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Research Report

# Effect of spirulina supplementation on growth, immunity, antioxidant status and pathomorphological perspectives in broilers exposed to dietary aflatoxin B1

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SUMMARY

The principal goal of this study was to evaluate the effect of Spirulina (SP) supplementation on growth, immunity, antioxidant status, liver and kidney functions, carcass criteria and pathomorphological perspectives in broilers given Aflatoxin B1(AFB1) in their diet. Two hundred (200) unsexed, 1-day-old broiler chicks (Ross 308) were divided into four treatment diet groups, each consisting of 50 birds. There were five replicates of each treatment (10 birds for each). Each group received specific dietary supplementation. Group 1 was fed a basal diet without additives. Group 2 was fed a diet with 2.5 mg/kg of AFB1. Group 3 was fed a diet with 1 g/kg of Spirulina. Group 4 was fed a diet with 2.5 mg/kg of AFB1 and 1 g/kg of Spirulina. The study lasted for six weeks (1–42 day). AFB1 toxicity significantly ($P < 0.05$) reduced final live body weight and weight gain whereas spirulina supplementation increased final body weight and weight gain. Moreover, spirulina supplementation significantly improved the feed conversion ratio ($P < 0.05$). AFB1 toxicity significantly ($P < 0.05$) decreased carcass weight and dressing percentage but spirulina supplementation significantly ($P < 0.05$) increased carcass weight, dressing percentage and giblet percentage relative to control. AFB1 toxicity significantly ($P < 0.05$) raised the blood concentrations of alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) ($P < 0.05$). Including SP in broiler diets contaminated with AFB1 decreased ALT, AST, and ALP concentrations relative to broilers intoxicated with AFB1 without SP ($P < 0.05$). The urea and creatinine concentrations were significantly elevated in broilers fed AFB1 diet with no SP ($P < 0.05$), but SP significantly lowered urea and creatinine concentrations ($P < 0.05$). AFB1 toxicity significantly ($P < 0.05$) increased the concentrations of total cholesterol, triglycerides, low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) however, the addition of SP resulted in a decrease in the concentrations of total cholesterol, triglycerides, low-density lipoprotein, and very low-density lipoprotein. AFB1 toxicity reduced the activity of SOD and GPx ($P < 0.05$). In contrast, SP elevated the SOD, GPx, catalase (CAT) and total antioxidant capacity (TAC) in broilers. Conversely, SP reduced the levels of MDA in broilers given AFB1, even though the levels of MDA was higher in broilers given AFB1 ($P < 0.05$). AFB1 toxicity significantly ($P < 0.05$) decreased the phagocytic index whereas dietary SP improved phagocytic activity and phagocytic index. In conclusion, using SP in broiler diets is

**Primary Audience:** These findings highlight the complex interplay between genetic, environmental, and physiological factors in post-hatch development and ascites susceptibility in broiler chickens. The results of this research can help breeders choose the right breeding strain and hatching place to reduce acidosis during breeding.

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considered a novel strategy for lowering the toxic impacts of AFB1 and improving growth, immunity, antioxidant status, liver and kidney function, carcass criteria and pathomorphological perspectives.

## Description of Problem

Food contaminated with different mycotoxins causes serious health problems and financial losses for livestock and poultry (Agag, 2004; Limaye et al., 2018). The present problem will also impact others who consume broiler meat because of the retention of mycotoxins in this meat (Wild and Gong, 2010; Alam et al., 2020). In the poultry industry, mycotoxicosis control is imperative as it poses a major threat to meat, and its derived products to maintain the safe food chain (Bilal et al., 2023). One of the harmful impacts of aflatoxin contamination in broiler chickens on the economy is a reduction in growth performance (Denli et al., 2009). Among the mycotoxins that are most commonly discovered in food include aflatoxin A, ochratoxin A, T-2 toxin, zearalenone, and nivalenol (Devegowda et al.,1998; Huwig et al., 2001). Fungi of the Aspergillus species produce a group of toxic compounds that are chemically identical and referred to as aflatoxins, namely aflatoxin (B1- B2- G1- G2) (Huff et al., 1986). *Aspergillus parasiticus* and *Aspergillus flavus* are the primary mycotoxigenic fungus responsible for the creation of aflatoxin (Dutta et al., 2001). Aflatoxin is produced by these harmful fungi when exposed to appropriate growing and storage conditions (Abidin et al., 2011). Aflatoxin B1 is the chemical that is most biologically active (Busby et al., 1981). It is very important to note that regulatory limits on AFB1 are 20 ppb, and animals subjected to low concentrations (1 mg/ kg diet) of aflatoxin in their diets may have adverse effects and cause immune system suppression, liver damage, and poorer reproductive outcomes (Agag, 2004; Denli et al., 2009). Aflatoxicosis in broilers diets causes enlargement of the pancreas, spleen, and liver, among other signs (Daghir, 2008).

Numerous studies have documented the benefits of nutritional supplements containing vitamins and electrolytes (Farghly et al., 2018; Abd El-Hack et al., 2018), probiotics (Salah et al., 2019a), prebiotics (Salah et al., 2019b), phytogenics and organic acids (Abu El Hammed et al., 2022; Abd Elzaher et al., 2023; Reda et al., 2024a), as well as other feed additives and management techniques (El-Tarabany et al., 2021; Ahmed-Farid et al., 2021; Reda et al., 2024b) in reducing the negative effects of environmental stress including aflatoxicosis on broiler chicken performance. Under these conditions, natural feed additives such as biomass or algal extracts rich in antioxidants and other bioactive components may enhance the resistance of birds to unfavorable or stressful environmental conditions (Salah et al., 2021c; Mohamed et al., 2024; Lestingi et al., 2024). *Spirulina platensis* (SP) is used as both a feed and nutritional additive in the field of aquaculture, cattle, and poultry industries. Spirulina is planted on a larger scale in multiple countries and can grow well in both freshwater and saltwater conditions. Dried spirulina is a highly nutritious food item due to its elevated protein level (260 to 770 g/kg), which makes up about 70 % of its dry weight, and its elevated fat content (10 to 140 g/kg) (Habib et al., 2008; Zahroojian et al., 2013). Moreover, spirulina has been discovered to possess a nutrient digestibility superior to feeds or other vegetable diets (Plaza et al., 2009; Alvarenga et al., 2011). As a result, spirulina can partially substitute conventional protein sources, especially soybean meal (Deepika et al., 2021). SP is an effective biological feed additive that boosts the immune system. It has been used successfully in aquafeed and may have the ability to decrease excessive oxidation (Rosas et al., 2019). According to recent studies conducted on animals, *S. platensis* may provide protection against the cytotoxic effects of substances like nephrotoxicity, cardiotoxicity, and hepatotoxicity. Because carotenoids are present, it is considered to have excellent antioxidant properties and be an incredibly powerful free radical scavenger (Arpita et al. 2014). According to Marin and Taranu (2012), AFB1 leads to excess free radicals, exacerbating DNA damage, cellular lipid peroxidation, and cell death. In broilers, adding SP mitigated the oxidative effects of AFB1 (Raju et al., 2005). Therefore, this study aimed to look at how spirulina can improve growth performance, antioxidant status, liver and renal functions, immunity, pathomorphological views, and serum biochemical markers of broilers while also mitigating aflatoxin B1′s detrimental impacts.

