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ROLE OF *Saccharomyces cerevisiae* H. IN ENHANCING GROWTH AND LIVER-KIDNEY HEALTH IN BROILERS FED NATURAL AFLATOXIN-CONTAMINATED DIET

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ABSTRACT

Sustainable broiler growth and health require the use of natural and biologically safe strategies. This study investigated the effects of incorporating two levels of *Saccharomyces cerevisiae* H. yeast into a diet naturally contaminated with aflatoxins on growth performance and hepatic and renal health indicators in broilers. A total of 200 one-day-old, unsexed Ross 308 chicks were randomly allocated to four treatment groups, with five replicates per group and 10 birds per replicate. Group T1 (negative control) received an uncontaminated basal diet. Group T2 (positive control) was fed a diet naturally contaminated with aflatoxins produced by *Aspergillus flavus* L. Groups T3 and T4 received the contaminated diet supplemented with 0.1 and 0.2% *S. cerevisiae*, respectively. Compared to T2, T4 exhibited significantly improved performance, with increases in body weight (8%, 5%), weight gain (9%, 4%), and feed intake (5%, 2%), as well as reductions in feed conversion ratio (3%, 2%) at the end of phases one and two, respectively. Hepatic function markers improved, with decreased AST (22%, 16%) and ALT (12%, 3%) levels on days 21 and 42, respectively. Renal function was also enhanced, as indicated by reduced serum creatinine (13%, 10%) and uric acid (9%, 5%) levels. Additionally, relative liver and kidney weights were reduced by 13 and 11%, respectively, in T4 by day 42. These findings suggest that 0.2% yeast supplementation is more effective than 0.1% in mitigating the adverse effects of natural aflatoxins in broilers.

Keywords: Biologically, hepatic function, performance, relative weights, renal function, weight gain, yeast.

INTRODUCTION

Aflatoxins are associated with both productive and economic consequences. Their presence in poultry feed and feed ingredients negatively affects production parameters, including feed intake, feed conversion ratio, weight gain, disease incidence, and overall bird performance, while also posing a risk of introducing these toxins into the food chain (Mishra and Swain, 2022). Numerous studies have documented the detrimental effects of aflatoxins on the performance of broiler chickens, with even low levels being associated with reduced body weight, decreased weight gain, and increased feed conversion ratio (Fochesato et al., 2023; Salako et al., 2022; Saminathan et al., 2018). The liver and kidneys appear to be particularly sensitive, as evidenced by elevated serum enzyme activities and increased concentrations

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of creatinine and uric acid, along with higher relative organ weights under varying levels of aflatoxin exposure–reflecting tissue damage and impaired physiological functions (Oloruntola et al., 2024; Ali and Mousa, 2023; Qing et al., 2022; Arif et al., 2020; Li et al., 2018; Barati et al., 2018; Prakoso et al., 2018). However, findings across studies have been inconsistent. Some reports indicate no significant effects on growth performance when birds are exposed to low concentrations of aflatoxins or when exposure duration is short (Cuccato et al., 2025; Paneru et al., 2024). Other investigations have indicated that the severity of the effects depends on the contamination level and exposure period, with adverse impacts emerging once certain thresholds are exceeded, influencing growth, feed efficiency, liver function, and antioxidant status (Bui et al., 2025; Zou et al., 2023). Overall, these findings reflect variability linked to contamination levels and experimental conditions, underscoring the need for comparative studies to better define tolerance limits and elucidate underlying mechanisms of toxicity.

