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Original research paper

AMELIORATIVE EFFECTS OF *SACCHAROMYCES CEREVISIAE* (H.) ON HEMATOLOGICAL- BIOCHEMICAL PARAMETERS IN BROILER CHICKENS FED WITH DIETS NATURALLY CONTAMINATED WITH AFLATOXIN

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Abstract: This study evaluated the effects of supplementing two levels of *Saccharomyces cerevisiae* (H.) yeast in broiler diets naturally contaminated with aflatoxins (32 ppb), produced by *Aspergillus flavus* L. on hematological and biochemical parameters. A total of 200 one-day-old Ross 308 chicks were randomly assigned to four groups, each with five replicates of 10 birds. The negative control group (T1) received an uncontaminated diet, whereas the positive control (T2) was fed an aflatoxin-contaminated diet without additives. Groups T3 and T4 received contaminated diets supplemented with *S. cerevisiae* at 0.1% and 0.2% levels, respectively. Feeding broilers, a naturally aflatoxin-contaminated diet (32 ppb) adversely affected hematological and biochemical parameters ($p \leq 0.01$), indicating compromised health and physiological status. Dietary supplementation, particularly at the 0.2% level, significantly improved hematological and biochemical profiles compared with the positive control ($p \leq 0.01$). Improvements were observed in red blood cell counts (26%, 17%), white blood cell counts (12%, 9%), hemoglobin concentrations (6%, 5%), total protein levels (33%, 13%), albumin (20%, 7%), and globulin (47%, 26%), along with reductions in heterophil-to-lymphocyte ratios (23%, 21%), glucose concentrations (4%, 3%), and total cholesterol (6%, 5%) on days 21 and 42, respectively. These results demonstrate that dietary supplementation with 0.2% *S. cerevisiae* effectively mitigates the adverse effects of natural aflatoxin contamination on hematological and biochemical parameters in broiler chickens.

Key words: *blood cell, natural aflatoxins, Ross 308 broiler, total cholesterol, yeast*

INTRODUCTION

Hematological parameters are widely used as indicators of the physiological and health status of animals, as they are influenced by factors such as age, sex, breed, stress, infectious diseases, and exposure to toxic compounds (Pessini et al., 2020). Among these toxins, aflatoxins—secondary metabolites mainly produced by *Aspergillus* fungi—are of major concern due to their detrimental effects on hematological and biochemical characteristics. These toxins induce oxidative stress through the generation of free radicals, thereby im-

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pairing cellular integrity and physiological functions (Jobe et al., 2023; Jahanian, Mahdavi, Asgary, Jahanian & Tajadini, 2019).

In broiler chickens, even low concentrations of naturally occurring aflatoxins (0.5–60 ppb) have been associated with impaired hematological and biochemical parameters, accompanied by reductions in red blood cell count and hemoglobin concentration, alterations in white blood cells, decreased levels of serum liver proteins, and elevated total cholesterol levels (Ejiofor et al., 2021; Fochesato et al., 2023; Bui et al., 2025). At higher concentrations (≥ 200 ppb), hematological and biochemical disturbances become more pronounced, including reduced red blood cell counts, increased leukocyte counts, alterations in the heterophil-to-lymphocyte ratio, and decreased levels of hepatic proteins, reflecting impaired liver function and metabolic pathways (Oloruntola et al., 2024; Ashry et al., 2022). These findings highlight the dose-dependent effects of aflatoxins on poultry physiology.

Biological detoxification strategies have gained increasing attention as safe and cost-effective alternatives to chemical binders, as they preserve the nutritional quality of feed without introducing harmful residues (Liu et al., 2022). Baker's yeast (*Saccharomyces cerevisiae*) is of particular importance due to its dual role as a growth promoter and immune modulator, in addition to its ability to bind mycotoxins and reduce their intestinal absorption (Lin et al., 2023). Recent evidence indicates that supplementing diets contaminated with aflatoxin with baker's yeast can improve hematological and biochemical parameters in broiler chickens, thereby enhancing their ability to cope with toxin-induced stress (Bui et al., 2025; Ashry et al., 2022; Hernández-Ramírez, Merino-Guzmán, Téllez-Isaías, Vázquez-Durán & Méndez-Albores, 2021). However, the extent of these protective effects may vary depending on the yeast inclusion level, toxin concentration, and duration of exposure.

