.05                   |



**Table 5:** The effect of treatment on blood picture.

| Groups/ week                 | Red Blood cells<br>Cellx10 <sup>6</sup> | White blood cells<br>Cellx10 <sup>3</sup> | Hemoglobin (Hb) g/dl    | Packed cell volume<br>(PCV) % |
|------------------------------|-----------------------------------------|-------------------------------------------|-------------------------|-------------------------------|
| Control                      | 45.26±0.7 <sup>ab</sup>                 | 50.82±1.02 <sup>ab</sup>                  | 8.80±0.12 <sup>b</sup>  | 29.48±0.50 <sup>a</sup>       |
| Group 2 ( <i>A. flavus</i> ) | 42.40±1.04 <sup>b</sup>                 | 49.70±0.75 <sup>b</sup>                   | 7.81 ±0.11 <sup>c</sup> | 25.40±1.14 <sup>b</sup>       |
| Group 3 ( <i>A. niger</i> )  | 46.41±1.27 <sup>a</sup>                 | 54.75±0.43 <sup>a</sup>                   | 9.91±0.07 <sup>a</sup>  | 31.10±0.17 <sup>a</sup>       |
| LSD                          | 3.11                                    | 3.017                                     | 0.640                   | 2.41                          |
| P value                      | <0.05                                   | <0.05                                     | <0.05                   | <0.05                         |

**Table 6:** The effect of treatment on differential Leucocyte count (%).

| Groups/week                  | Heterophil               | Lymphocyte               | Eosinophil              | Basophil  | Monocytes |
|------------------------------|--------------------------|--------------------------|-------------------------|-----------|-----------|
| Control                      | 27.91±0.12 <sup>a</sup>  | 64.61±0.14 <sup>b</sup>  | 0.65±0.11 <sup>b</sup>  | 0.71±0.01 | 6.64±0.17 |
| Group 2 ( <i>A. flavus</i> ) | 25.17±0.41 <sup>b</sup>  | 66.94±2.91 <sup>a</sup>  | 0.78 ±0.13 <sup>a</sup> | 0.75±0.03 | 6.84±0.44 |
| Group 3 ( <i>A. niger</i> )  | 26.99±0.71 <sup>ab</sup> | 65.01±2.81 <sup>ab</sup> | 0.86±0.14 <sup>a</sup>  | 0.75±0.04 | 6.75±0.30 |
| LSD                          | 1.12                     | 2.04                     | 0.11                    | 0.170     | 1.301     |
| P value                      | <0.05                    | <0.05                    | <0.05                   | N.S       | N.S       |

**Table 7:** The effect of treatment on Lipid profile (µg/dl).

| Groups/ week                 | cholesterol              | Triglycerides | HDL                      | LDL         | vLDL       |
|------------------------------|--------------------------|---------------|--------------------------|-------------|------------|
| Control                      | 136.51±1.17 <sup>a</sup> | 49.55±1.14    | 23.31±1.09 <sup>ab</sup> | 102.11±1.14 | 9.91±0.83  |
| Group 2 ( <i>A. flavus</i> ) | 133.41±1.90 <sup>b</sup> | 48.56±1.81    | 22.50 ±0.70 <sup>b</sup> | 100.31±1.21 | 9.71±0.72  |
| Group 3 ( <i>A. niger</i> )  | 133.90±1.71 <sup>b</sup> | 54.50±3.09    | 24.61±1.90 <sup>a</sup>  | 99.20±0.017 | 10.90±2.14 |
| LSD                          | 2.10                     | 7.35          | 2.11                     | 3.24        | 5.31       |
| P value                      | <0.05                    | N.S           | <0.05                    | N.S         | N.S        |

**Table 8:** The effect of treatment on biochemical parameters.

| Groups/ week                 | Total protein<br>g/dl  | Albumin<br>g/dl        | Globulin g/dl           | AST u/l                   | ALT u/l                  | Urea mg/dl | Creatinine Mg/<br>dl    |
|------------------------------|------------------------|------------------------|-------------------------|---------------------------|--------------------------|------------|-------------------------|
| Control                      | 3.33±0.42 <sup>b</sup> | 1.42±1.14 <sup>a</sup> | 1.91±0.31 <sup>ab</sup> | 120.36±14.12 <sup>b</sup> | 401.34±3.74 <sup>b</sup> | 3.18±11.21 | 0.41±13.22 <sup>b</sup> |
| Group 2 ( <i>A. flavus</i> ) | 3.01±0.12 <sup>b</sup> | 1.31±0.43 <sup>b</sup> | 1.70±0.36 <sup>b</sup>  | 129.31±13.98 <sup>a</sup> | 411.60±6.14 <sup>a</sup> | 3.21±14.2  | 0.47±17.12 <sup>a</sup> |
| Group 3 ( <i>A. niger</i> )  | 3.63±0.1 <sup>a</sup>  | 1.44±0.4 <sup>a</sup>  | 2.19±0.3 <sup>a</sup>   | 122.14±15.0 <sup>ab</sup> | 404.19±3.8 <sup>ab</sup> | 3.27±20.1  | 0.43±21.0 <sup>ab</sup> |
| LSD                          | 0.857                  | 0.10                   | 0.411                   | 8.51                      | 5.314                    | 2.150      | 0.055                   |
| P value                      | <0.05                  | <0.05                  | <0.05                   | <0.05                     | <0.05                    | N.S        | <0.05                   |

