

RESEARCH ARTICLE

Dietary Supplementation with *Lactiplantibacillus plantarum* AB11 Mitigates Aflatoxin-Induced Toxicity and Enhances Growth Performance, Blood Biochemistry, Immune Gene Expression, and Cecal Microbiota in Japanese Quail

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ARTICLE HISTORY (26-1043)

Received: August 19, 2026
Revised: September 25, 2026
Accepted: September 26, 2026
Published online: September 28, 2026

Key words:

Aflatoxin
Antifungal
Antioxidant
Gut Health
Probiotic

ABSTRACT

Aflatoxins are among the most prevalent mycotoxins in poultry feed, significantly impairing livestock health and productivity by inducing growth depression, hepatotoxicity, and immunosuppression. This study aimed to identify a natural mitigation strategy by isolating and characterizing probiotic bacteria with antifungal and antioxidant potential to mitigate aflatoxin-induced toxicity in quails. Forty bacterial isolates from dairy samples were screened for antifungal activity against *Aspergillus niger* and *Fusarium oxysporum*. The isolated *Lactiplantibacillus plantarum* AB11 (*L. plantarum* AB11) was identified as *Lactiplantibacillus plantarum* by morphological and biochemical tests and confirmed by MALDITOF. *In-vitro*, AB11 exhibited considerable probiotic traits, including high survival under acidic conditions (91.2% at pH 2.5) and bile salts (85.3% at 0.3%). AB11 (400 µg/mL) also showed dose-dependent antioxidant activity, reaching 90% DPPH scavenging. A 21-day *in vivo* trial on 210 eight-day-old Japanese quails allocated to seven dietary treatments evaluated aflatoxin-contaminated feed with or without *L. plantarum* AB11 or Triton X 100. The inclusion of *L. plantarum* AB11 (400 mg/kg) in the quails' diet markedly alleviated aflatoxin-induced damage, improving growth performance, feed efficiency, liver function (lower AST, ALT, MDA), antioxidant status (higher SOD, GSH, CAT), and immunity (higher IgG, IgA). At the molecular level, *L. plantarum* AB11 downregulated aflatoxin-induced overexpression of immune genes, *CYP1A1*, *AHR1*, *IL-1β*, and *Casp-3*, and beneficially modulated the gut microbiota by reducing pathogenic microbes while enriching lactic acid bacteria. These findings indicate that *L. plantarum* AB11 is a promising dietary probiotic for protecting poultry against aflatoxin toxicity.

To Cite This Article: Alamoudi SA, Ashkan MF, Alqahtani AN, AlOmari A, Bouqellah NA, Osailan R, Al-Najjar MAA, Aldawood N, Alamri SA, Althubiani SA, Alshammari NAH, Al-Rasheed M and Elakkad HA, 2026. Dietary supplementation with *Lactiplantibacillus plantarum* AB11 mitigates aflatoxin-induced toxicity and enhances growth performance, blood biochemistry, immune gene expression, and cecal microbiota in Japanese quail. Pak Vet J, 46(9): 2282-2292. <http://dx.doi.org/10.29261/pakvetj/2026.218>

INTRODUCTION

Certain mold species, particularly *Aspergillus flavus*, *A. parasiticus*, *A. nomius* and *Penicillium puberulum*, naturally produce aflatoxins (AFs) as secondary metabolic byproducts (Benkerroum, 2020). Dietary exposure to aflatoxins, especially aflatoxin B1 (AFB1), has been associated with higher mortality rates, decreased performance, more hepatic lesions, and increased immunosuppression in various poultry species (Mahmood *et al.*, 2017). Quails possess numerous advantages that render them valuable among poultry species; these encompass their delectable taste, high nutritional content, rapid growth, and elevated productivity. Furthermore, quail eggs are reported to be abundant in crude protein (13%) and vitamin B1. Additionally, quail farming provides considerable economic benefits due to its low investment requirements and early production, as quails are renowned for reaching sexual maturity swiftly and generating substantial egg output in the initial stages (Zabiulla *et al.*, 2021).

Reducing antibiotic reliance, probiotics, prebiotics, and natural compounds act as potential alternatives to antibiotics to reduce microbial dispersal, promoting environmental sustainability and socioeconomic resilience in animal production; further research on their functional roles is needed to address knowledge gaps and advance safe, sustainable animal agriculture (Hasen *et al.*, 2024; Atuahene *et al.* 2025; Pewan *et al.* 2025). Probiotics are defined by the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) as live microorganisms that, when administered in adequate amounts, confer health benefits to the host by helping maintain a balanced gut microbiota. They support intestinal health by promoting beneficial microbial populations, limiting the growth of harmful organisms and contributing to physiological functions such as metabolic regulation and improved lactose digestion (Sebouai *et al.*, 2024). In recent decades, the decline in antibiotic use in livestock production has highlighted probiotics as promising alternatives (Salem *et al.*, 2023). They improve feed intake, increase weight gain, and promote animal health while helping to reduce antimicrobial resistance.