Despite the growing awareness of the risks posed by naturally occurring aflatoxins, few studies have addressed their effects on hematological and biochemical parameters under conditions of natural contamination or evaluated biological strategies to mitigate their impacts. In commercial poultry production systems, birds are more frequently exposed to low and chronic concentrations of mycotoxins rather than the high doses typically employed in experimental studies. Accordingly, this study emphasizes exposure levels of naturally occurring aflatoxins that are more representative of field conditions and aligns with the goal of evaluating practically applicable mitigation strategies. Therefore, the present study was designed to investigate the ameliorative effects of dietary supplementation with two inclusion levels of *S. cerevisiae* in broiler chickens fed naturally contaminated diets with aflatoxins, with a focus on hematological and biochemical parameters across different growth phases, and to determine the optimal inclusion level for effective mitigation of their effects.

MATERIALS AND METHODS

Animal care

The study was conducted in accordance with the guidelines of the Ethics Committee of the Department of Animal Production, Faculty of Agricultural Engineering, and approved by the Latakia University Council, Lattakia, Syria (Decision No. 3917, Session No. 23).

Experimental design

A total of 200 one-day-old unsexed Ross-308 broiler chicks were obtained from a private hatchery. Each chick was individually weighed to record the initial body weight, then randomly assigned to four groups (50 birds per group), with five replicates per group (10 birds per replicate). The experiment was conducted over 42 days (March 28 - May 8, 2024) at a private farm in Tartous Governorate, Syria.

Management and care

Prior to the experiment, the care barn was prepared following strict biosecurity measures, which were enforced throughout the study period. The rearing system was semi-open, with a 5 cm thick wood shavings bedding at a stocking density of 10 birds/m².

Brooding temperature was maintained at 33–35 °C during the first two days, and then gradually de-creased by 3 °C per week until the end of the experiment, using thermostatically controlled heaters. Lighting, was continuous for 24 hours during the first week, and subsequently reduced to 22h/day until day 42.

Preparation of aflatoxin-contaminated feed mixtures

A fungal isolate of *A. flavus* was obtained from poultry feed at the Plant Protection Department laboratories, Faculty of Agricultural Engineering, University of Latakia, Syria. Commercial broiler feed, confirmed to be free of fungi and aflatoxins, was used as the basal substrate. Fungal isolation was performed on Potato Dextrose Agar (PDA; Babio®, Jinan, Shandong, China) using standard serial dilution and culture methods (Pitt & Hocking, 2009). The absence of aflatoxins was verified using high-performance liquid chromatography with fluorescence detection (HPLC-FLD; Shimadzu®, LC-20AT, Kyoto, Japan). The mobile phase consisted of distilled water, methanol, and acetonitrile (60:20:20, v/v) at a flow rate of 1 ml/min, with a MACHEREY-NAGEL® C18 chromatography column (Düren, Germany) maintained at 40 °C. Detector settings were excitation wavelength 365 nm and emission wavelength 435 nm. Aflatoxin quantification was performed using NKBIO® AFT-D205F1 kits, following standard protocols (Ahmed & Elbashir, 2023).

Subsequently, 10 ml of fungal spore suspension containing 10^6 spores/ml was added to a portion of the feed mixture, which was incubated for two weeks at 28–30 °C. Aflatoxin concentrations in the contaminated feed were determined according to standard protocols (Ahmed & Elbashir, 2023), with a total level of 32 ppb detected. The contaminated feed mixture was then stored under refrigeration (Haier®, HRF-718DW, Qingdao, Shandong, China) until use.

Feeding system

A balanced diet was provided to the experimental bird groups in two phases (starter and finisher) to fulfill their nutritional requirements, in accordance with the recommenddations of the National Research Council (NRC, 1994), as presented in Table 1. The birds were randomly assigned to four experimental groups as follows: T1 (negative control), fed a diet free of contamination and additives; T2 (positive control), fed a diet contaminated with aflatoxins by incorporating the naturally contaminated feed mixture produced by *A. flavus* into the basal diet; and T3 and T4, fed diets contaminated with aflatoxin and supplemented with *S. cerevisiae* yeast at levels of 0.1% and 0.2%, respectively.

Sterile tubes containing the anticoagulant ethylenediaminetetraacetic acid (EDTA) (ZL®, EDTA, Jinan, Zhengzhou, China), used to prepare thin blood smears for microscopic examination to determine red and white blood cell counts and the percentages of lymphocytes, heterophils, monocytes, eosinophils, and basophils, as well as the heterophil-to-lymphocyte (H/L) ratio. These samples were also used to measure hemoglobin concentration and packed cell volume.