Biological detoxification methods are considered safe, as they preserve both the nutritional value and sensory characteristics of feed (Liu et al., 2022). Baker's yeast (*Saccharomyces cerevisiae* H.) has been demonstrated to be an effective mycotoxin adsorbent, capable of reducing toxin absorption from the gastrointestinal tract during digestion (Borovikova et al., 2016; Pereyra et al., 2018). Several studies have highlighted the positive role of yeast in improving growth performance and the health of vital organs in broiler chickens exposed to aflatoxins. The inclusion of *S. cerevisiae* cell walls has been shown to enhance body weight gain and feed intake while reducing feed conversion ratio and serum concentrations of creatinine and urea (Ashry et al., 2022; Hernández-Ramírez et al., 2021). Similarly, supplementation with different doses demonstrated effectiveness in lowering the relative weights of the liver and kidneys and improving growth indices compared with control groups (Arif et al., 2020; Śliżewska et al., 2019). Yeast supplementation has also been reported to decrease serum levels of hepatic enzymes AST and ALT in birds fed contaminated diets (Ejiofor et al., 2021). However, some lower doses (1 g kg^{-1}) failed to produce the desired mitigating effects on growth performance under aflatoxin challenge (Bui et al., 2025). These findings indicate that the efficacy of yeast depends on both the level of contamination and the inclusion rate, underscoring the need for further studies to establish optimal application thresholds.

Most previous studies have focused on the role of yeast in mitigating the adverse effects of high concentrations of purified aflatoxins, whereas limited research has addressed its effectiveness against natural aflatoxins originating from mold-contaminated feed. A study conducted by Jeff-Agboola (2014) demonstrated that diets contaminated with fungal strains (*Aspergillus flavus* L. and *A. parasiticus* S.), producing aflatoxins at concentrations of 0.5 ppb and 1 ppb, resulted in a greater reduction in average body weight in broiler chickens compared to diets containing equivalent concentrations of purified aflatoxins. The significance of this study lies in emphasizing the adverse effects of low concentrations of aflatoxins produced by *A. flavus*-contaminated feed, and in exploring the role of yeast as a safe and cost-effective biological strategy to mitigate aflatoxin-induced damage in broilers–thus contributing to sustainable poultry production. Accordingly, this study aimed to evaluate the effects of supplementing two levels of *S. cerevisiae* yeast in a diet naturally contaminated with aflatoxins on growth performance and pathological indicators of liver and kidney function throughout the broiler growth phases, and to determine the optimal yeast inclusion level.

MATERIALS AND METHODS

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 initial body weight and then randomly allocated to four experimental groups (50 birds per group), with five replicates per group and 10 birds per replicate. The experiment was conducted over a period of 42 d during the spring season of 2024 at a private poultry farm located in Tartous Governorate, Syria.

Management and care

The facility was prepared in advance following standard biosecurity protocols, which were strictly maintained throughout the experimental period. Birds were reared in a semi-open housing system on wood shavings bedding with a thickness of 5 cm, at a stocking density of 10 birds m^{-2}. The temperature in the brooding area was maintained at 33–35°C during the first two days and then gradually reduced by 3 °C per week until the end of the trial, with thermostats used for temperature regulation. Lighting was provided continuously (24 h d^{-1}) during the first week, followed by 22 h of light per day until the conclusion of the experiment at 42 d of age.

Preparation of aflatoxin-contaminated feed mixtures

A fungal isolate of *A. flavus* was obtained from poultry feed at the laboratories of the Plant Protection Department, Faculty of Agricultural Engineering, University of Tishreen. The feed mixture used for inoculation was procured from a private supplier and tested to ensure it was free from fungal contamination. Fungal isolation was performed on Potato Dextrose Agar (PDA) medium (Babio®, Potato Dextrose Agar Medium (USP), Jinan, Shandong, China), using serial dilution techniques in accordance with the standard method described by Pitt and Hocking (2009).

To verify the absence of aflatoxins, high-performance liquid chromatography (HPLC) (Shimadzu®, LC-20AT, Kyoto, Japan) equipped with a fluorescence detector (FLD) was used. The mobile phase consisted of distilled water, methanol, and acetonitrile (60:20:20, v/v/v), with a flow rate of 1 mL min^{-1}. A C18 chromatography column (MACHEREY-NAGEL (MN)®, Düren, Nordrhein-Westfalen, Germany) was maintained at 40 °C. The FLD was set to an excitation wavelength of 365 nm and an emission wavelength of 435 nm.