Lactobacillus species are widely recognized for their significance in food fermentation and functional feed design. Among nearly 150 LAB species, *L. plantarum* and *Bifidobacterium longum* are distinguished by their notable tolerance to environmental stress and their suitability for laboratory propagation (Liu *et al.*, 2024). The resulting lactic acid production decreases intestinal pH, suppressing harmful bacteria and supporting gut integrity (Zeng *et al.*, 2018). These interactions contribute to improved poultry health, immunity, and meat quality.

Probiotics, therefore, serve as tools to manipulate gut microbial communities, improving both animal health and product quality in commercial production systems (Mârza *et al.*, 2025). Nevertheless, excessive probiotic supplementation can diminish these advantages, likely due to nutrient competition between hosts and microbes or the overproduction of bacteriocins with self-inhibitory effects (Sebouai *et al.*, 2024). Administering probiotics early in the animal's life enhances their colonization potential and

prevents enteric pathogens from establishing themselves (Mârza *et al.*, 2025).

Among the numerous naturally occurring biological toxins that pose a threat to human and animal health, aflatoxins hold a particularly significant position. Aflatoxin B1 (AFB1) is considered the most hazardous and prevalent member of this group, while the remaining compounds G1, B2, and G2 are comparatively less toxic (Shabeer *et al.*, 2022). Acute exposure to high levels of AFB1 can precipitate severe illness, emesis, and mortality, while chronic low-dose intake is strongly associated with hepatocarcinogenesis (Huybrechts *et al.*, 2024).

Although European climatic conditions are generally less favorable for mycotoxin production than in regions such as North America and Asia, cereal contamination remains an ongoing concern (Kos *et al.*, 2023). Current European Union regulations specify a maximum permissible concentration of 20 µg/kg for aflatoxin (AFB1) in poultry feed to protect both avian and, indirectly, human health. The majority of experimental studies have documented only modest improvements following intervention, and robust, consistent results are comparatively uncommon (Salama *et al.*, 2024). The considerable variability in both aflatoxin exposure levels and duration indicates a pressing need for further systematic investigation (Salama *et al.*, 2024).

Mycotoxin detoxification may proceed through biotransformation by the host's microbiota or through binding to microbial cell wall components, including those of bacteria and yeast (Xu *et al.*, 2022). Probiotics have demonstrated a remarkable ability to sequester diverse xenobiotics, including mycotoxins, *via* adsorption to cell wall structures, thus reducing their bioavailability (Ghadaksaz *et al.*, 2022). Residual aflatoxins may accumulate in edible animal tissues, posing a direct threat to public health. The utilization of probiotics as dietary adjuncts has been suggested as a strategy for mitigating aflatoxin contamination through binding or bio-transforming mycotoxins; however, their efficacy in quails remains limited and inconclusive (Lázaro *et al.*, 2024). As a versatile generalist, *L. plantarum* exhibits an outstanding ability to adapt to diverse ecological environments. It has been documented across environments as varied as dairy products and the intestinal tracts of diverse animal species, including poultry (Mejía-Caballero *et al.*, 2025).

The previous studies have supported the use of dairy-derived *L. plantarum* strains in poultry applications, demonstrating that their robust functional attributes, including tolerance to low pH and bile salts and the production of antimicrobial agents, underpin their effectiveness in such settings (Yilmaz *et al.*, 2022). In addition, *L. plantarum* has been found to have anti-mycotoxin impacts by different modes of action, including binding to toxins; some strains bio-transform mycotoxins, enhance the gut barrier and boost gut immunity (Muhammad *et al.*, 2026). Therefore, in this study, *L. plantarum* AB11's functional properties (acid/bile tolerance and antimicrobial production) are often retained across different origins. Based on its promising *in vitro* probiotic characteristics, a dairy-derived strain was selected to examine how it influences

growth performance and carcass properties in quails under aflatoxin stress. The findings are intended to support the design of feasible interventions to protect poultry health and sustain production efficiency in mycotoxin-contaminated environments.

MATERIALS AND METHODS

Isolation, screening, and identification of the selected isolate: Different dairy samples were procured from the local market. Lactic acid bacteria (LAB) were selectively cultured on de Man, Rogosa and Sharpe (MRS) agar. Ten grams from each dairy sample were homogenized in 90 mL of peptone buffer to prepare an initial 10^{-1} dilution, which was subsequently subjected to tenfold serial dilution up to 10^{-7} . Aliquots from each dilution level were plated onto MRS agar and incubated at 37°C for 24 hours. The antifungal activity of all *Lactobacillus* isolates was tested by the agar well diffusion assay using cell-free supernatant (CFS). CFS was obtained from 24-h *Lactobacillus* cultures by centrifugation ($10,000 \times g$, 10 min, 4°C) and filtration through 0.22- μ m syringe filters. Aliquots (100 μ L) of CFS were placed into wells (8 mm diameter) cut into PDA plates previously seeded with fungal spores. Plates were incubated at 28°C for 3–5 days, and inhibition zones were measured.