Table 1.
Feed ingredients used, and chemical composition

Ingredients	**Starter (1-21 days) %**	**Finisher (22-42 days) %**
Yellow corn	55	59
Soybean meal	39.2	34.68
Soy oil	2	2.5
Dicalcium phosphate	2.15	2.1
Calcium carbonate (limestone)	0.86	0.87
Free methionine	0.18	0.15
Iodized table salt	0.4	0.4
Choline chloride	0.1	0.1
Vitamin Mix*	0.1	0.1
Mineral Mix**	0.1	0.1
Total	100	100
Chemical composition		
Crude protein	22%	18%
Metabolizable energy	2850 Kcal/kg	2950 Kcal/kg

**The vitamin mix included per 1 kg of ready-made feed: 13,000 IU vitamin A, 5000 IU vitamin D3, 80 mg vitamin E, 4 mg vitamin K3, 6 mg vitamin B1, 8 mg vitamin B2, 4 mg vitamin B6, 0.02 mg vitamin B12, 0.12 mg biotin, 2 mg folic acid, 85 mg nicotamide, 22 mg pantothenic acid*

***The mineral mix included per 1 kg of ready-made feed: 120 mg manganese, 100 mg zinc, 40 mg iron, 20 mg copper, 1 mg iodine, 0.3 mg selenium*

Table 2.
Immunization program

Day	Vaccine	Route
7	Newcastle disease (Clone 30)[1]	Drinking water
	Infectious bronchitis (H 120)[2]	Drinking water
14	Gamboro (Gamboro. TM)[3]	Drinking water
21	Newcastle disease (Clone 30)	Drinking water
32	Newcastle disease (Clone 30)	Drinking water

[1]KBNP, Himmvac®, Dalguban N Plus, Ansan, Gyeonggi-do, South Korea, [2]KBNP, Himmvac®, Dalguban B+Q Live Vaccine, Ansan, Gyeonggi-do, South Korea, [3]KBNP, Himmvac®, Dalguban IBD Live Vaccine, Ansan, Gyeonggi-do, South Korea

Sterile tubes without anticoagulant (EDTA-free), used for serum biochemical analyses (total protein, albumin, globulin, total cholesterol, and glucose). Tubes were placed at an angle to facilitate serum separation.

Tubes were transported to the laboratory in an ice-cooled container (Remi Elektrotechnik Ltd®, Refrigerated Tube Holders, Mumbai, Maharashtra, India). Samples designated for serum biochemical analyses were centrifuged (Remi Elektrotechnik Ltd®, R-8C, Mumbai, Maharashtra, India) at 3500 rpm for 5 minutes to obtain clear serum. The serum was aliquoted into Eppendorf tubes (Eduscope®, Eppendorf, Delhi, India), appropriately labeled, and stored in a freezer (Remi Elektrotechnik Ltd®, RLR Series, Mumbai, Maharashtra, India) at −15 to −20 °C until hematological and biochemical analyses were performed.

Red and white blood cell count

Red blood cell (RBC) and white blood cell (WBC) counts were performed using a modified Neubauer hemocytometer (Microsil®, Improved Neubauer Hemocytometer, Ambala, Haryana, India). A small volume of blood was mixed with Turk's solution (Himedia®, Turk's Solution, Mumbai, Maharashtra, India) as the diluent, and the mixture was gently agitated horizontally for a few minutes. Twenty microliters of the diluted blood sample were then loaded into a clean, dry counting chamber of the hemocytometer. Care was taken to ensure that the chamber space between the ruled area and the cover glass was completely filled without overflow.

The filled chamber was left undisturbed for 3 minutes to allow the cells to settle. Using a 40× light microscope (Olympus®, SZX16, Tokyo, Kanagawa, Japan), total RBCs and WBCs were manually counted in the Neubauer hemocytometer grid (four corner squares and the central square within the central counting area). The RBC count was calculated by multiplying the total number by 1000, while the WBC count was determined using the following equation (Onyishi, Oguine, Nwani, Aguzie, & Nwani, 2017): Total white blood cell count = (white blood cell count X 200) / 4.