## LIPID PROFILE

Fungal treatments influenced the blood lipid profile, with the total cholesterol concentration significantly higher ( $p<0.05$ ) in the control group compared to the second group. Additionally, the concentration of high-density lipoproteins (HDL) was higher in the third group compared to the second group, as shown in Table 7.

## BIOCHEMICAL PARAMETERS

As shown in Table 8, the concentrations of total blood proteins, albumin, and globulin in the third group were significantly higher ( $p<0.05$ ) compared to the second group. Additionally, the concentrations of the enzymes AST and ALT were significantly higher ( $p<0.05$ ) in the second group compared to the third group. The concentration of creatinine was significantly higher ( $p<0.05$ ) in the second group compared to the control group. However, the

concentration of blood urea was not significantly affected by the treatment.

## DISCUSSION

The improvement in the productive performance of broilers in the third group, as shown in Tables 1, 2, and 4, reflects the positive effects of *A. niger* on the gastrointestinal tract. This fungus enhances nutrient utilization particularly carbohydrates, polysaccharides, and enzymes by improving their digestion and releasing bioactive compounds that promote chick growth (Muhammed and Oloyede, 2010). *A. niger* also improves the digestion of complex substances due to its probiotic-like action (Muhammed and Oloyede, 2006), mediated by enzymes it secretes, such as pectinase, carbohydrase, lactase, invertase, and acid protease (Nagashima *et al.*, 1999). These enzymes boost

protein digestion and mineral absorption, both crucial for growth, weight gain, and overall health (Nelson and Cox, 2005). These findings align with those of Muhammed and Oloyede (2010), Omid *et al.* (2022), and Saleh *et al.* (2011), who reported increased broiler weights after *A. niger* supplementation.

The improved feed conversion ratio observed in the third group may also result from enhanced feed utilization due to *A. niger*'s enzymatic activity, consistent with previous findings in various animals (Muhammad *et al.*, 2000; Omid *et al.*, 2022; Kayode *et al.*, 2008).

In contrast, the second group showed a significant ( $p < 0.05$ ) decline in productive traits compared to the control and third groups, likely due to the harmful effects of aflatoxins produced by *A. flavus* (Freire *et al.*, 1996). This result agrees with earlier studies by Jeff-Agboola (2014), Nazarizadeh *et al.* (2019), and Khaleghipour *et al.* (2019), which reported negative impacts of aflatoxins on poultry performance.

Significant heterogeneity ( $p < 0.05$ ) was observed in the blood parameters, particularly a decrease in the second group, likely due to the effects of aflatoxins secreted by *A. flavus* (Anwar *et al.*, 2001). Blood parameters are key indicators of an animal's health, and reductions in hemoglobin, packed cell volume (PCV), and erythrocyte count have similarly been reported in layer hens fed *A. flavus*-contaminated feed (Fawaz *et al.*, 2022). Aflatoxins are known to trigger inflammation and cellular damage, contributing to these hematological disruptions, as also observed in turkey chicks (Javed *et al.*, 2005), with findings supported by Basmacioglu *et al.* (2005).

In contrast, Table 5 shows a significant increase ( $p < 0.05$ ) in blood parameters in the third group. These findings align with Al-Kassie *et al.* (2008), who reported that probiotics significantly enhanced hemoglobin levels in broilers, improving oxygen delivery and reducing hypoxia risk. *A. niger* may contribute to better oxygen saturation and support the development and protection of red blood cells and their hemoglobin content (Pavlidis *et al.*, 2007). Similar results were reported by Hao *et al.* (2020), who observed a significant increase in blood parameters in chicks supplemented with *A. niger* at different dietary levels (0.25%, 0.5%, 0.75%, and 1%).