**Table 1**
Composition and chemical analysis of the experimental diets (starter and finisher diets).

| Items | Starter | Finisher |
|---|---|---|
| Ingredients (%) | | |
| Yellow Corn | 50.53 | 59.25 |
| Soybean meal | 38.50 | 33.50 |
| Soybean oil | 0.30 | 1.40 |
| Bran | 7.50 | 3.00 |
| Mono Calcium phosphate | 1.00 | 0.90 |
| Limestone | 1.30 | 1.20 |
| Vit-min Premix* | 0.30 | 0.30 |
| NaCl | 0.30 | 0.30 |
| DL Methionine | 0.11 | 0.10 |
| L-Lysine | 0.11 | 0.01 |
| Choline Chloride 60 % | 0.05 | 0.04 |
| Total | 100 | 100 |
| Calculated analysis** (%) | | |
| Crude protein | 23.00 | 20.00 |
| ME Kcal/kg diet | 2900 | 3100 |
| Calcium | 1.00 | 0.90 |
| Phosphorus (Available) | 0.48 | 0.45 |
| Lysine | 1.40 | 1.20 |
| Methionine+Cysteine | 0.92 | 0.72 |
| Crude Fiber | 3.43 | 2.88 |
| Linoleic acid | 1.50 | 20.40 |

* Growth vitamin and Mineral premix Each 2.5 kg consists of:Vit A 12000, 000 IU; Vit D3, 2000, 000 IU; Vit. E. 10 g; Vit k3 2 g; Vit B1, 1000 mg; Vit B2, 49 g; Vit B6, 105 g; Vit B12, 10 mg; Pantothenic acid, 10 g; Niacin, 20 g, Folic acid, 1000 mg; Biotin, 50 g; Choline Chloride, 500 mg, Fe, 30 g; Mn, 40 g; Cu, 3 g; Co, 200 mg; Si, 100 mg and Zn, 45 g.

** Calculated according to NRC (1994).

## Materials and methods

### *Preparation of aflatoxins, and spirulina*

The Central Laboratory of Agricultural Product Residues, Agriculture Pesticides Residues Centre, Dokki, Egypt, provided *Aspergillus flavus* MD 341. To produce aflatoxin B1, the fungus was cultured on liquid media with 20 % sucrose and 2 % yeast extract for 8 days. The resulting aflatoxin B1 concentration in the media was calculated by applying the following procedures (A.O.A.C, 1990). The diet with aflatoxins underwent analysis, confirming and determining the existence of primary aflatoxins as previously described. Dietary Spirulina microalgae powder was purchased from ABCHEM company for pharmaceutical raw materials 11 Saad Zaghlol St, Toril El-Gedida, Mansoura, Egypt.

### *Housing, and birds*

The trial was conducted at the Faculty of Veterinary Medicine, particularly in the Nutrition and Clinical Nutrition Department, at New Valley University in Egypt. The chicks were reared in an open-ventilated house in wire cages (120 × 50 × 50 $cm^3$, width x length x height, respectively). Throughout the trial period, chickens were provided unrestricted access to both water and feed. The basal diet was prepared following the NRC (1994) guidelines to fulfill the nutritional needs listed in Table 1. Prior to use, each batch of the basal feed was analyzed for aflatoxin and ochratoxin to ensure their levels were within acceptable limits. The testing parameters consisted of maintaining a constant temperature range of 22–25 °C, maintaining air humidity levels between 55 and 65 %, and ensuring sufficient ventilation. The chicks were one day old and had similar average body weights in all groups. The experiment follows the regulations established by the New Valley University Ethics Committee for the utilization of experimental animals. Throughout the experiment, all birds were kept in identical environmental conditions and were treated to the same sanitary treatments.

*Experimental design, diets, and treatments*

The research employed a randomized complete block design. The experiment included four treatments, with each treatment repeated five times. Each replication consisted of ten birds, giving an overall total of 200 broiler chicks (Ross 308). The study lasted for six weeks, during which the treatment groups were organized as follows: Group 1 was fed a diet only. Group 2 was fed a diet with 2.5 mg/kg of AFB1. Group 3 was fed a diet with 1 g/kg of Spirulina. Group 4 was fed a diet with 2.5 mg/kg of AFB1 and 1 g/kg of Spirulina. The avian specimens were all grown under the same conditions and given a balanced diet that met their nutritional requirements, as specified in NRC (1994) standards (Table 1). Feed and water were offered *ad libtum*.

*Growth performance*

All birds were weighed weekly to calculate body weight (BW). In addition, body weight gain (BWG) was assessed continuously for the entire length of the experiments. Furthermore, feed consumption was evaluated throughout the experimental periods in a replicated way to determine the feed conversion ratio. FCR was calculated based on the following formula $(FCR) = \frac{Feed\ consumed\ (g)}{weight\ gain(g)}$

*Carcass traits*

At the end of the trial, a random selection of 5 birds was made for each replicate. The birds were weighed and slaughtered by hand to study their carcass traits. The weight of the animal's body after slaughter and its internal organs, such as the giblets, gizzard, heart, liver, and abdominal fat, were measured and stated as % relative to the total weight. Furthermore, the dressing percentage was calculated.

*Biochemical parameters*

By the end of this study, the bird samples were gently taken from the wing vein, and blood samples were taken sterilely into clean tubes. Afterward, the samples were let to clot and centrifuged at 4000 rpm per minute for 10 min. The obtained serums were stored until they were prepared for examination. The spectrophotometric measurement of several variables was performed using kits provided by Biodiagnostic Company (Giza, Egypt). The parameters considered were aspartate transaminase (AST), alanine transaminase (ALT), triglycerides, total cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL), very low-density lipoprotein (VLDL) levels. The levels of superoxide dismutase (SOD), malondialdehyde (MDA), total antioxidant capacity (TAC), catalase enzyme (CAT) and glutathione peroxidase (GPx) in plasma samples were determined by utilizing commercially available kits and a spectrophotometer produced by Shimadzu, Japan.