Differential white blood cell count

Differential white blood cell count (DWBC) was performed by preparing blood smears from anticoagulated samples using the slide-to-slide technique. Smears were fixed with methyl alcohol (Himedia®, Methanol, Mumbai, Maharashtra, India), stained with Giemsa stain (Himedia®, Giemsa Stain [Modified], Product Code: S013, Mumbai, Maharashtra, India), rinsed, and air-dried. A drop of cedar oil (Fusion Biotech®, Microscope Immersion Oil, Delhi, India) was applied to the lower third of the blood film, which was then examined under a microscope. Initial observation was made at 10× magnification, followed by differential counting at 40× magnification using the zigzag method. Data were recorded manually, and the absolute number of each leukocyte type was calculated according to Onyishi et al. (2017) as follows:

Absolute count % = Percentage of white blood cells counted x Total white blood cells / 100

Packed cell volume (PCV)

PCV was determined by filling anticoagulated blood samples into heparinized capillary tubes (Dr. Morepen®, Hemoglobin Capillary Tubes [Microhematocrit Tubes – Heparinized/Non-Heparinized], New Delhi, Delhi, India) up to three-quarters of their volume and sealing one end with a sealant. The sealed tubes were centrifuged using a microhematocrit centrifuge (REMI®, RM-12C Plus, Mumbai, Maharashtra, India) at 12,000 rpm for 5 minutes. The sedimented blood cells were then read as percentages using a microhematocrit reader (Borosil®, Micro Hematocrit Capillary Tube Reader, Mumbai, Maharashtra, India) (Onyishi et al., 2017).

Hemoglobin (Hb) concentration

Hemoglobin concentration was measured using a commercial kit (BioSystems®, HEMOGLOBIN A1C-DIRECT [HbA1C-DIR], Barcelona, Catalonia, Spain). Twenty microliters of blood were added to 4 ml of the kit solution and allowed to stand for 5 minutes to reach room temperature, lyse red blood cells, and release hemoglobin. Then, 3.5 ml of the prepared solution was transferred to a spectrophotometer (Shimadzu®, UV-1900i, Kyoto, Japan) for absorbance measurement at 540 nm (Onyishi et al., 2017).

Total protein content

Total protein was measured using the enzymatic Biuret method with a commercial kit (BioSystems®, PROTEIN [TOTAL], Barcelona, Catalonia, Spain). Absorbance readings were carried out using a spectrophotometer at 545 nm (Zaia, Marques, & Zaia, 2023).

Albumin content

Albumin concentration was determined by the bromocresol green colorimetric assay using a commercial kit (BioSystems®, ALBUMIN, Barcelona, Catalonia, Spain). Absorbance was measured at 630 nm (Schmidt, Paulillo, Locatelli-Dittrich, Beltrame, & de Oliveira, 2013).

Globulin content

Globulin concentration was calculated by subtracting the albumin concentration from the total protein concentration (Burtis, Ashwood, Bruns, & Tietz, 2012).

Total cholesterol content

Total cholesterol was measured using an enzymatic method with a commercial kit (BioSystems®, CHOLESTEROL, Barcelona, Catalonia, Spain), and absorbance was recorded at 500 nm (Torres-Gámiz, Rodríguez, Páez-Hernández, & Galán-Vidal, 2023).

Glucose content

Glucose concentration was determined using an enzymatic method with a commercial kit (BioSystems®, GLUCOSE, Barcelona, Catalonia, Spain), and absorbance was measured at 505 nm (Torres-Gámiz et al., 2023).

Statistical analysis

Data were analyzed using SPSS v25 (IBM Corporation, IBM SPSS Statistics, Armonk, New York, USA). One-way ANOVA was performed across the fully randomized groups, followed by Duncan's multiple range test for pairwise comparisons (George & Mallery, 2018). The percentage change between two groups was calculated using the following formula:

Percentage Change % = (Base Value – Secondary Value / Absolute Value of Base Value) X 100

RESULTS AND DISCUSSION

Hematological indicators

The results presented in Table 3 indicate significant alterations in the blood parameters of birds in the positive control group (T2) fed a diet naturally contaminated with aflatoxins compared to the negative control group (T1). On days 21 and 42, significant decreases ($p \leq 0.01$) were observed in RBC count (24%, 27%), hemoglobin concentration (7%, 6%), PCV (5%, 4%), WBC count (13%, 9%), and lymphocytes (13%, 10%), respectively. In contrast, significant increases ($p \leq 0.01$) were noted in the percentages of heterophils (18%, 16%), monocytes (35%, 26%), eosinophils (38%, 16%), basophils (57%, 66%), and the heterophil-to-lymphocyte (H/L) ratio (36%, 31%) on days 21 and 42, respectively.