Aflatoxins were quantified using commercial analysis kits (NKBIO®, AFT-D205F1, Jinan, Shandong, China) according to standardized procedures (Ahmed and Elbashir, 2023). Subsequently, 10 mL of a fungal spore suspension (10^6 spores mL^{-1}) was added to a portion of previously uncontaminated feed, which was then incubated at 28–30 °C for two weeks to induce aflatoxin production. Following incubation, samples from this portion were collected to determine the final aflatoxin concentration, which was measured at 32 ppb. The aflatoxin-contaminated feed was stored under refrigeration (Haier®, Hrf-718DW, Qingdao, Shandong, China) to maintain the aflatoxin levels and was incorporated daily into the diets of the respective bird groups throughout the experimental period to ensure a consistent exposure level.

Feeding system

Balanced diets were formulated and provided in two phases–starter and finisher–according to the nutritional requirements outlined by 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 basal diet free from aflatoxins and additives.

T2 (Positive Control): Fed a diet contaminated with aflatoxins (32 ppb) by incorporating a naturally contaminated feed mixture produced by *A. flavus* into the basal diet.

T3 and T4: Fed aflatoxin-contaminated diets supplemented with *Saccharomyces cerevisiae* yeast at inclusion levels of 0.1 and 0.2%, respectively.

Table 1. Composition of the experimental diets and their calculated chemical analysis (% as-fed basis).

Ingredient	Starter (1-21 days), %	Finisher (22-42 days), %
Yellow corn	55.0	59.0
Soybean meal	39.2	34.68
Soy oil	2.0	2.5
Dicalcium phosphate	2.15	2.1
Calcium carbonate (limestone)	0.86	0.87
DL-Methionine (free methionine)	0.18	0.15
Iodized table salt	0.4	0.4
Choline chloride	0.1	0.1
Vitamin Mix1	0.1	0.1
Mineral Mix2	0.1	0.1
Total	100	100
Calculated chemical composition		
Crude protein (%)	22.0	18.0
Metabolizable energy (Kcal kg^{-1})	2,850	2,950

[1] The vitamin premix provided per kilogram of finished feed contained: 13,000 IU vitamin A, 5,000 IU vitamin D_3, 80 mg vitamin E, 4 mg vitamin K_3, 6 mg vitamin B_1, 8 mg vitamin B_2, 4 mg vitamin B_6, 0.02 mg vitamin B_{12}, 0.12 mg biotin, 2 mg folic acid, 85 mg niacin (nicotinamide), and 22 mg pantothenic acid.

[2] The mineral premix provided per kilogram of finished feed contained: 120 mg manganese, 100 mg zinc, 40 mg iron, 20 mg copper, 1 mg iodine, and 0.3 mg selenium.

Health and vaccination programs

Strict biosecurity measures were enforced throughout the experimental period. Access to the facility was limited to authorized farm personnel, who adhered daily to standardized biosecurity protocols. Birds were vaccinated against major poultry diseases according to the schedule outlined in Table 2.

Growth performance standards

Upon arrival at the farm, chicks in all replicate groups were individually weighed, and average body weight was then recorded weekly and on days 21 and 42 following a 3-h feed withdrawal. Weighing was conducted using a precision electronic scale (NANBEI®, JD5000-2, Henan, Zhengzhou, China) with a maximum capacity of 5,000 g. Other production parameters were calculated as follows (Arif et al., 2020):

Average weight gain during each phase (g) = Average body weight at the end of the phase (g) − Average body weight at the beginning of the phase (g)

Average feed intake per bird (g) = Total feed consumed (g) ÷ Number of birds per replicate

Feed conversion ratio (FCR) = Average feed intake per bird (g) ÷ Average weight gain (g)

Pathological biochemical indicators of liver and kidney functions

Blood sample collection

Blood samples were collected following standard poultry sampling guidelines (Morishita, 2019). On days 21 and 42, blood was drawn from the wing vein of fifteen birds per group (3 birds per replicate) using 3- or 5-mL syringes (Hi-Tech®, Uttar Pradesh, India). The collected samples were transferred into sterile, additive-free Vacutainer tubes (Ajosha®, Mumbai, Maharashtra, India), without anticoagulant ethylenediaminetetraacetic acid (EDTA) (ZL®, Jinan, Zhengzhou, China) for subsequent biochemical analyses of AST, ALT, creatinine, and uric acid. Tubes were placed in an inclined position to facilitate serum separation.