Isolates were classified as showing strong inhibitory activity when they produced a mean inhibition zone ≥ 10 mm against both *A. niger* and *F. oxysporum*. Only those isolates meeting these criteria were selected for further characterization.

Selected isolates were preserved in MRS broth containing 20% (v/v) glycerol at -80°C until use.

The selected isolates were evaluated for biochemical and physiological characteristics. Isolates exhibiting Gram-positive, catalase-negative bacilli morphology typical of LAB were further subjected to protein level characterization by MALDI-TOF spectroscopy (Rachwai *et al.*, 2024).

After 24 hours of cultivation, bacterial cultures were harvested by centrifugation at 8,000 rpm for 10 minutes. The recovered cell pellets were subsequently washed with phosphate-buffered saline (PBS, pH 7.2) to remove residual growth medium. The final cell suspensions were standardized to 10^6 colony-forming units (CFU) per milliliter before experimental inoculation.

Assessment of probiotic potential and safety profile:

In-vitro acid and bile salt resistance assays were conducted to characterize the gastrointestinal survival capacity of the selected isolates, following the methodology previously described by Sahadeva *et al.* (2011). The acid tolerance of each isolate was examined by inoculating 1 mL of culture into 9 mL of LB broth at pH 2.5, then incubating at 37°C for 3 hours. Bacterial growth was monitored hourly by measuring optical density at 650 nm (OD_{650}), and the initial cell density of all samples was normalized to $\text{OD}_{650} = 0.08 \pm 0.05$ to ensure comparability across treatments. To evaluate bile salt resistance following acid tolerance testing, 100 μ L of overnight culture was transferred to fresh LB broth containing 0.3% (w/v) bile salts. At 0, 60, 120, 180, and 240 min post-inoculation, 100 μ L samples were aseptically

plated on LB agar to monitor viability. Survival under bile stress was scored qualitatively as positive (growth) or negative (no growth). Acid tolerance and survival rates were then calculated using the formula below.

$$\text{Survival rate (\%)} = \frac{\text{OD after treatment}}{\text{OD before treatment}} \times 100 \quad (1)$$

To evaluate the hemolytic activity of the selected isolate which streaked onto blood agar containing 5% (v/v) human blood to assess hemolytic activity using the streak plate technique. Plates were incubated at 37°C for 24 hours and examined macroscopically for hemolytic activity. Alpha hemolysis was characterized by a greenish discoloration surrounding the colony, indicating partial erythrocyte lysis, while beta hemolysis was indicated by a well-defined clear zone reflecting complete red blood cell lysis.

Gamma hemolysis was recorded when no lytic zone was observed. A *Staphylococcus aureus* strain with confirmed hemolytic activity was used as a positive control to validate the assay. The antibiotic resistance profiles of selected isolates were determined using the Kirby-Bauer disc diffusion method. Bacterial suspensions were adjusted to 10^6 CFU/g and spread uniformly onto suitable solid nutrient agar. Antibiotic discs (30 μ g) including tetracycline, azithromycin, erythromycin, ceftriaxone, and gentamicin were placed onto inoculated plates and incubated at 42°C for 48 hours. The diameters of inhibition zones were measured and classified as sensitive, intermediate, or resistant according to CLSI/EUCAST guidelines.

In-vitro biological activity assessment

Antioxidant: The *in-vitro* antioxidant activity of *L. plantarum* AB11 was determined by assessing its capacity to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals at serial concentrations of 15, 30, 50, 100, 200 and 400 μ g/mL, following the methodology previously described by Abdel-Moneim *et al.* (2022). Reaction mixtures containing 0.5 mL ethanolic DPPH and 1 mL isolate suspension were incubated in the dark for 30 minutes, after which absorbance was measured at 517 nm. The IC_{50} value, representing the concentration at which 50% of DPPH radicals were scavenged, was calculated from the resulting inhibition curve (El-Saadony *et al.*, 2022). The ability of *L. plantarum* AB11 to scavenge DPPH radicals was expressed as percentage inhibition, calculated as follows:

$$\text{DPPH scavenging activity (\%)} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100 \quad (2)$$

Antifungal: The antifungal efficacy of isolate *L. plantarum* AB11 was determined by the agar disc diffusion method. Bacterial suspensions were prepared at serial concentrations of 15, 30, 50, 100, 200, and 400 μ g/mL. Sterile 8-mm filter paper discs were submerged in each concentration for 60 min for saturation, then aseptically placed onto the surface of potato dextrose agar (PDA) plates pre-inoculated with poultry-pathogenic fungi *Aspergillus niger*, *A. fumigatus*, *Fusarium*

oxyssporum and *F. moniliforme* at a standardized concentration of 1.5×10^6 CFU/mL. Following the application of the discs, the plates were incubated to ensure fungal proliferation. After incubation, the diameter of the inhibition zone appearing on the disc was measured (mm).