Birds in group T3, which received aflatoxin-contaminated feed, supplemented with 0.1% *S. cerevisiae*, exhibited partial improvement in hematological parameters. RBC count increased by 14% and 9%, hemoglobin by 3% and 2%, PCV by 2% at both time points, WBC counts by 6% and 4%, and lymphocytes by 6% and 4% on days 21 and 42, respectively. The percentages of heterophils (6%, 5%), monocytes (10%, 8%), eosinophils (11%, 5%), basophils (14%, 14%), and the H/L ratio (11%, 11%) decreased. The increases in RBC count and PCV were significant ($p \leq 0.01$) at day 42, whereas changes in hemoglobin and PCV at day 21, as well as WBC and its components, were not statistically significant ($p \geq 0.01$) compared with the T2 group.

Birds in group T4, fed aflatoxin-contaminated feed supplemented with 0.2% *S. cerevisiae*, showed more significant improvement compared to the positive control group T2. On days 21 and 42, RBC counts increased by 26% and 17%, hemoglobin by 6% and 5%, PCV by 5% and 4%, WBC counts by 12% and 9%, and

lymphocytes by 13% and 10%. Simultaneously, the percentages of heterophils (13%, 12%), monocytes (23%, 19%), eosinophils (25%, 13%), basophils (33%, 37%), and the H/L ratio (23%, 21%) decreased. Administration of aflatoxin-contaminated diets (T2) to broiler chickens resulted in marked hematological alterations relative to the negative control group (T1) (Table 3).

On days 21 and 42, red blood cell count, hemoglobin concentration, and packed cell volume decreased, indicating liver damage that reduced its ability to support erythropoiesis (Liu et al., 2020). Similarly, white blood cell count and lymphocyte percentage declined, while the proportions of heterophils, monocytes, eosinophils, basophils, and the heterophil-to-lymphocyte ratio increased, reflecting the ability of aflatoxins to suppress immunity and enhance susceptibility to secondary infections (Ochieng et al., 2021).

Moreover, the elevated H/L ratio indicated oxidative damage induced by free radical generation due to aflatoxin exposure (Jobe et al., 2023; Bin-Jumah et al., 2020). In contrast, dietary supplementation with *S. cerevisiae* partially (0.1%, T3) or significantly (0.2%, T4) restored hematological parameters toward normal values.

Table 3.
Selected hematological parameters of broiler chickens in experimental groups

Blood Indicators	Day	Treatments (Mean ± SD)			
		T1	T2	T3	T4
Red blood cells (RBC) (10^6/mm^3)	21	2.32 ± 0.078^{d}	1.77 ± 0.075^{a}	2.03 ± 0.081^{b}	2.24 ± 0.086^{c}
	42	2.77 ± 0.065^{c}	2.31 ± 0.063^{a}	2.52 ± 0.064^{b}	2.70 ± 0.066^{c}
Hemoglobin (g/dl)	21	12.81 ± 0.589^{b}	11.96 ± 0.592^{a}	12.27 ± 0.586^{ab}	12.69 ± 0.588^{b}
	42	13.70 ± 0.509^{b}	12.94 ± 0.513^{a}	13.22 ± 0.511^{ab}	13.59 ± 0.505^{b}
Packed cell volume (PCV) (%)	21	28.67 ± 0.561^{c}	27.19 ± 0.553^{a}	27.75 ± 0.560^{ab}	28.45 ± 0.573^{bc}
	42	30.87 ± 0.547^{b}	29.48 ± 0.538^{a}	29.95 ± 0.519^{a}	30.65 ± 0.562^{b}
White blood cells (WBC) (10^3/mm^3)	21	21.56 ± 0.279^{d}	18.81 ± 0.277^{a}	19.90 ± 0.279^{b}	21.10 ± 0.306^{c}
	42	26.14 ± 0.341^{c}	23.69 ± 0.334^{a}	24.63 ± 0.333^{b}	25.80 ± 0.349^{c}
Lymphocytes (%)	21	64.03 ± 2.864^{b}	55.67 ± 3.222^{a}	59.12 ± 3.112^{a}	63.05 ± 2.938^{b}
	42	66.18 ± 4.729^{b}	59.48 ± 5.255^{a}	61.98 ± 5.033^{ab}	65.47 ± 4.821^{b}
Heterophils (%)	21	27.11 ± 3.581^{a}	31.97 ± 4.132^{b}	29.91 ± 3.889^{ab}	27.68 ± 3.619^{a}
	42	23.82 ± 2.965^{a}	27.72 ± 3.249^{b}	26.25 ± 3.133^{ab}	24.29 ± 3.015^{a}
Monocytes (%)	21	4.54 ± 1.144^{a}	6.13 ± 1.300^{b}	5.49 ± 1.227^{ab}	4.75 ± 1.160^{a}
	42	4.86 ± 0.613^{a}	6.13 ± 0.685^{b}	5.66 ± 0.670^{ab}	4.97 ± 0.634^{a}
Eosinophils (%)	21	2.98 ± 0.663^{a}	4.12 ± 0.788^{b}	3.67 ± 0.742^{ab}	3.11 ± 0.699^{a}
	42	3.74 ± 2.426^{a}	4.35 ± 2.681^{b}	4.12 ± 2.577^{ab}	3.80 ± 2.466^{a}
Basophils (%)	21	1.34 ± 0.570^{a}	2.11 ± 0.671^{b}	1.81 ± 0.614^{ab}	1.41 ± 0.591^{a}
	42	1.40 ± 0.460^{a}	2.32 ± 0.514^{b}	1.99 ± 0.494^{ab}	1.47 ± 0.472^{a}
H/L ratio (%)	21	0.42 ± 0.055^{a}	0.57 ± 0.077^{b}	0.51 ± 0.068^{ab}	0.44 ± 0.061^{a}
	42	0.36 ± 0.035^{a}	0.47 ± 0.044^{b}	0.42 ± 0.038^{b}	0.37 ± 0.036^{a}