*Pathomorphological perspectives*

Upon the conclusion of the trial, three birds from each group were euthanized using carbon dioxide and subsequently dissected. Prior to euthanasia, the weight of all the animals was determined. Thorough postmortem examinations were immediately performed on the birds, and significant abnormalities were documented. In addition, tissue samples of the liver and kidneys were extracted and stored in 10 % neutral buffered formalin for 72 h for histological analysis. Following fixation, the samples underwent dehydration using increasing concentrations of ethanol, subsequently, the specimens were purified using xylene and then embedded in paraffin. Tissue sections measuring five micrometers in thickness were cut using a microtome and then placed onto a glass slide. Hematoxylin and eosin (H&E) staining was applied to the slides and analyzed by a Leika DM500 light microscope to observe histological alterations (Suvarna et al., 2018).

**Table 2**
Effect of dietary Spirulina supplement on body weight and body weight gain of broilers exposed to aflatoxins.

| Parameter | Age (days) | Experimental groups | | | | P-value |
|---|---|---|---|---|---|---|
| | | CON[1] | AFB1[2] | SP[3] | AFB1+SP[4] | |
| Body weight (g) | 1 | 41 ± 0.57 | 41 ± 0.57 | 40.7 ± 0.88 | 40.6 ± 0.89 | 0.976 |
| | 14 | 398 ± 8.3 | 395 ± 4.3 | 418 ± 3.7 | 402 ± 7.3 | 0.229 |
| | 28 | 1340 ± 19.1[a] | 1260 ± 16.4[b] | 1396 ± 18.9[a] | 1348 ± 15.8[a] | 0.019 |
| | 42 | 2210 ± 20.8[b] | 2023 ± 18.8[c] | 2413 ± 20.5[a] | 2185 ± 22.6[b] | 0.001 |
| Body gain (g) | 1–14 | 357 ± 6.2 | 354 ± 5.5 | 377 ± 3.6 | 361 ± 4.4 | 0.251 |
| | 15–28 | 941 ± 14.8 | 865 ± 16.1 | 978 ± 14.8 | 947 ± 17.7 | 0.075 |
| | 29–42 | 870 ± 13.1[b] | 763 ± 12.6[c] | 1016 ± 14.7[a] | 837 ± 18.4[bc] | 0.001 |
| | 1–42 | 2169 ± 21.3[b] | 1982 ± 19.8[c] | 2372 ± 18.5[a] | 2144 ± 24.9[b] | 0.001 |

*1Control group, [2]Aflatoxin-exposed group, [3]Spirulina-supplemented group. [4]Spirulina+Aflatoxin group, [a,b,c]Means with different superscripts in each row are significantly differed.

*Immunohistochemical staining of caspase-3*

Paraffin sections from the liver and kidneys were stained by immunohistochemistry (IHC) as stated by Hsu et al. (1981) using mouse monoclonal anti-caspase-3 Ab [ABM1C12] at 5 µg/ml dilution, Cambridge, UK., Abcam. Tissue sections from each experimental group were washed with water and dewaxed. Staining was then performed by the DAB chromogenic agent (Expose mouse and rabbit specific HRP/DAB detection kit, Abcam; Ready-to-use; Cat. #: ab80436). Hematoxylin counterstaining was carried out. All tissue sections were inspected by a microscope (leika DM 500) associated with a Swift digital camera.

*Statistical analysis*

The statistical analysis was conducted using the IBM SPSS software (Version 16.0; IBM Corp., NY, USA). The data was analyzed using ANOVA, specifically the one-way model of analysis of variance. A significance level of $P < 0.05$ was employed to detect significant variations between the groups, and Tukey post hoc test was utilized to calculate the probabilities.

## Results and discussion

*Growth performance*

Table 2 presents the influence of dietary Spirulina supplements on broilers' body weight and weight gain exposed to Aflatoxins. Results showed that AFB1 significantly decreased the body weight and body weight gain compared to the control treatment. While co-administration

**Table 3**
Effect of dietary Spirulina supplement on feed intake and feed conversion ratio of broilers exposed to aflatoxins.

| Parameter | Age (days) | Experimental groups | | | | *P*-value |
|---|---|---|---|---|---|---|
| | | CON[1] | AFB1[2] | SP[3] | AFB1+SP[4] | |
| Feed intake (g) | 1–14 | 715 ± 3.8[a] | 650 ± 3.9[d] | 681 ± 4.6[b] | 665 ± 3.5[c] | 0.001 |
| | 15–28 | 1584 ± 5.8[a] | 1440 ± 4.9[d] | 1490 ± 5.3[c] | 1510 ± 5.6[b] | 0.001 |
| | 29–42 | 1706 ± 6.9[a] | 1575 ± 7.3[b] | 1550 ± 7.8[c] | 1590 ± 6.7[b] | 0.001 |
| | 1–42 | 4007 ± 7.7[a] | 3660 ± 8.3[d] | 3720 ± 7.9[c] | 3760 ± 8.2[b] | 0.001 |
| Feed conversion ratio (g feed/g gain) | 1–14 | 2.01 ± 0.03 | 1.84 ± 0.04 | 1.80 ± 0.03 | 1.84 ± 0.04 | 0.077 |
| | 15–28 | 1.68 ± 0.03 | 1.67 ± 0.04 | 1.52 ± 0.03 | 1.59 ± 0.03 | 0.114 |
| | 29–42 | 1.96 ± 0.06[a] | 2.06 ± 0.07[a] | 1.53 ± 0.05[b] | 1.90 ± 0.07[a] | 0.001 |
| | 1–42 | 1.84 ± 0.02[a] | 1.85 ± 0.02[a] | 1.56 ± 0.02[c] | 1.75 ± 0.03[b] | 0.001 |

*1Control group, [2]Aflatoxin-exposed group, [3]Spirulina-supplemented group. [4]Spirulina+Aflatoxin group, [a,b,c]Means with different superscripts in each row are significantly differed.

of spirulina with AFB1 improved growth performance parameters when compared to AFB1 alone group. Results also showed that spirulina 1g/kg diet significantly recorded the highest value of body weight and body weight gain compared to the control treatment during the last 2 weeks. As presented in Table 3, group 2 supplemented by AFB1 2.5mg/kg diet presented a significant decline ($P = 0.001$) in feed consumption rate, and the control treatment presented the highest feed consumption rate. Furthermore, dietary Spirulina treatments significantly ($P = 0.001$) enhanced the FCR and group 3 supplemented with spirulina 1g/kg diet showed the best results (1.56 %) followed by group 4 supplemented with spirulina 1g/kg diet and AFB1 2.5mg/kg diet in contrast the group 2 showed the worst level (1.85 g feed/g gain).