For the *in-vivo* feeding trial, the probiotic was incorporated into the feed on a dry weight basis. The *L. plantarum* AB11 biomass was produced by culturing, harvesting by centrifugation, washing, and then freeze-drying. The resulting freeze-dried powder was standardized to a known viable count (CFU/g). The dietary inclusion levels of 200 and 400 mg/kg correspond to approximately 1×10^8 and 2×10^8 CFU/kg of feed, respectively.

Animals and experimental design: Aflatoxin biosynthesis was achieved by inoculating the toxigenic reference strain *Aspergillus parasiticus* NRRL 2999 onto fresh potato dextrose agar (PDA) supplemented with quail feed extract, following the established procedure described by Shotwell *et al.* (1966). The aflatoxin-contaminated feed was processed by autoclaving, followed by drying and fine grinding to produce a homogeneous powder. Aflatoxin levels were quantified via high-performance liquid chromatography (HPLC) at the Central Laboratory for Mycotoxins and Food Safety, National Research Center, Egypt. The resulting feed powder was subsequently incorporated into a quail basal diet at calculated inclusion rates to achieve final dietary aflatoxin concentrations of 200 and 400 ppb.

Triton X-100 is a nonionic surfactant, chemically known as octylphenol ethoxylate, with the general formula $C_{14}H_{22}O(C_2H_4O)_n$, where 'n' averages around 9.5 ethylene oxide units, creating a balance of a hydrophobic (water-fearing) t-octylphenyl group and a hydrophilic (water-loving) poly (ethylene oxide) chain, making it an excellent detergent, emulsifier, and wetting agent.

A total of 210 eight-day-old Japanese quail chicks were randomly allocated into seven dietary treatment groups, each comprising 30 birds housed in three replicate pens of 10 birds per pen. All birds were individually weighed at the commencement of the 21-day experimental period, with body weights recorded weekly thereafter. The seven treatment groups were: G-1, a basal diet with no additives (control); G-2, a basal diet contaminated with 400 ppb aflatoxin (BAF 400); G-3, a basal diet supplemented with 200 mg of *L. plantarum* AB11 per kilogram of feed; G-4, a basal diet supplemented with 400 mg of *L. plantarum* AB11 per kilogram of feed; G-5, a basal diet supplemented with 400 mg of Triton X-100 per kilogram of feed; G-6, a basal diet co-treated with 400mg of AB11 per kilogram and 400 ppb aflatoxin; and G-7, a basal diet co-treated with 400 mg of Triton X-100 per kilogram and 400 ppb aflatoxin.

The experimental basal diet was formulated to meet or exceed the nutrient requirements for Japanese quails as established by the National Research Council (NRC) and was deliberately formulated without antibiotic growth promoters. Each treatment group was housed in separate pens on a deep-litter system with fresh wood-shaving bedding. Environmental parameters, including photoperiod, relative humidity, and ambient temperature,

were maintained at levels optimal for quail growth and welfare throughout the experimental period. Clean drinking water and experimental feed were provided *ad libitum* to all birds throughout the trial. All animal procedures were conducted in accordance with the institutional animal ethics guidelines of Zagazig University, ensuring humane treatment and minimizing pain, suffering, distress, and lasting harm. The study was reviewed and approved by the Ethics Committee of Zagazig University, Zagazig, Egypt (approval no. ZU-IACUC/2/F/313/2023). The number of animals was minimized in accordance with the 3Rs principle (replacement, reduction, and refinement), while maintaining sufficient statistical power to yield valid and reproducible findings.

Assessment of growth performance: Growth performance was evaluated by monitoring live body weight (LBW) at the onset of the experiment (day 8) and weekly, with final body weight (FBW) determined at the end of the 21-day period. Feed intake (FI) was measured on a replicate basis, and the following derived parameters were calculated: body weight gain (BWG) as the difference between FBW and initial weight; feed conversion ratio (FCR) as total feed consumed per unit of weight gained; performance index (PI) according to the formula; and growth rate (GR) as daily weight gain. All calculations were performed in accordance with the methodologies established by Brody and Lardy (1946);

Body weight gain (BWG) = Final weight (FW) – Initial weight (IW) (3)

Growth rate (GR) = $(LBW_{21} - LBW_8) / [0.5 \times (LBW_8 + LBW_{21})]$ (4)

Performance index (PI) = $\frac{BWG}{FCR}$ (5)