$^{a-d}$Means in a row marked different letters indicate significant differences at the 1% level. T1: Negative control (free of contamination, additives), T2: Positive control (contaminated with 32ppb aflatoxins), T3: T2 + 0.1% S. cerevisiae yeast, T4: T2 + 0.2% S. cerevisiae yeast*

Birds in the T4 group showed no-table increases in red blood cell count, hemoglobin concentration, and lymphocyte percentage, along with a reduced heterophil-to-lymphocyte ratio compared with the positive control group (T2).

These improvements are attributed to bioactive components derived from yeast, such as β-glucans, which enhance immune modulation, and trace minerals (e.g., selenium and iron), which support erythropoiesis and antioxidant enzyme activity (Fathima, Shanmugasundaram & Sifri, 2023; Trivedi & Barve, 2021; Ghazalah, Abdel-Hamid, Abdelaleem & ELnaggar, 2020; Onofre, Bertoldo, Abatti & Refosco, 2017).

The present study corroborates multiple studies reporting a general improvement in hematological and immune parameters after yeast supplementation of aflatoxin-contaminated diets. However, it differs from previous studies in terms of the natural aflatoxin concentration, the evaluation periods (21 and 42 days), and the two different yeast inclusion levels (0.1% and 0.2%). One study reported increases in red blood cell count, hemoglobin concentration, white blood cell count, and lymphocyte percentage, accompanied by decreases in heterophils, monocytes, and eosinophils in broiler chickens fed a diet contaminated with aflatoxin at 250 ppb and supplemented with yeast cell wall on day 35 of the trial (Ashry et al., 2022). Similarly, supplementation with *S. cerevisiae* at 1010 cells/kg of feed to diets contaminated with naturally occurring aflatoxins (20–60 ppb) improved hematological indices, as indicated by elevated lymphocyte and basophil percentages and neutrophil reduction (Bui et al., 2025). Another study also demonstrated comparable improvements in red blood cell count, hemoglobin concentration, and packed cell volume, while white blood cell counts decreased in broiler chickens fed naturally contaminated diets with aflatoxin (53.27 ppb) supplemented with 2 g/kg of yeast (Ejiofor et al., 2021).

Biochemical indicators

The results presented in Table 4 demonstrate significant alterations in the biochemical profile of broiler chickens fed aflatoxin-contaminated diets. Birds in the positive control group (T2) compared to the negative control group (T1) showed a significant decrease ($p \leq 0.01$) in total protein concentration (33%, 17%), albumin concentration (22%, 10%), and globulin concentration (44%, 28%), while a significant increase ($p \leq 0.01$) was observed in glucose concentration (6%, 5%) and total cholesterol concentration (9%, 8%) on days 21 and 42, respectively.

Conversely, birds in group T3, which received aflatoxin-contaminated feed supplemented with 0.1% yeast, exhibited partial improvement in serum biochemical indicators. Total protein increased by 17% and 7%, albumin by 11% and 3%, and globulin by 22% and 14% on days 21 and 42, respectively. The increases were significant ($p \leq 0.01$) for total protein, while changes in albumin and globulin on day 21 were not significant ($p \geq 0.01$). Glucose concentration decreased by 2% at both time points, and total cholesterol decreased by 3% at both time points. The decrease was significant ($p \leq 0.01$) for glucose on day 42 and for total cholesterol on day 21, while it was not significant ($p \geq 0.01$) for glucose on day 21 and total cholesterol on day 42, compared with the positive control group T2.