The samples were then transported in an ice-cooled container (Remi Elektrotechnik Ltd®, Mumbai, Maharashtra, India) to the laboratory. Serum was obtained by centrifuging the tubes in a refrigerated centrifuge (Remi Elektrotechnik Ltd®, R-8C, Mumbai, Maharashtra, India) at 3,500 rpm for 5 min. The clear serum was aliquoted into labeled Eppendorf tubes (Eduscope®, Delhi, India) and stored at −15 to −20 °C in a freezer (Remi Elektrotechnik Ltd®, RLR Series, Mumbai, Maharashtra, India) until biochemical assays were performed.

Laboratory diagnosis to measure biochemical indicators

Measurement of enzyme activity (AST, ALT)

Enzyme activities of AST and ALT were determined using a colorimetric assay with a commercial kit (BioSystems®, Barcelona, Catalonia, Spain). Absorbance readings were taken using a spectrophotometer (Shimadzu®, UV-1900i, Kyoto, Japan) at a wavelength of 340 nm. Enzyme concentrations were calculated following the method described by Gella et al. (1985).

Determination of creatinine and uric acid

Calibration was performed using a commercial kit (BioSystems®, Barcelona, Catalonia, Spain), and absorbance readings were taken with a spectrophotometer at a wavelength of 220–230 nm, following the method described by Khajehsharifi et al. (2013).

Relative weights of liver and kidney

At the end of the experiment (day 42), fifteen birds from each group (three birds per replicate) were dissected. The livers and kidneys were carefully removed and weighed using a sensitive electronic scale (FURI®, FRH, Fujian, China) with a capacity of up to 500 g. The relative organ weights were calculated using the following formula (Arif et al., 2020):

Table 2. Immunization program.

Day	Vaccine	Route of administration
7	Newcastle disease (Clone 30)[1]	Drinking water
	Infectious bronchitis (H120)[2]	Drinking water
14	Gumboro disease (TM strain)[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.

Relative organ weight (liver, kidney) % = (organ weight / live weight of bird) × 100

Statistical Analysis

Data were statistically analyzed using SPSS software version 25 (IBM Corp., Armonk, NY, USA). A one-way analysis of variance (ANOVA) was performed to evaluate the effects of the experimental treatments in a completely randomized design. When significant differences were detected ($p \leq 0.05$ or $p \leq 0.01$), Duncan's multiple range test was applied to separate the means and identify pairwise differences among treatment groups (George and Mallery, 2018). All data were expressed as means ± standard deviation of the mean (SEM). The percentage change between two groups was calculated using the following formula:

Percentage Change % = (Base Value - Secondary Value / Absolute Value of Base Value) × 100.

Significance levels were set at 5% for growth performance and 1% for liver and kidney function indicators and relative organ weights.

RESULTS AND DISCUSSION

Growth performance standards

The results presented in Table 3 demonstrate a significant decline in the productive performance of broilers fed a diet contaminated with natural aflatoxins (positive control group T2) compared to the negative control group (T1). Specifically, there was a significant decrease ($p \leq 0.05$) in average body weight by 13 and 9%, average weight gain by 14 and 7%, and average feed intake by 11 and 5%, alongside a significant increase ($p \leq 0.05$) in the feed conversion ratio by 4 and 2% during the starter (first) and finisher (second) phases, respectively.

Conversely, birds in group T3, which were fed an aflatoxin-contaminated diet supplemented with 0.1% *S. cerevisiae* yeast, showed improvements in production parameters. Average body weight increased by 11 and 5%, and average feed intake increased by 11 and 5%. Average weight gain improved by 3 and 2%, and the feed conversion ratio showed slight improvements of 1 and 0.5%. However, these differences were statistically significant ($p \leq 0.05$) only for body weight, weight gain, and feed intake, but not for feed conversion ratio during the starter phase, compared to group T2.

The greatest improvement was observed in group T4, which received the aflatoxin-contaminated feed supplemented with 0.2% yeast. In this group, average body weight increased by 8 and 5%, average weight gain by 9 and 4%, and average feed intake by 5% and 2%, while the feed conversion ratio decreased by 3 and 2% at the end of the first and second phases, respectively. All these changes were significant ($p \leq 0.05$) compared to the positive control group T2.