Higher levels of Aflatoxin B1 (1–5 mg/kg) have been shown to be hepatotoxic to broiler chickens, causing pathological liver lesions (Fawaz et al., 2022). Since aflatoxin B1 frequently occurs in feedstuffs and causes serious health issues and significant financial losses for the poultry industry, most research is concentrated on decontaminating and detoxifying feed from this toxin (Bilal et al., 2023). Our findings illustrated that spirulina treatment 1gm/kg diet significantly ($p < 0.05$) improved body weight, weight gain and FCR. Moreover, it significantly ($p < 0.05$) decreases the negative effect of aflatoxicosis. On the other hand aflatoxicosis negatively affect body weight, weight gain and FCR. Our finding agrees with Ibrahim et al. (2018), who significantly improved various parameters by adding spirulina to drinking water at doses of 0.5, 1, and 2 g/l for 4 weeks. The observed improvements included higher average body weight gain, enhanced overall health, raised chick viability %, improved FCR, enhanced feed efficiency, elevated European Production Efficiency values, and greater relative weights of carcass and internal organs. Additionally, the addition of spirulina results in a significant decline in abdominal fat. This result indicates a decrease in chicks' abdominal fat when consumed SP enriched diet. This result is in agreement with Abed al. (2023) who found that birds fed 1 or 2 g spirulina/kg diet achieved the lowest value of abdominal fat of broiler chickens. Spirulina also demonstrated strong aflatoxin-binding properties in broiler breeders (Manafi et al.,2011; Arpita et al., 2014). However, there was no beneficial effect observed on feed consumption (Raju et al., 2004). As for the mechanisms that may explain the protective efficacy of Spirulina against the toxic effects induced by AFB1 in chickens, a significant contribution likely derives from the multiple biological properties of the microalga (immunomodulatory, antioxidant, antiapoptotic, prebiotic, hepatoprotective) (Abdelnour et al., 2024). Furthermore, Spirulina microalga has been found to act as an aflatoxin-binder, reducing the absorption of mycotoxin in the gastrointestinal tract (Abdelnour et al., 2024). In addition, the vitamins contained in Spirulina play an important detoxifying role (Abdelnour et al., 2024; Lestingi et al. 2024).

**Table 4**
Effect of dietary Spirulina supplement on carcass traits of broilers exposed to aflatoxins.

| Item | Experimental groups | | | | *P*-value |
|---|---|---|---|---|---|
| | CON[1] | AFB1[2] | SP[3] | AFB1+SP[4] | |
| Carcass weight (g) | 1711 ± 22.7[b] | 1530 ± 21.5[c] | 1903 ± 18.8[a] | 1675 ± 19.3[b] | 0.001 |
| Dressing % | 77.4 ± 0.56[ab] | 75.6 ± 0.71[c] | 78.9 ± 0.61[a] | 76.7 ± 0.52[bc] | 0.013 |
| Giblet % | 4.43 ± 0.08[ab] | 4.70 ± 0.05[a] | 4.26 ± 0.06[b] | 4.50 ± 0.09[ab] | 0.050 |
| Liver % | 1.63 ± 0.06[bc] | 1.83 ± 0.05[a] | 1.51 ± 0.05[c] | 1.73 ± 0.04[ab] | 0.018 |
| Gizzard % | 2.33 ± 0.08 | 2.30 ± 0.05 | 2.37 ± 0.09 | 2.27 ± 0.06 | 0.878 |
| Heart % | 0.47 ± 0.04 | 0.57 ± 0.03 | 0.40 ± 0.03 | 0.51 ± 0.04 | 0.270 |
| Abdominal fat % | 1.57 ± 0.04[a] | 1.53 ± 0.02[a] | 1.26 ± 0.02[b] | 1.60 ± 0.05[a] | 0.002 |

*1Control group, [2]Aflatoxin-exposed group, [3]Spirulina-supplemented group. [4]Spirulina+Aflatoxin group, [a,b,c]Means with different superscripts in each row are significantly differed.

*Carcass traits*

The findings of carcass traits in the present study are reported in Table 4. The finding revealed that chicks treated with 1 g Spirulina had significantly ($P = 0.001$, $P = 0.013$, $P = 0.050$) improved carcass yield, dressing %, and weight of giblets relative to live weight. In contrast, the finding illustrated that chicks fed with 2.5 mg AFB1 had significantly ($P = 0.05$, $P = 0.018$) an increased weight of giblets and liver % relative to live weight, respectively. Moreover, the result illustrated a significant variation in abdominal fat % and group 3 supplemented with Spirulina revealed the lowest percentage. However, Spirulina did not have any

**Table 5**
Effect of dietary Spirulina supplement on blood biochemical indices of broilers exposed to Aflatoxins.

| Item | Experimental groups | | | | *P*-value |
|---|---|---|---|---|---|
| | CON[1] | AFB1[2] | SP[3] | AFB1+SP[4] | |
| ALT (IU/L)[5] | 24.33 ± 1.76[b] | 41.0 ± 2.30[a] | 23.33 ± 1.20[b] | 38.34 ± 2.18[a] | 0.001 |
| AST (IU/L)[6] | 147.6 ± 2.90[b] | 172.0 ± 2.65[a] | 144.3 ± 2.84[b] | 162.0 ± 2.77[a] | 0.002 |
| ALP (IU/L)[7] | 171.4 ± 3.21[b] | 191.3 ± 4.62[a] | 170.6 ± 3.81[b] | 174.6 ± 4.25[b] | 0.006 |
| Uric acid (mg/dL) | 4.10 ± 0.07[c] | 6.34 ± 0.08[a] | 4.02 ± 0.09[c] | 5.34 ± 0.12[b] | 0.001 |
| Creatinine (mg/dL) | 0.92 ± 0.02[c] | 1.85 ± 0.04[a] | 0.89 ± 0.02[c] | 1.32 ± 0.05[b] | 0.001 |
| T.Cholesterol (mg/dL) | 183.0 ± 5.85[c] | 357.6 ± 6.21[a] | 179.1 ± 5.09[c] | 259.7 ± 6.11[b] | 0.001 |
| Triglycerides (mg/dL) | 140.7 ± 4.35[c] | 215.4 ± 4.53[a] | 138.5 ± 3.80[c] | 181.8 ± 4.12[b] | 0.001 |
| HDL (mg/dL)[8] | 64.02 ± 2.82[a] | 49.40 ± 2.58[b] | 67.66 ± 2.99[a] | 57.66 ± 2.89[ab] | 0.021 |
| LDL (mg/dL)[9] | 74.84 ± 2.84[c] | 124.72 ± 3.27[a] | 71.17 ± 2.11[c] | 96.51 ± 3.12[b] | 0.001 |
| VLDL (mg/dL)[10] | 37.62 ± 2.84[c] | 65.43 ± 2.67[a] | 36.96 ± 2.81[c] | 52.19 ± 2.76[b] | 0.005 |