Blood biochemistry and liver function analysis: At the termination of the experiment (day 21), five birds were randomly selected from the groups and anesthetized using an animal anesthesia apparatus, with independent channel control enabling precise gas-flow delivery to the induction chamber at 0–2.0 L/min. Blood was collected into clean, dry centrifuge tubes devoid of anticoagulant. After clotting, blood samples were centrifuged at 3,000 rpm for 15 minutes; the harvested serum was aliquoted and stored at -20°C until biochemical analysis. The enzymatic activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were estimated (Agina *et al.*, 2017). Subsequent to euthanasia, the liver was meticulously removed, rinsed in cooled 0.9% (w/v) saline to eliminate leftover blood, gently blotted, and weighed to ascertain both absolute and relative organ weight. The tissue was rapidly frozen and maintained at -70°C pending analysis. For oxidative stress evaluation, hepatic homogenates were prepared and analyzed for the enzymatic activities of superoxide dismutase (SOD) and catalase (CAT), the antioxidant capacity indicator reduced glutathione (GSH), and the lipid peroxidation marker malondialdehyde (MDA), all determined using commercially available diagnostic kits according to the manufacturer's instructions (Agina *et al.*, 2017).

Total antioxidant capacity (TAC) was measured according Agina *et al.* (2017). Immunoglobulin markers in serum were quantified by sandwich ELISA using species-specific capture/detection antibodies with an HRP-streptavidin-TMB reporter system. Reactions were stopped with acid and read at 450 nm, with immunoglobulin concentrations interpolated from standard curves generated using certified calibrators (Agina *et al.*, 2017).

Gene expression: For molecular analysis, total RNA was isolated from the ileal mucosa, with tissue samples precisely harvested from the segment between Meckel's diverticulum and the ileocecal junction. Following extraction and ethanol precipitation, RNA pellets were dissolved in DEPC-treated water to inactivate any contaminating RNases. RNA concentration and purity were quantified spectrophotometrically, with an A_{260}/A_{280} ratio of ~ 2.0 accepted as indicative of high-quality, protein-free RNA, according to the methodology established by Saif and Khan (2022).

Quantitative gene expression analysis was conducted by real-time PCR (qPCR) following reverse transcription. To ensure proper unfolding, which is crucial for reliable downstream reactions and helps the audience trust the method's effectiveness, 3 μ g of total RNA per sample was denatured at 70°C for 5min using a Bio-Rad T100™ Thermal Cycler. First-strand cDNA was synthesized in a reaction mixture containing 0.5ng oligo(dT) primers, 2 μ L of 10 $\times$ reverse transcription buffer, 2 μ L of 10mM dNTPs and 1 μ L of Moloney murine leukemia virus (M-MLV) reverse transcriptase at a concentration of 100 units. The reaction occurred at 42°C for 60 minutes, followed by heat inactivation at 70°C for 10 min. Relative mRNA expression levels were assessed by qPCR with SYBR Green detection and quantified using the $2^{-\Delta\Delta CT}$ method, normalized to a consistently expressed housekeeping gene. Gene-specific primer sequences are provided in Table 1. For selecting *CYP1A1*, *AHR1*, *IL-1 β* and *Casp-3*, these genes represent key pathways in aflatoxin-induced toxicity: *AHR1* is a critical transcription factor activated by AFB1; *CYP1A1* is a downstream enzyme responsible for AFB1 bioactivation; *IL-1 β* is a central pro-inflammatory cytokine; and *Casp-3* is a key executioner of apoptosis and these genes selection allows us to assess the molecular impact of aflatoxin and the protective effect of AB11 on these fundamental pathways. Gene expression levels were compared across samples using normalized Ct values and standard comparative quantification methods.

Table 1: Primer sequences and characteristics for selected defense-related genes

Target gene	Primer sequence (5'→3')	Product length (bp)	Accession number
<i>CYP1A1</i>	F: CTACAGGCAGCTGTGGATGAG R: GACAGGGCGGTTGTACAGT	198	XM_015872789
<i>AHR1</i>	F: GACAGGGCGGTTGTACAGT R: TTCAAACGGTCCCTGTGCCTC	118	XM_015853813
<i>IL-1β</i>	F: CTGCCAGAAGGAAATGCCAA R: TGTCAGGAGCAGGGTTTGG	164	NM_204524.2
<i>Casp-3</i>	F: TGGCGATGAAGGACTCTTCT R: AGAGTTTGGGTTTCCCTGCT	155	NM_204725.1

CYP1A1: Cytochrome P450 Family I Subfamily A Member 1; AHR1: aryl hydrocarbon receptor 1; IL-1 β : Interleukin 1 Beta; Casp-3: Caspase-3. F: forward primer, R: reverse primer, bp: base pair.

Quantitative analysis of intestinal microbes:

Immediately following euthanasia, the ileal segment of the small intestine, spanning from Meckel's diverticulum to the ileocecal junction, was excised and transferred aseptically to a sterile workspace. Intestinal contents were carefully collected, homogenized in sterile glass containers, and either processed immediately or stored at 4°C for a brief period until microbiological analysis. Total bacterial count (TBC), total mold and yeast count (TMYC), coliforms, and presumptive *Lactobacillus* species were enumerated by the spread plate technique using appropriate selective and differential media, following the standardized protocols previously described by Abd El-Wahab *et al.* (2022). All microbial counts were transformed to base-10 logarithms and reported as log₁₀ colony-forming units (CFU) per gram of intestinal sample to normalize the distribution of bacterial and fungal population data.