Numerous studies have shown that aflatoxins, even at low levels (0.51 – 20 ppb), lead to reduced body weight, impaired weight gain, and increased feed conversion ratio in broiler chickens (Fochesato et al., 2023; Saminathan et al., 2018). Zou et al. (2023) indicated that

Table 3. Growth performance parameters of broiler chickens across different treatment groups.

Time of Rearing	Treatments*			
	T1	T2	T3	T4
Body weight, g		Mean ± SD		
21 Day	911.15 ± 2.34d	788.67 ± 2.46a	810.46 ± 2.71b	852.21 ± 2.21c
Day 42	2771.77 ± 6.61d	2523.22 ± 6.69a	2565.26 ± 6.46b	2651.99 ± 6.45c
Body weight gain, g		Mean ± SD		
1 – 21 Day	868.60 ± 1.31d	746.19 ± 1.32a	767.96 ± 1.57b	809.70 ± 1.10c
22 – 42 Day	1860.62 ± 4.31d	1734.55 ± 4.25a	1754.79 ± 3.78b	1799.79 ± 4.28c
Feed intake, g		Mean ± SD		
1 – 21 Day	1187.85 ± 29.88d	1061.77 ± 28.53a	1080.43 ± 28.35b	1117.89 ± 28.10c
22 – 42 Day	3567.16 ± 28.58d	3402.80 ± 28.96a	3426.40 ± 28.39b	3467.50 ± 28.65c
Feed conversion ratio %		Mean ± SD		
1 – 21 Day	1.37 ± 0.03a	1.42 ± 0.04b	1.41 ± 0.03b	1.38 ± 0.03a
22 – 42 Day	1.92 ± 0.01a	1.96 ± 0.01d	1.95 ± 0.01c	1.93 ± 0.01b

[a, b, c, d]: Different letters within the same row indicate significant differences at the 5% level ($p \leq 0.05$). * T1: Negative control (free of contamination and additives); T2: Positive control (contaminated with aflatoxins); T3: T2 supplemented with 0.1% *S. cerevisiae* yeast; T4: T2 supplemented with 0.2% *S. cerevisiae* yeast.

adverse effects become evident at concentrations exceeding 45 ppb, whereas Bui et al. (2025) observed growth reduction at 60 ppb. However, other studies have reported no significant effects on growth performance in birds exposed to low concentrations of aflatoxins or to short durations (Cuccato et al., 2025; Paneru et al., 2024). These discrepancies are attributed to differences in contamination levels, exposure duration, and the age of the birds at assessment. The decline in performance in aflatoxin-contaminated groups is associated with metabolic disturbances, inhibited protein synthesis, and damage to the intestinal mucosa, leading to reduced nutrient absorption efficiency (Liu et al., 2019; Monson et al., 2015).

In contrast, yeast-supplemented groups exhibited marked improvements in body weight and feed conversion, consistent with several studies confirming the efficacy of *S. cerevisiae* or its cell walls in mitigating toxic effects when added at levels ranging from 0.5 to 3.57 g kg^{-1} in diets contaminated with 180–350 ppb aflatoxin (Ashry et al., 2022; Hernández-Ramírez et al., 2021; Arif et al., 2020; Bushwereb et al., 2019; Mendieta et al., 2018). However, low doses (1 g kg^{-1}) did not achieve the desired improvement in growth performance (Bui et al., 2025). These findings indicate that the effectiveness of yeast depends on both the inclusion level and the contamination severity, contributing to enhanced metabolism, nutrient absorption, and alleviation of oxidative stress, in addition to its ability to bind aflatoxin molecules or degrade them into less toxic metabolites (Liu et al., 2022).