*1Control group, [2]Aflatoxin-exposed group, [3]Spirulina-supplemented group. [4]Spirulina+Aflatoxin group, [5]Alanine transaminase, [6]Aspartate transferase, [7]Alkaline phosphatase, [8]High-density lipoprotein, [9]Low-density lipoproteins, [10]Very-low-density lipoproteins, [a,b,c]Means with different superscripts in each row are significantly differed.

impact on feed consumption, FCR, leg abnormality scores, and the weights of the liver, giblets, spleen, and abdominal fat (Raju et al., 2004; Abd Elzaher et al., 2023). SP did not alter the liver weights or the stages of kidney, proventriculus, or gizzard lesions in broiler breeder hens, regardless of whether they were incorporated alone or in a mixture with different quantities of AF (Manafi et al., 2011). The present results align with the findings reported by Fathi (2018), who demonstrated that the inclusion of spirulina had a significant positive impact on slaughter features at 38 days of age of broilers, but total giblets (liver, heart, and gizzard), were significantly influenced. Adding spirulina to the diet significantly enhances the measurements of broiler carcasses, as proven by Bellof and Alarcon (2013). In addition, Kaoud (2012), Abd Elzaher et al.(2023) demonstrated a significant enhancement in carcass yield, breast yield, and bursa weight relative to live weight in the group treated with Spirulina. The boost in carcass yield and breast muscle percentage can be attributed to the higher nutritional composition of spirulina, which includes all important amino acids.

*Serum biochemical parameters*

*Liver and kidney functions and lipid profile*

The impacts of dietary Spirulina supplements on the blood biochemical indices of broilers subjected to Aflatoxins are demonstrated in Table 5. The findings illustrated that birds exposed to 2.5 mg AFB1 had significantly ($P = 0.001$, $P = 0.002$, $P = 0.006$) raised ALT, AST and ALP concentrations in comparison to control and other groups. Moreover, AFB1 toxicity significantly ($P = 0.001$) increases uric acid and creatinine concentrations in the serum and group 2 supplemented with AFB1 2.5 mg /kg diet revealed the highest results. Furthermore, our result illustrated that the group treated with spirulina revealed a significant reduction ($P = 0.001$, $P = 0.001$, $P = 0.001$, $P = 0.005$) in total cholesterol, triglycerides, LDL, and VLDL. On the other hand group 2 supplemented with AFB1 2.2mg/kg diet revealed a significant elevation in total cholesterol, triglycerides, LDL, and VLDL. In contrast, group 2 exposed to AFB1 2.5mg/kg illustrated significantly decreased HDL, and group 3 supplemented with spirulina at 1gm/kg diet increased HDL level ($P = 0.021$).

The alteration of physiological indices can affect characteristics related to the health of birds (Arif et al., 2022; Alagawany et al., 2024). The outcomes of our study illustrated that AFB1 treatments significantly increased liver enzymes (ALT, AST and ALP) in broilers. SP demonstrated a significant ($P < 0.05$) reduction in serum ALT, AST and ALP activity within the entire research duration. This demonstrates the beneficial impact of SP in mitigating the detrimental effects of aflatoxicosis on enzyme activity. The increase in serum ALT, AST and ALP activity during aflatoxicosis can be related to the degeneration of hepatocytes and the consequent release of enzymes into the bloodstream. Elevated ALT, AST and ALP activity are the most accurate indicators of liver injury in cases of aflatoxicosis (Salahi et al., 2011). Aflatoxin produces alteration in fat, the metabolism of carbohydrates, and protein (Ledoux et al., 1999). The vacuolar degeneration of hepatocytes may result from the dysfunction of lipid metabolism and disruptions in ATP pumps. The presence of cytoplasmic vacuolation, located perilobularly, in birds fed with AF has been documented in prior research (Abo-aziza et al., 2022). Aflatoxins harm hepatocytes, preventing their regeneration and reducing the metabolic activity in the cell, This results in the buildup of metabolic substances. Hyperplasia of the bile ductal epithelium may result from the direct impact of AF on biliary epithelial cells, as suggested by Abdel-Wahhab et al. (2002). Supplementing SP to birds fed an AF diet improved most liver lesions, demonstrating its protective function (El-Shall et al., 2023).

Apoptosis is an energy-reliant mechanism that depends on molecular signaling through various receptors, ultimately resulting in the activation of caspases (Fotouh et al., 2024). The present study illustrated strong expression of cleaved caspase-3 in hepatocytes in AF fed group. These reactions were reduced when challenged by SP.

**Table 6**
Effect of dietary Spirulina supplement on antioxidant activity of broilers exposed to Aflatoxins.

| Item | Experimental groups | | | | *P*-value |
|---|---|---|---|---|---|
| | CON[1] | AFB1[2] | SP[3] | AFB1+SP[4] | |
| MDA (nmol/g)[5] | 14.42 ± 1.71[b] | 24.46 ± 1.79[a] | 16.55 ± 1.61[b] | 20.91 ± 1.38[ab] | 0.033 |
| SOD (u/g)[6] | 374.1 ± 4.64[b] | 215.5 ± 4.53[c] | 439.5 ± 3.47[a] | 324.6 ± 3.16[b] | 0.001 |
| GPx (u/g)[7] | 81.35 ± 3.53[a] | 44.40 ± 3.58[b] | 85.10 ± 3.75[a] | 58.79 ± 2.79[b] | 0.003 |
| CAT (nmol/g)[8] | 38.17 ± 3.17 | 28.07 ± 2.70 | 35.63 ± 2.47 | 34.85 ± 2.63 | 0.139 |
| TAC (nmol/g)[9] | 138.3 ± 6.55[a] | 102.1 ± 6.64[b] | 139.2 ± 6.73[a] | 115.3 ± 5.59[ab] | 0.023 |

*1Control group, [2]Aflatoxin-exposed group, [3]Spirulina-supplemented group. [4]Spirulina+Aflatoxin group, [5]Malondialdehyde, [6]Superoxide dismutase, [7]Glutathione Peroxidase, [8]Catalase;[9]Total antioxidant capacity, [a,b,c]Means with different superscripts in each row are significantly differed.