Statistical analysis: Data are presented as mean $\pm$ standard error (SE). The normality of distribution and homogeneity of variances were confirmed prior to analysis. Differences among the seven treatment groups were evaluated by one-way analysis of variance (ANOVA), followed by Tukey's Honestly Significant Difference (HSD) post hoc test for multiple pairwise comparisons. Statistical significance was set at $p < 0.05$ for all tests. All analyses were performed using IBM SPSS Statistics for Windows, version 26.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Screening and identification of the antifungal-

producing isolate: Forty presumptive lactic acid bacteria were isolated from dairy sources, coded AB1 through AB40, and initially screened for antifungal efficacy against *Aspergillus niger* and *Fusarium oxysporum*. Six isolates, AB1, AB2, AB9, AB11, AB22 and AB35, demonstrated marked antagonistic activity against both fungal targets. The forty isolates were subsequently subjected to phenotypic characterization using standard biochemical tests as outlined in Bergey's Manual of Determinative Bacteriology. Isolate AB11, which produced the largest inhibition zones (35.5 mm against *A. niger* and 29.8 mm against *F. oxysporum*), was selected as the lead candidate for species-level identification by MALDI-TOF MS and was examined microscopically and macroscopically. The isolate appeared as Gram-positive, motile, endospore-forming rods occurring individually. Colonies on MRS agar were flat, round, translucent, with irregular edges and pale cream pigmentation. These phenotypic findings supported the preliminary identification of isolate AB11 as *Lactiplantibacillus plantarum*. MALDI-TOF MS analysis confirmed isolate AB11 as *Lactiplantibacillus plantarum*, with a species-level identification score of 2.25 (> 2.00).

The tests of probiotics

Effect of bile salt and low pH: The isolate *L. plantarum* AB11, which exhibited the greatest inhibitory activity against the target fungal pathogens *Aspergillus niger* and *Fusarium oxysporum*, was advanced for comprehensive characterization. Beyond its antifungal properties, *L. plantarum* AB11 demonstrated exceptional

gastrointestinal tract survivability, as evidenced by survival rates of 91.2% following 3-hour exposure to pH 2.5 and 85.3% after incubation in 0.3% bile salts (Table 2). These findings indicate that *L. plantarum* AB11 possesses both potent antifungal activity and the physiological robustness required for transit through and potential colonization of the avian gastrointestinal environment. Notably, *L. plantarum* AB11 combined the highest antifungal activity with stress resilience, thereby establishing itself as a promising probiotic candidate.

Table 2: Screening the selected isolates based on their inhibition zones against *Aspergillus niger* and *Fusarium oxysporum*, and survival at low pH and bile salt (0.3%)

Isolate	Antifungal Activity (Inhibition Zone in mm)		Stress Tolerance (Survival Rate in %)	
	<i>Aspergillus niger</i>	<i>Fusarium oxysporum</i>	pH 2.5	Bile Salt (0.3%)
AB2	32.0±1.2 ^b	26.9±1.3 ^b	84.1±2.5 ^b	77.4±2.1 ^b
AB5	28.3±0.9 ^c	26.2±1.0 ^b	78.3±2.7 ^c	68.5±2.4 ^c
AB9	26.5±1.1 ^e	23.5±0.9 ^c	72.4±2.2 ^{cd}	67.1±2.0 ^c
AB11	35.5±1.0 ^a	29.8±1.1 ^a	91.2±2.0 ^a	85.3±1.8 ^a
AB22	27.1±0.8 ^d	24.1±1.2 ^c	74.0±2.5 ^{cd}	67.8±2.2 ^c
AB35	25.9±1.1 ^e	21.7±1.2 ^d	69.0±2.8 ^d	65.9±2.3 ^d

Data are presented as mean ± SD. Different lowercase letters in the same column indicate the significant differences.

Antibiotic resistance and hemolytic activity: Table 3 reveals Gentamicin as the most effective antibiotic (inhibition zones ranging from 11.0±0.8 to 27.5±1.7 mm) and Tetracycline as the least effective (7.2±0.4 to 18.9±1.1 mm). Isolate AB35 was the most susceptible overall (27.5±1.7 mm for Gentamicin), while AB11 exhibited the highest resistance (e.g., 7.2±0.4 mm for Tetracycline). Notably, the hemolytic isolates (AB9, AB22, AB35) were more susceptible to antibiotics, whereas the non-hemolytic strains (AB2, AB5, AB11) exhibited greater resistance, indicating an inverse relationship between hemolytic activity and antimicrobial resistance.