Serum biochemical indicators of liver function (AST, ALT)

The results presented in Table 4 indicate a significant increase ($p \leq 0.01$) in liver enzyme levels in the serum of birds in the positive control group (T2) compared to the negative control group (T1). Specifically, the AST levels increased by 43 and 23%, and ALT levels by 22 and 5% at the end of the first and second phases, respectively. Conversely, supplementation with 0.1% yeast in group T3 led to improvements in liver enzyme activity in aflatoxin-contaminated birds, with AST levels decreasing by 9 and 7%, and ALT levels by 6 and 2%. The reduction in AST was statistically significant ($p \leq 0.01$), whereas changes in ALT were not significant ($p \geq 0.01$) compared to the positive control group. A more pronounced improvement was observed in group T4, which received 0.2% yeast supplementation; AST levels decreased by 22 and 16%, and ALT levels by 12 and 3% on days 21 and 42, respectively. These reductions were significant ($p \leq 0.01$) relative to group T2.

These findings align with previous studies reporting that supplementation with yeast at 2 g kg^{-1} of feed naturally contaminated with mold and aflatoxin at 53.27 ppb significantly reduced serum levels of liver enzymes (AST, ALT) in broiler chickens at 10 weeks of age (Ejiofor et al., 2021). Similarly, Hernández-Ramírez et al. (2021) observed decreased AST and ALT enzyme levels at day 21 in broilers fed a diet contaminated with 500 ng g^{-1} aflatoxin and supplemented with 0.05% yeast. Conversely, Fochesato et al. (2023) reported that yeast supplementation did not reduce liver enzyme levels in broilers fed a diet contaminated with 506.14 ng kg^{-1} aflatoxin at the end of the fifth week.

The elevated serum levels of liver enzymes observed in the T2 positive control group may be attributed to oxidative stress induced by aflatoxin metabolism in the liver (Jobe et al., 2023). This oxidative damage results in hepatocellular injury and necrosis, increasing the permeability of cell membranes, which consequently leads to the leakage of enzymes such as AST and ALT into the bloodstream, thereby elevating their serum concentrations (Ali and Mousa, 2023; Mekuria et al., 2023).

Conversely, the marked reduction in these enzyme levels in the treated groups (T3, T4)

Table 4. Serum levels of liver enzymes (AST and ALT) in broiler chickens across treatment groups.

Serum Biochemical Indicators	Day	Treatments (Mean ± SD)			
		T1	T2	T3	T4
AST (U/L)	21	141.11 ± 2.086a	202.36 ± 2.910d	183.31 ± 2.043c	157.23 ± 2.172b
	42	239.52 ± 1.827a	294.77 ± 2.620d	275.24 ± 2.996c	247.95 ± 3.425b
ALT (U/L)	21	15.43 ± 0.913a	18.86 ± 0.932c	17.72 ± 0.925bc	16.58 ± 0.919ab
	42	44.35 ± 1.043a	46.78 ± 1.057c	45.99 ± 1.047bc	45.21 ± 1.041ab

[a, b, c, d]: Different letters within the same row indicate significant differences at the 1% level ($p \leq 0.01$). * T1: Negative control (free of contamination and additives); T2: Positive control (contaminated with aflatoxins); T3: T2 supplemented with 0.1% *S. cerevisiae* yeast; T4: T2 supplemented with 0.2% *S. cerevisiae* yeast.

reflects hepatic protection conferred by *S. cerevisiae* through binding aflatoxin molecules and reducing their bioavailability (Istiqomah et al., 2019; Afzal et al., 2022; Zanganeh et al., 2025). The more limited changes observed in ALT compared to AST likely result from the primary mechanism being aflatoxin binding rather than direct antioxidant activity, coupled with ALT's specificity as an indicator of acute hepatic injury, whereas AST is more sensitive to chronic or extensive tissue damage. These protective effects are further supported by the high selenium content in yeast, which activates glutathione peroxidase and reduces lipid peroxidation in cell membranes, while also contributing to tissue regeneration through stimulation of thyroid hormones (Mustafa, 2015; Gul et al., 2021).