The urea concentration is used to measure the quantity of degraded protein in the liver and the liver's ability to remove excess blood urea with the assistance of the kidney (Hester et al., 1992; Fotouh et al., 2020). Furthermore, elevated levels of creatinine indicate the presence of damaged creatine in the muscle and the kidney's capacity to filter it into creatinine for excretion in urine (Watson et al., 2002). The current investigation demonstrated increasing levels of urea and blood creatinine in broilers subjected to AFB1, indicating kidney impairment. The kidney is an organ that requires a significant amount of energy and includes a large number of mitochondria, making it disposed to oxidative destruction. The overproduction of reactive oxygen species (ROS) in renal cells produces mitochondrial malfunction, depletion of ATP, impairment of membrane transporters, and disruption of cytoplasmic proteins (Abdel-Daim et al., 2020a). This study showed reduced renal tubular function as the outcome of all these occurrences. The pathomorphological alterations of kidneys are mainly due to the direct effect of the toxin on different components leading to congestion, hemorrhages, degeneration of tubular epithelium and nephrotoxicity. The nephrotoxicity may be due to disturbances in the transport function system in tubular cells specially proximal tubular function. Also, the increasing levels of metabolites in nephrons lead to nephrotoxicity (Sharma et al., 2011). Higher concentrations of certain substances mostly induce tubular necrosis, while smaller doses of the same substances predominantly result in accelerated apoptosis (Fotouh et al., 2024). Our findings indicated that SP treatments reduced total cholesterol, triglyceride and LDL levels. Safari et al. (2020) showed that SP is a powerful antioxidant that prevents the creation of harmful free radicals and suppresses the oxidation of lipids immediately and even after sixty days. Furthermore, prior research conducted on broilers has shown that the supplementation of Spirulina to their diet leads to decreased levels of blood cholesterol, triglycerides, total lipids, and LDL (Moustafa et al., 2021; Abdel-Moneim et al., 2022). Furthermore, the supplement of Spirulina to the diet leads to a decrease in plasma cholesterol levels (Abd Elzaher et al., 2023). The hypolipidemic impact of SP can be related to a dose-dependent reduction in lipase activity in the pancreas (Xalxo et al., 2013) and an elevation in lipid peroxidation (Hwang et al.,2011).

*Antioxidant status*

The impacts of dietary Spirulina supplements on the antioxidant activity of broilers exposed to Aflatoxins are illustrated in Table 6. The findings illustrated that group 3 supplementation with spirulina at 1gm/kg diet significantly increased SOD, GPx, and TAC levels and significantly reduced MDA levels ($P = 0.033$). In contrast, group 2 exposed to AFB1 2.5mg/kg illustrated significantly decreased SOD, GPx, and TAC levels and significantly raised MDA levels ($P = 0.033$). Moreover, our result revealed no significant changes ($P = 0.139$) in CAT levels and

**Table 7**
Effect of dietary Spirulina supplement on some immunological indices of broilers exposed to aflatoxins.

| Item | Experimental groups | | | | P-value |
|---|---|---|---|---|---|
| | CON[1] | AFB1[2] | SP[3] | AFB1+SP[4] | |
| Phagocytic activity | 34.99 ± 3.30 | 27.81 ± 3.43 | 52.22 ± 3.77 | 33.45 ± 3.27 | 0.117 |
| Phagocytic index | 0.37 ± 0.03[ab] | 0.20 ± 0.02[c] | 0.50 ± 0.04[a] | 0.27 ± 0.03[bc] | 0.010 |

[*1]Control group, [2]Aflatoxin-exposed group, [3]Spirulina-supplemented group. [4]Spirulina+Aflatoxin group, [a,b,c]Means with different superscripts in each row are significantly differed.

group 2 exposed to AFB1 2.5mg/kg revealed the lowest level, but, the control treatment revealed the highest levels. The data of our research illustrated that the contamination by AFB1 significantly decreased SOD, GPx and TAC levels in broilers. but SP supplementation ameliorates the negative impact of aflatoxicosis and increases the antioxidant status. The antioxidant action of spirulina may be due to its varied range of antioxidant components, including ß-carotene, astaxanthin, phycocyanin, tocopherol, selenium, phenolic acids (Chu, 2011; El-Desoky et al., 2013). The Spirulina-supplemented group exhibited significantly elevated levels of TAC, GPx, and SOD in this study. GPx and SOD are commonly recognized as enzymatic antioxidants that remove free radicals in cells (Abdel-Wahhab et al., 2005). Spirulina treatment has been observed to adjust the redox status of birds in both typical and difficult conditions. In a trial stated by Park et al. (2018), the levels of GPx and SOD in birds showed a linear increase when they were treated with Spirulina at doses of 0.25 % - 0.5 % - 0.75 % - 1 %. Spirulina, when included in the diet, has been found to improve the opposing impacts of heat stress on the broiler oxidant/antioxidant balance. This is achieved by reducing the concentration of MDA and boosting the action of SOD and TAC, also the concentration of reduced glutathione (GSH) (Moustafa et al., 2021). The outcomes were boosted by the scavenging impact of Spirulina on hydroxyl radicals, which can be attributed to its C-phycocyanin concentration (Estrada et al., 2001). C-phycocyanin exhibits significantly higher antioxidant activity compared to vitamin E and vitamin C. It is approximately sixteen times more potent than vitamin E and twenty times more powerful than vitamin C. (Romay et al., 2000).

*Immunity*

The impacts of dietary Spirulina supplements on some immunological indices of broilers subjected to Aflatoxins are illustrated in Table 7. The findings revealed no significant differences ($P = 0.117$) in Phagocytic activity and group 3 supplemented with spirulina at 1gm/kg diet revealed the highest level, while group 2 treated with AFB1 revealed the lowest result. Moreover, our result illustrated significant variation ($P = 0.010$) in Phagocytic index and group 3 supplemented with spirulina at 1gm/kg diet revealed the highest level while group 2 treated with AFB1 revealed the lowest result.

Our study found that including *Spirulina platensis* in the broilers' diet improved their immune indices, including phagocytic activity and phagocytic index. The incorporation of *Spirulina platensis* as a binder in this work aligns with the results of Fernandez et al. (1994), who illustrated that including Spirulina at a concentration of 0.02 % in the diet enhances the cellular immunological response. In contrast, broilers fed with 300 AF did not have any noticeable benefit. There are numerous findings by Pasha et al. (2007) on the usefulness of *Spirulina platensis* in improving the immunological condition of birds fed with AF. Spirulina is a potent immunomodulatory agent that effectively enhances the function of the immune system. Animal studies have demonstrated that the blue-green natural protein pigment phycocyanin enhances lymphocyte function and promotes blood formation. Phycocyanin likely enhances immunity, hence potentially preventing several degenerative organ disorders. SP contains several bioactive elements, including vitamins, minerals, carotenoids, and polysaccharides, that function as immunostimulants to boost immunity (Rosas et al., 2019). In addition, Spirulina boosts mucosal immunity by promoting the development of IgA and IgE antibodies and supporting healthy microbiota (Khan et al., 2005; Shokri et al., 2014). The detrimental impact of 300 (ppb) aflatoxin on the growth rate of birds and the weights of their lymphoid organs could potentially be reduced to some extent by adding spirulina at a concentration of 0.05 % (Raju et al., 2004). Incorporating Spirulina (0.02 %) into the feed resulted in beneficial outcomes for the growth, ready-to-cook yields, bursa weight, and immune response of birds exposed to aflatoxin (300 ppb). Immunohistochemical staining of the Caspas-3 antigen in the AF group revealed a strong presence of fine brown granules located predominantly in the cytoplasm of the epithelial lining in convoluted tubules. The intensity of this reaction was diminished in the SP-challenged group.