Birds in group T4, fed aflatoxin-contaminated feed supplemented with 0.2% yeast, showed greater improvement. Total protein increased by 33% and 13%, albumin by 20% and 7%, and globulin by 47% and 26% on days 21 and 42, respectively, and these increases were significant ($p \leq 0.01$). Glucose concentration decreased by 4% and 3%, and total cholesterol decreased by 6% and 5% on days 21 and 42, respectively, with the decreases being significant ($p \leq 0.01$) compared with the positive control group T2.

Serum biochemical profiles were also negatively affected by aflatoxin exposure (T2), as evidenced by decreases in total protein, albumin, and globulin, along with increases in glucose and total cholesterol concentrations (Table 4).

These alterations reflect impaired hepatic protein synthesis and disruptions in carbohydrate and lipid metabolism. Aflatoxins are metabolized by hepatic cytochrome P450 enzymes into reactive epoxide derivatives, which bind to macromolecules such as nucleic acids, proteins, and phospholipids, while also promoting oxidative stress through the sequential activation of free radical species, thereby compromising metabolic and structural integrity (Jobe et al., 2023; Damiano et al., 2022; Wang, Cheng & Yu, 2021).

Table 4.
Selected biochemical indicators of broiler chicken serum in the experimental groups

Serum biochemical indicators	Day	Treatments (Mean ± SD)			
		T1	**T2**	**T3**	**T4**
Total protein (g/dl)	21	2.80 ± 0.090^{d}	1.87 ± 0.072^{a}	2.18 ± 0.078^{b}	2.49 ± 0.084^{c}
	42	4.95 ± 0.025^{d}	4.13 ± 0.031^{a}	4.40 ± 0.028^{b}	4.67 ± 0.026^{c}
Albumin (g/dl)	21	1.54 ± 0.174^{b}	1.20 ± 0.177^{a}	1.33 ± 0.154^{ab}	1.45 ± 0.172^{b}
	42	3.14 ± 0.035^{d}	2.83 ± 0.039^{a}	2.92 ± 0.038^{b}	3.03 ± 0.044^{c}
Globulin (g/dl)	21	1.34 ± 0.164^{c}	0.75 ± 0.163^{a}	0.91 ± 0.138^{ab}	1.10 ± 0.139^{b}
	42	1.81 ± 0.044^{d}	1.30 ± 0.039^{a}	1.48 ± 0.033^{b}	1.64 ± 0.041^{c}
Glucose (mg/dl)	21	273.04 ± 8.736^{a}	290.11 ± 8.387^{c}	284.42 ± 8.498^{bc}	278.73 ± 8.611^{ab}
	42	222.28 ± 2.635^{a}	233.97 ± 2.923^{d}	230.09 ± 2.804^{c}	226.22 ± 2.691^{b}
Total cholesterol (mg/dl)	21	223.12 ± 3.646^{a}	243.20 ± 3.912^{d}	236.51 ± 3.819^{c}	229.82 ± 3.731^{b}
	42	153.50 ± 3.775^{a}	166.50 ± 4.169^{c}	162.15 ± 4.021^{bc}	157.81 ± 3.874^{ab}

$^{a-d}$Means in a row marked with different letters indicate significant differences at the 1% level. T1: Negative control (free of contamination, additives), T2: Positive control (contaminated with 32 ppb aflatoxins), T3: T2 + 0.1% S. cerevisiae yeast, T4: T2 + 0.2% S. cerevisiae yeast*

In contrast, supplementation with *S. cerevisiae*, particularly at 0.2% led to marked improvements in serum biochemical parameters. The increases in total protein, albumin, and globulin concentrations indicate enhanced liver function, while the reductions in glucose and cholesterol levels suggest improved metabolic regulation. These effects may be attributed to the antioxidant and immunomodulatory properties of yeast, including the ability of β-glucans to reduce oxidative stress and blood cholesterol, and the role of yeast-derived inositol phospholipids in regulating glucose metabolism (Fathima et al., 2023; Sudiana, Kuswendi, Dewi & Balia, 2021; Gonzalez-Uarquin, Rodehutscord & Huber, 2020).

The findings of this study are consistent with several reports that demonstrated increases in total protein, albumin, and globulin concentrations (Ashry et al., 2022; Hernández-Ramírez et al., 2021; Ejiofor et al., 2021).