Serum biochemical indicators of kidney function (creatinine, uric acid)

The data presented in Table 5 demonstrated a significant increase ($p \leq 0.01$) in the concentrations of both creatinine and uric acid in the blood serum of birds in the positive control group (T2) compared to the negative control group (T1). Specifically, creatinine levels increased by 25 and 16%, and uric acid levels increased by 13 and 7% on days 21 and 42, respectively. Conversely, supplementation with 0.1% yeast in the T3 group, fed an aflatoxin-contaminated diet, improved these parameters, with creatinine concentrations decreasing by 6 and 5%, and uric acid concentrations decreasing by 4 and 2%. The reduction in uric acid was statistically significant ($p \leq 0.01$), whereas the decrease in creatinine was not significant ($p \geq 0.01$) compared to the positive control group (T2). Greater improvements were observed in the T4 group, which received 0.2% yeast supplementation, with creatinine concentrations decreasing by 13 and 10%, and uric acid concentrations decreasing by 9 and 5% on days 21 and 42, respectively; these decreases were significant ($p \leq 0.01$) compared to T2.

Previous studies have reported similar findings. For example, supplementation with *S. cerevisiae* cell walls in a diet contaminated with aflatoxin at 250 ppb resulted in decreased serum concentrations of creatinine and uric acid in broiler chickens at 35 d of age (Ashry et al., 2022). Likewise, a reduction in creatinine and uric acid levels was observed in broilers fed an aflatoxin-contaminated diet at 300 ppb supplemented with *S. cerevisiae* yeast (Abou-Zeid et al., 2015).

The elevated serum concentrations of creatinine and uric acid observed in birds from the positive control group (T2) may result from aflatoxin accumulation in the kidneys, leading to renal damage and increased levels of kidney function biomarkers, such as creatinine and uric acid, which indicate impaired kidney function (Li et al., 2018). Additionally, oxidative stress plays a key role in aflatoxin-induced kidney injury, as aflatoxin exposure elevates reactive oxygen species (ROS) levels, disrupting cellular redox homeostasis and causing oxidative stress-mediated renal damage (Umaya et al., 2021; Yang et al., 2023; Ofori-Attah et al., 2024).

The improvement in serum creatinine and uric acid concentrations observed in birds from groups T3 and T4 may be attributed to glycine, an amino acid present in yeast (Sun et al., 2019), which exerts antioxidant effects by reducing lipid peroxidation in cell membranes and enhancing the activity of antioxidant enzymes, including superoxide dismutase (SOD), glutathione (GSH), catalase (CAT), and glutathione S-transferase (GST) (Mabrouk and Cheikh, 2016).

Relative weight of liver and kidney

The results presented in Table 6 indicate significant changes ($p \leq 0.05$) in the average relative weights of the liver and kidneys in birds from the positive control group (T2) compared to the negative control group (T1). Specifically, the relative weights of the liver and kidney increased by 23 and 19%, respectively, in T2 birds

Table 5. Serum levels of creatinine and uric acid in experimental bird groups.

Serum Biochemical Indicators	Day	Treatments (Mean ± SD)			
		T1	T2	T3	T4
Creatinine (mg dl^{-1})	21	0.46 ± 0.049a	0.58 ± 0.042c	0.54 ± 0.040bc	0.51 ± 0.041ab
	42	0.51 ± 0.051a	0.59 ± 0.049c	0.56 ± 0.047bc	0.53 ± 0.063ab
uric acid (mg dl^{-1})	21	6.74 ± 0.047a	7.65 ± 0.040d	7.34 ± 0.044c	6.94 ± 0.054b
	42	7.43 ± 0.046a	7.94 ± 0.073d	7.77 ± 0.061c	7.51 ± 0.053b

[a, b, c, d]: Different letters within the same row indicate significant differences at the 1% level ($p \leq 0.01$). * T1: Negative control (free of contamination and additives); T2: Positive control (contaminated with aflatoxins); T3: T2 supplemented with 0.1% *S. cerevisiae* yeast; T4: T2 supplemented with 0.2% *S. cerevisiae* yeast.

Table 6. Relative weights of liver and kidney in birds from experimental groups.