As for differential leukocyte counts, Table 6 indicates a significant increase ( $p < 0.05$ ) in lymphocyte percentage in the second group, which may reflect the immunological stress caused by aflatoxin exposure. Aflatoxins contribute to anemia, cellular damage, and reduced red and white blood cell counts, consistent with the findings of Jeff-Agboola (2014). The elevated eosinophil levels in the second group could be attributed to hypersensitivity or inflammatory

responses triggered by aflatoxins or fungal elements (Betina, 1989; Oguz *et al.*, 2000), further supported by Anjorin and Cyriacus (2014).

The concentration of high-density lipoproteins (HDL) significantly increased ( $p < 0.05$ ) in the third group compared to both the control and second groups (Table 6), suggesting the beneficial role of *A. niger* in lowering harmful cholesterol levels (Al-Kassie *et al.*, 2008; Kim *et al.*, 2003). This effect may be attributed to the fungus's influence on the enzyme HMG-CoA reductase, which is critical in cholesterol biosynthesis and is known to be inhibited by fungal metabolites (Hajjaj *et al.*, 2005). Similarly, Srinivasan *et al.* (2022) demonstrated reduced cholesterol levels in broilers supplemented with *A. niger* and probiotics, consistent with the findings of Saleh *et al.* (2011).

Further, *A. niger* may reduce hepatic cholesterol synthesis by suppressing liver enzyme activity and decreasing bile secretion (Tufan and Bolacali, 2017). Its role in enhancing short-chain fatty acid production in the intestine may also contribute to lower cholesterol formation in the liver (Tang *et al.*, 2017). Increased HDL levels and reduced cholesterol could also stem from reduced lipid absorption in the intestine due to microbial activity stimulated by *A. niger* (Abul-Lateef *et al.*, 2019; Awais *et al.*, 2019). This may involve beta-fructan secretion by probiotic-associated fungi (Saleh *et al.*, 2011; Hao *et al.*, 2020).

In contrast, the decrease in cholesterol observed in the second group may result from the toxic effects of aflatoxins produced by *A. flavus* (Solis *et al.*, 2019). Liu *et al.* (2018) similarly reported reduced cholesterol levels in broilers exposed to aflatoxins, likely due to impaired liver function and lipid metabolism.

The concentration of total protein, albumin, and globulin in the blood of the third group increased significantly ( $p < 0.05$ ) compared to both the second and control groups, as shown in Table 7. This improvement may be attributed to the role of *A. niger* in enhancing the utilization of organic compounds in the feed, particularly proteins (Abul-Lateef *et al.*, 2019). In contrast, protein levels in the second group (*A. flavus*) were significantly lower ( $p < 0.05$ ) than those in the control group, likely due to the toxic effects of aflatoxins (Zou *et al.*, 2023).

However, despite this reduction, the chicks' vital functions were not severely affected, possibly due to the low fungal dosage and relatively short rearing period (35 days), which the liver may have managed without major dysfunction (Lafi *et al.*, 2010). These findings are consistent with those of Yunus *et al.* (2011), who also observed minimal physiological disruption at lower aflatoxin exposure levels

over short durations.

Fungi can influence liver cells in multiple ways, either negatively or positively, depending on the fungal species and the bioactive compounds or toxins they produce (Ozer et al., 2008). The significant increase ( $p < 0.05$ ) in liver enzyme levels observed in the second group (*A. flavus*) may be attributed to liver cell damage and increased cellular breakdown (Yunus et al., 2011). Exposure of broiler chicks to mycotoxins is known to reduce blood protein levels while elevating liver enzymes (Soares et al., 1989). In this study, the observed signs of anemia further suggest possible liver fibrosis, likely caused by aflatoxin exposure (Zou et al., 2023). These findings are consistent with Liu et al. (2018b), who reported decreased total protein and albumin levels, alongside increased liver enzyme activity, in broilers administered aflatoxins derived from *A. flavus*.

The elevated creatinine levels observed in the second group (*A. flavus*) may be attributed to the effects of aflatoxin secretion, which can cause damage to kidney tissues. This damage likely impairs the kidneys' ability to effectively eliminate metabolic waste from the body (Khaleghipour et al., 2019). These findings are consistent with those of Ukoha et al. (2023), who also reported increased blood creatinine concentrations in birds fed aflatoxin-contaminated feed.

## CONCLUSIONS

According to the study, *A. flavus* negatively affects broiler growth and health parameters, while *A. niger* shows beneficial effects. Therefore, *A. niger* may be further explored using molecular techniques before being applied in commercial poultry production.

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## NOVELTY STATEMENT

This article is one of the most recent articles in the country where it was conducted.

## AUTHOR'S CONTRIBUTION

The researchers contributed to the completion of this work with regular group work.

## CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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