However, some studies observed no significant effects on certain indicators such as total cholesterol and glucose in 21-day-old broiler chickens fed a diet contaminated with 500 ng/g of aflatoxin and supplemented with 0.05% yeast (Hernández-Ramírez et al., 2021), whereas a significant reduction in total cholesterol was reported elsewhere (Ejiofor et al., 2021). These outcomes differ from the present study, which also varied from previous reports in terms of natural aflatoxin concentrations, yeast inclusion levels, and the two evaluation periods.

CONCLUSIONS

This study demonstrated that feeding broiler chickens naturally aflatoxin-contaminated diets (32 ppb) adversely affected hematological and biochemical parameters, indicating compromised health and physiological status. Supplementation with *Saccharomyces cerevisiae*, particularly at 0.2%, effectively mitigated these negative effects, restoring hematological and biochemical profiles toward normal values. These findings highlight the potential of yeast supplementation as a practical, safe, and cost-effective strategy to reduce the risk of aflatoxin toxicity in poultry production.

Despite its relevance, this study has certain limitations. The trial was conducted under controlled farm conditions with a fixed contamination level (32 ppb), which may not fully reflect the variability of aflatoxin exposure in commercial settings. Additionally, only two yeast inclusion levels were tested, and other potential dosages or yeast-derived products (e.g., cell wall fractions, β-glucans) were not evaluated. Furthermore, the study focused solely on hematological and biochemical indices, while other trait changes were not assessed.

Future studies should investigate a broader range of yeast inclusion levels, explore different yeast-based products, and assess long-

term impacts under diverse environmental and management conditions. Analyses of additional traits could also provide deeper insights into the mechanisms by which yeast mitigates aflatoxin toxicity.

AUTHOR CONTRIBUTIONS

Conceptualization, A.M.M.; Methodology, A.M.M., M.S.T. and Q.A.A.; Investigation, formal analysis, validation, M.S.T. and Q.A.A.; writing-original draft preparation, A.M.M.; Writing-review and editing, A.M.M., Q.A.A. and M.S.T.

DATA AVAILABILITY STATEMENT

Data contained within the article.

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CONFLICT OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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UTICAJ *SACCHAROMYCES CEREVISIAE* (H.) NA POBOLJŠANJE HEMATOLOŠKIH I BIOHEMIJSKIH PARAMETARA KOD BROJLERA HRANJENIH HRANOM PRIRODNO KONTAMINIRANOM AFLATOKSINOM

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Sažetak: Ova studija je ispitivala efekte suplementacije dve koncentracije kvasca *Saccharomyces cerevisiae* (H.) u ishrani brojlera prirodno kontaminiranoj aflatoksinima (32 ppb), proizvedenim od strane *Aspergillus flavus* L. na hematološke i biohemijske parameter brojlera. Ukupno 200 jednodnevnih pilića Ross 308 nasumično je raspoređeno u četiri grupe, svaka sa pet replika od po 10 jedinki. Negativna kontrolna grupa (T1) je dobijala nekontaminiranu ishranu, dok je pozitivna kontrola (T2) hranjena hranom kontaminiranom aflatoksinima bez dodataka. Grupe T3 i T4 dobijale su kontaminiranu hranu suplementovanu kvascem *S. cerevisiae* u dva nivoa dodatka (0,1% i 0,2%). Hranjenje brojlera prirodno kontaminiranom ishranom (32 ppb) negativno je uticalo na hematološke i biohemijske parametre ($p \leq 0,01$), ukazujući na narušeno zdravstveno i fiziološko stanje životinja. Suplementacija ishrane, naročito na nivou od 0,2%, značajno je poboljšala hematološke i biohemijske profile u poređenju s pozitivnom kontrolom ($p \leq 0,01$). Poboljšanja su zabeležena u broju eritrocita (26%, 17%), broju leukocita (12%, 9%), koncentracijama hemoglobina (6%, 5%), ukupnom sadržaju proteina (33%, 13%), albumina (20%, 7%) i globulina (47%, 26%), uz smanjenje odnosa heterofili/limfociti (23%, 21%), koncentracije glukoze (4%, 3%) i ukupnog holesterola (6%, 5%) u 21om i 42om danu ogleda. Rezultati pokazuju da suplementacija ishrane sa 0,2% *S. cerevisiae* efikasno ublažava nepovoljne efekte prirodne aflatoksinske kontaminacije na hematološke i biohemijske parametre kod brojlera.

Ključne reči: *krvne ćelije, prirodni aflatoksini, Ross 308 brojler, ukupni holesterol, kvasac*

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