Relative weights	Day	Treatments (Mean ± SD)			
		T1	T2	T3	T4
Relative weight of the liver, %	42	2.61 ± 0.030a	3.20 ± 0.029d	2.98 ± 0.025c	2.77 ± 0.031b
Relative weight of the kidney, %	42	0.77 ± 0.032a	0.92 ± 0.036d	0.88 ± 0.035c	0.82 ± 0.034b

[a, b, c, d]: Different letters within the same row indicate significant differences at the 1% level ($p \leq 0.01$). * T1: Negative control (free of contamination and additives); T2: Positive control (contaminated with aflatoxins); T3: T2 supplemented with 0.1% *S. cerevisiae* yeast; T4: T2 supplemented with 0.2% *S. cerevisiae* yeast.

compared to those in T1. Conversely, significant reductions ($p \leq 0.05$) were observed in the relative weights of these organs in broilers fed an aflatoxin-contaminated diet supplemented with 0.1% *S. cerevisiae* yeast (T3), with decreases of 7% in liver weight and 4% in kidney weight compared to T2 birds. The improvement was more pronounced in birds receiving 0.2% yeast supplementation (T4), where relative liver and kidney weights decreased by 13 and 11%, respectively, compared to the values observed in T2.

Several studies have reported similar findings. For instance, broiler feed contaminated with aflatoxin at 100 ppb and supplemented with 2.5 or 3.57 g kg^{-1} of *S. cerevisiae* yeast as a biodegrading agent resulted in improved relative weights of the liver and kidneys, with reductions observed compared to the control group (Arif et al., 2020). Additionally, the negative effects of a diet contaminated with aflatoxin at 1 ppm were mitigated by the inclusion of 0.2% of two different antitoxins, primarily derived from the cell walls of *S. cerevisiae* yeast, which reduced the relative liver weight (Oliveira et al., 2015). Similarly, another study demonstrated that supplementing a yeast probiotic to a diet containing 1 ppm aflatoxin restored the relative weights of the liver and kidneys to levels comparable to the control group, while also reducing these organ weights in broilers fed a diet contaminated with 5 ppm aflatoxin (Sli'zewska et al., 2019).

The increase in the relative liver weight observed in the positive control group (T2) may be attributed to oxidative stress. Aflatoxin is known to promote the generation of free radicals in the body, leading to oxidative stress (Jobe et al., 2023). This oxidative damage can cause lipid accumulation within the liver by impairing lipid transport, ultimately resulting in hepatomegaly and an increased relative liver weight (Shannon et al., 2017; Aikore et al., 2019). Similarly, the significant increase in kidney weight in birds from the T2 group can be explained by the nephrotoxic effects of aflatoxins and their metabolites, which affect various structures of the nephron prior to excretion in the urine. Aflatoxin-induced nephrotoxicity has been linked to thickening of the glomerular basement membrane, reduced glomerular filtration rate, decreased urine output, and induction of apoptosis, as demonstrated in animal models (Wang et al., 2022; Owumi et al., 2023).

The improvement in the relative weights of the liver and kidneys observed in groups T3 and T4 may be attributed to the antifungal properties of the yeast and its ability to bind aflatoxins (Istiqomah et al., 2019). Additionally, the high selenium content in yeast (Onofre et al., 2017) plays a crucial role in DNA repair, endocrine regulation, immune function, and other physiological processes due to its potent free radical scavenging capacity. This antioxidant activity protects cellular membranes and prevents malignant cellular transformations (Ofori-Attah et al., 2024). Consequently, these effects lead to improved biochemical serum indicators, reduced hepatic fat accumulation, and thus a decrease in relative liver weight. Similarly, enhanced kidney function results in a reduction in relative kidney weight.

CONCLUSIONS

Supplementation of the aflatoxin-contaminated diet with 0.2% *S. cerevisiae* yeast led to a more pronounced improvement in growth performance parameters, including increases in average body weight, weight gain, and feed intake, accompanied by a reduction in feed conversion ratio. Furthermore, indicators of liver and kidney health showed significant enhancement, as reflected by improved biochemical markers of hepatic and renal function. Additionally, the relative weights of the liver and kidneys exhibited greater normalization compared to the 0.1% yeast supplementation group under the experimental conditions of this study.

Conflict of interest

No conflict of interest declared.

Author contributions

All authors contributed to every stage of the research, including literature review,

methodological design, interpretation of results, and approval of the final version of the manuscript.

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