Table 3: Antibiotic resistance and hemolytic activity of selected isolates

Isolate	Antibiotic (30µg)					Hemolytic activity
	Erythromycin	Azithromycin	Tetracycline	Ceftriaxone	Gentamicin	
AB2	12.3±0.8 ^e	13.5±0.9 ^e	10.1±0.6 ^e	15.2±1.0 ^e	16.8±1.1 ^e	-
AB5	14.6±1.0 ^d	15.8±1.0 ^d	12.7±0.7 ^d	17.9±1.2 ^d	19.5±1.3 ^d	-
AB9	18.2±1.2 ^b	19.5±1.3 ^b	16.3±0.9 ^b	22.1±1.4 ^b	24.0±1.5 ^b	+
AB11	8.5±0.5 ^f	9.1±0.6 ^f	7.2±0.4 ^f	10.3±0.7 ^f	11.0±0.8 ^f	-
AB22	16.5±1.1 ^c	17.8±1.2 ^c	14.8±0.8 ^c	20.5±1.3 ^c	21.8±1.4 ^c	+
AB35	20.5±1.4 ^a	22.1±1.5 ^a	18.9±1.1 ^a	25.3±1.6 ^a	27.5±1.7 ^a	+

Data are inhibition zone diameters (mm ± SD; n=3). Statistical letters within a row indicate significant differences (P<0.05).

Biological activities

Antioxidant: Fig. 1 summarizes the antioxidant activity of *L. plantarum* AB11 isolated by scavenging DPPH free radicals at concentrations of 15-400µg/mL. The antioxidant activity of isolate *L. plantarum* AB11 increased in a statistically significant, concentration-dependent manner (P<0.05), ranging from approximately 35% at 15µg/mL to ~90% at 400µg/mL. This dose-response relationship indicates a direct correlation between isolated concentration and free radical scavenging capacity. Bars bearing different lowercase letters differ significantly from one another (P<0.05), confirming that the observed concentration-dependent trends are statistically robust and not attributable to random variation. The findings demonstrated the robust antioxidant potential of the *L. plantarum* AB11 isolate, particularly at 400µg/mL.

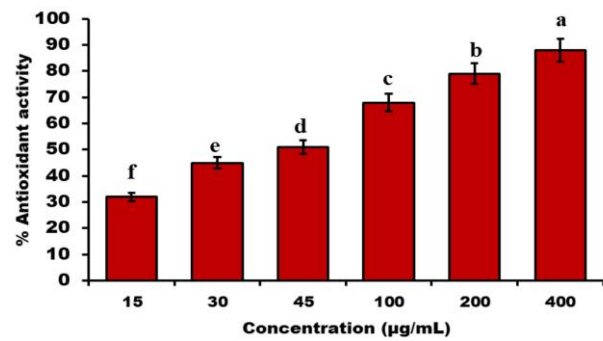


Fig. 1: Antioxidant activity of *Lactobacillus plantarum* AB11 isolate against DPPH free radicals. Lowercase letters above columns indicate significant differences (P<0.05). All error bars represent the standard error of the mean (SEM) based on biological replicates (n=3 pens per treatment).

Antifungal: The antagonistic activity of *L. plantarum* AB11 against *Aspergillus niger*, *A. fumigatus*, *Fusarium oxysporum*, and *F. moniliforme* is detailed in Table 4. A clear concentration-dependent response was observed, with inhibition zone diameters varying with concentration. At the highest tested concentration, AB11 produced inhibition zones exceeding 30mm against both *Fusarium* species, whereas they remained below 30 mm against both *Aspergillus* species. These findings demonstrated the broad-spectrum, concentration-dependent antifungal potential of the *L. plantarum* AB11 isolate, suggesting its potential use as a natural biocontrol agent or as an adjunct for controlling fungal pathogens and their toxins.

In-vivo experiment

Growth performance parameters of aflatoxin challenged quails: Table 5 investigates the positive impact of *L. plantarum* AB11 on growth markers of quails exposed to aflatoxin. The parameters evaluated included LBW, FBW, BWG, FI, FCR, GR, and PI. The results showed that groups given *L. plantarum* AB11, especially at 400 mg/kg (G-4, G-5), had higher final body weight (BW), BWG, GR and PI than the control and the aflatoxin-only group (G-2). These groups also exhibited lower FCRs, indicating better feed efficiency. G-4 exhibited a 28.10% increase in body weight gain and a 20.93% reduction in FCR, followed by G-5. G-6 showed a 23.64% increase in BWG along with an 18.60% reduction in FCR, while G-7 recorded a 21.46% increase in BWG and a 17.67% reduction in FCR. The dietary AB11 mitigated the adverse effects of aflatoxin on growth in quails, with the higher dose providing the most significant benefits for growth and performance.