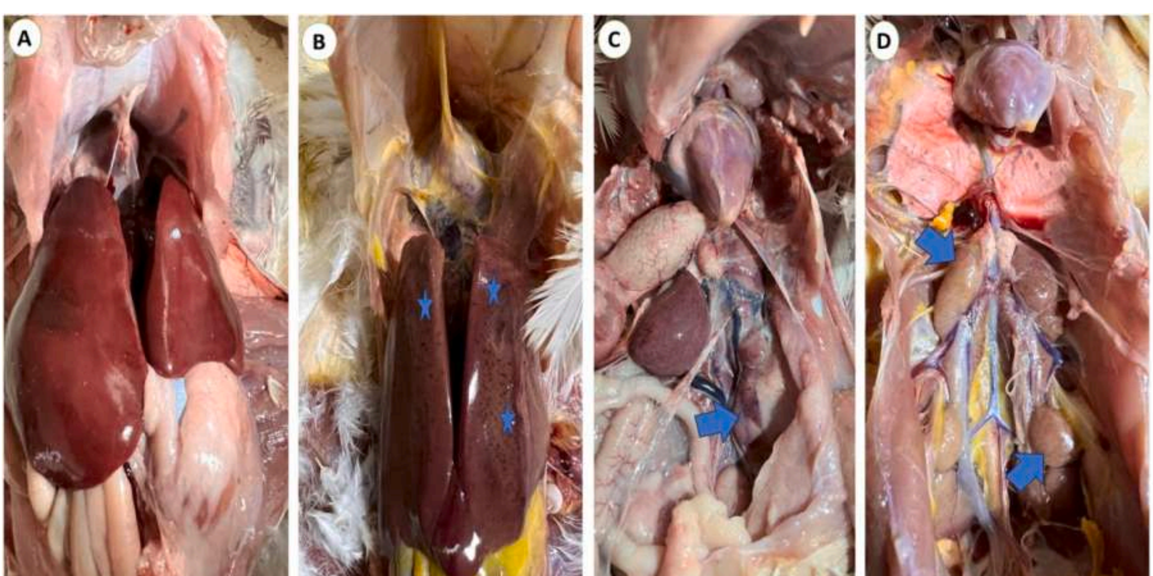


**Fig. 1.** Gross lesion from liver and kidneys of broilers (42 days old) experimentally fed aflatoxin. (A) control group showing normal liver (B) aflatoxin fed group showing severely enlarged liver with petechial hemorrhages on the surface (stars). (C) control group showing normal kidney (arrow). (D) aflatoxin fed group showing enlarged and congested kidneys (arrows).

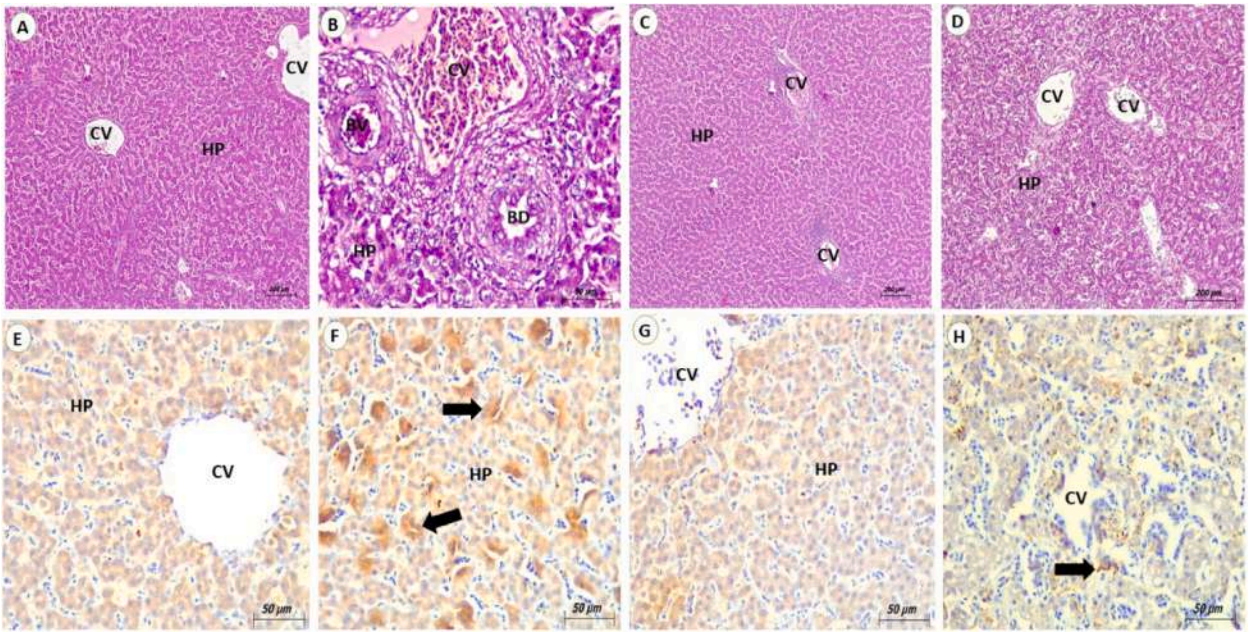


**Fig. 2.** Liver from broilers experimentally fed aflatoxin. A and C: from control and SP groups showed normal hepatic parenchyma (HP) radiating around central vein (CV) (H&E, scale bar: 200). B: from AF group showed congestion of central vein and sinusoids (CV). Severe degeneration of hepatic parenchyma (HP) and bile ductuli (BD). (H&E, scale bar: 50). D: from AF + SP group showed mild vacuolar degeneration of hepatic parenchyma (HP) and sinusoidal congestion (H&E, scale bar: 200). E and G: no any positive reaction for caspases-3 (IHC stain, scale bar: 50). F: strong positive reaction for caspases-3 in hepatocytes all over hepatic parenchyma (HP) (IHC stain, scale bar: 50). H: mild positive reaction for caspases-3 in hepatocytes around central vein (CV) (IHC stain, scale bar: 50).

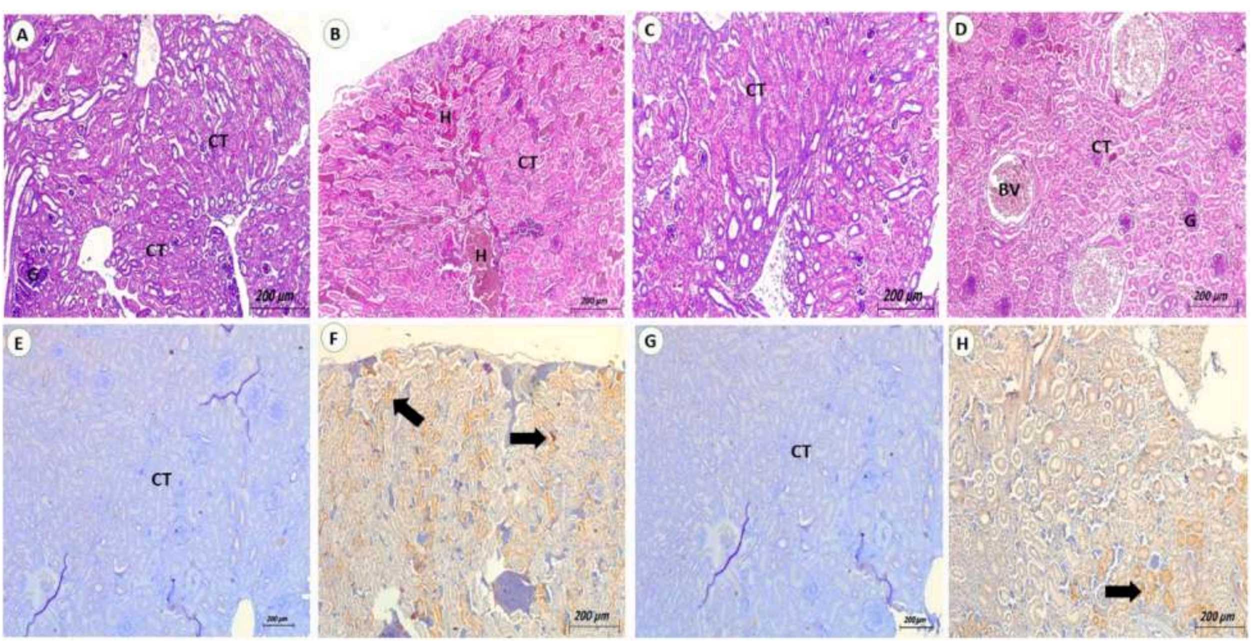


**Fig. 3.** Kidneys from broilers experimentally fed aflatoxin. A and C: from control and SP groups showed normal renal tissue; convoluted tubules (CT) and glomeruli (G) (H&E, scale bar: 200). B: from AF group showed severe congestion and hemorrhages (H) with tubular atrophy (CT). (H&E, scale bar: 50). D: from AF + SP group showed mild vacuolar degeneration of convoluted tubules (CT) and congestion of blood vessels (BV) (H&E, scale bar: 200). E and G: no any positive reaction for caspases-3 (IHC stain, scale bar: 200). F: strong positive reaction for caspases-3 in lining epithelium of tubules (arrows) (IHC stain, scale bar: 200). H: mild positive reaction for caspases-3 in some renal tubules (arrow) (IHC stain, scale bar: 200).

*Pathomorphological perspectives*

*Gross findings*

The livers and kidneys of the control and SP groups illustrated normal morphological appearance. Some livers and kidneys were severely enlarged with petechial hemorrhages on the surface in the AF group (Fig. 1A & B). For the AF + SP group, only mild enlargement in some livers were noticed.

*Histopathological and immunohistochemical findings*

Liver sections from the control and SP group revealed normal hepatic parenchyma. Hepatocytes are arranged in cords extending from central veins and divided by regular sinusoids (Fig. 2A&C). The AF group exhibits vacuolar degeneration of hepatocytes, central vein congestion, and sinusoid dilation. Areas of coagulative necrosis were observed in hepatocytes, accompanied by the presence of newly developed blood vessels. some cases showed moderate hyperplasia of the bile tract epithelium with severe degeneration of its wall (Fig. 2B). infiltration of lymphocytic cells was more intense and occurred in interlobular areas. Regarding to AF + SP group, amelioration of most microscopical lesions was noted. Only mild degenerative changes and congestion of hepatic parenchyma were seen (Fig. 2D). The immunohistochemical expression of Caspas-3 was not detected in both the control and SP groups (Fig. 2E&G). Hepatocytes exhibited detectable expression of cleaved caspase-3 specially around portal triads in AF group (Fig. 2F). We observed a low level of immune response in hepatocytes, specifically in the AF + SP group, as indicated by the presence of cleaved caspase-3 (Fig. 2H). Regarding kidney examination both control and SP groups revealed normal histological structure, in which the nephron consists of the glomerulus and the renal tubules (Fig. 3A&C). In AF group, severe hemorrhage and congestion were noticed all over the renal tissues. Vacuolar degeneration of renal tubular epithelium associated with tubular atrophy and increased glomerular cellularity (Fig. 3B). For the AF + SP group, all the lesions were diminished except mild congestion and vacuolar degeneration of some renal tubules. (Fig. 3D). The immunoreactivity of the Caspas-3 antigen was not detected in the control and SP groups (Fig. 3E&G). Strong reaction in AF group appeared as fine brown granules mostly in the cytoplasm of the epithelial lining of both proximal and distal convoluted tubules (Fig. 2F). Mild immunoreactivity of Caspas-3 was recorded in AF + SP group in some renal tubules (Fig. 3H).

## Conclusion and applications

1. The results of the present study showed that Aflatoxins B1 has adverse effects on the performance and health of broiler chickens.
2. The use of *Spirulina platensis* in broiler diets is a novel strategy to reduce the harmful effects of aflatoxin B1 and improve growth, immunity, antioxidant status, liver and kidney functions, and pathomorphological perspectives.

## Institutional review board statement

The ethical statement conformed to the guidelines set up by the Ethical Committee of the Egyptian Research for the Use and Care of Laboratory Animals, as established by New Valley University (NVREC/02/3/5/2024/16).

## Declaration of competing interest

The authors declare no conflict of interest.

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*Disclosures*

The authors declare that there is no conflict of interest regarding the publication of this paper.

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