Table 4: Antifungal activity of the *Lactobacillus plantarum* AB11 isolate at concentrations (15, 30, 45, 100, 200 and 400 µg/ml) against tested fungi (inhibition zone diameter; mm ± SD)

Tested fungi	AB11 (µg/mL) / Inhibition Zone Diameters (mm)					
	15	30	45	100	200	400
<i>Aspergillus niger</i>	12.5±0.8 ^f	15.8±1.1 ^e	18.2±1.0 ^d	22.4±1.2 ^c	26.7±1.3 ^b	30.5±1.5 ^a
<i>Aspergillus fumigatus</i>	13.8±1.0 ^f	16.9±0.9 ^e	19.5±1.1 ^d	23.1±1.3 ^c	27.3±1.4 ^b	31.8±1.6 ^a
<i>Fusarium oxysporum</i>	11.2±0.7 ^f	13.5±0.8 ^e	15.9±0.9 ^d	18.4±1.0 ^c	21.8±1.1 ^b	25.9±1.2 ^a
<i>Fusarium moniliforme</i>	12.1±0.9 ^f	15.2±0.8 ^e	17.8±1.0 ^d	21.5±1.1 ^c	25.1±1.3 ^b	29.3±1.4 ^a

Data are mean inhibition zone diameters (mm ± SD; n = 3). Different superscript letters (a-f) within rows denote significant differences between concentrations (P<0.05).

Blood biochemistry markers: The ameliorative potential of dietary *L. plantarum* AB11 against aflatoxin-induced systemic perturbations is detailed in Table 6, which includes serum biochemical markers, hepatic function indicators, tissue oxidative stress biomarkers, and humoral immune indices in treated quails. Notably, groups given AB11, especially G-4 and G-5, demonstrated significant ($P<0.05$) improvements in liver health, with remarkably lower levels of AST, ALT and MDA, along with increased antioxidant enzyme activities, higher levels of SOD, GSH and CAT. The AB11 supplementation reduced oxidative stress and enhanced liver protection. Additionally, it showed higher levels of immunoglobulin G (IgG) and A (IgA), indicating a positive effect on humoral immunity. When compared with the infected control group (G-2), group G-4 showed a 38.66% decrease in AST, a 51.28% decrease in ALT, and a 60.00% decrease in MDA, indicating significant improvements in liver function and oxidative stress markers. Additionally, immune markers in G-4 increased, with IgG up by 34.25% and IgA by 53.57%. Group G-6 also exhibited notable benefits, with an 18.56% rise in IgG and a 27.51% rise in IgA, along with decreases of 27.51% in AST, 30.77% in ALT, and 35.71% in MDA.

Gene expression against aflatoxin challenge in quails:

Fig. 2 illustrates the fluctuations of key defense-related genes in Japanese quails after aflatoxin exposure and the impact of subsequent treatment with *L. plantarum* AB11 at various dietary levels, compared to a commercial fungicide. Notably, aflatoxin exposure (G-2) significantly increased ($P<0.05$) *CYP1A1*, *AHRI*, *IL-1 β* and *Casp-3*, indicating heightened xenobiotic metabolism, inflammation, and

apoptosis. Treatment with AB11, especially at higher doses (G-4 and G-5), resulted in notable reductions in these genes compared to the infected group, as reflected by lower fold change values. In the treated group (G-4) compared to the infected group (G-2), the transcript levels of key defense genes were markedly 2288 upregulated: *CYP1A1* by 71.43%, *AHRI* by 79.49%, *IL-1 β* by 82.93% and *Casp-3* by 85.71%. This indicates that AB11 supplementation mitigates aflatoxin-induced oxidative stress, inflammation, and cellular apoptosis at the molecular level, thereby reinforcing its protective role against the toxin's effects. The decrease in gene expression in the AB11 groups, approaching control levels or even dropping below those observed in fungicide-treated birds, highlights the efficacy of probiotic intervention in maintaining cellular equilibrium in challenged quails.

Microbial count: Fig. 3 illustrates the protective role of *L. plantarum* AB11 against aflatoxin (AFB1)-induced microbial shifts in quails. AFB1 exposure alone (T2) elevated total bacteria (7.5 log CFU/g) and coliforms (6.5 log CFU/g) while depleting lactic acid bacteria (LAB, 1.5 log CFU/g). Supplementation with AB11 at 400 mg/kg (T4) most effectively reversed these effects, yielding the lowest total bacteria (5.8 log CFU/g) and coliforms (2.5 log CFU/g), and the highest LAB counts (5.5 log CFU/g). The combination of AB11 with AFB1 (T6) also provided strong protection, reducing coliforms to ~3.8 log cfu/g and restoring LAB to ~5.2 log CFU/g. Additionally, all treatments (T3–T7) significantly reduced total mold and yeast counts compared to the control and AFB1 groups (from 6.5 down to 4.5–5.5 log CFU/g). Overall, AB11 effectively mitigates AFB1 toxicity and promotes beneficial gut bacteria.

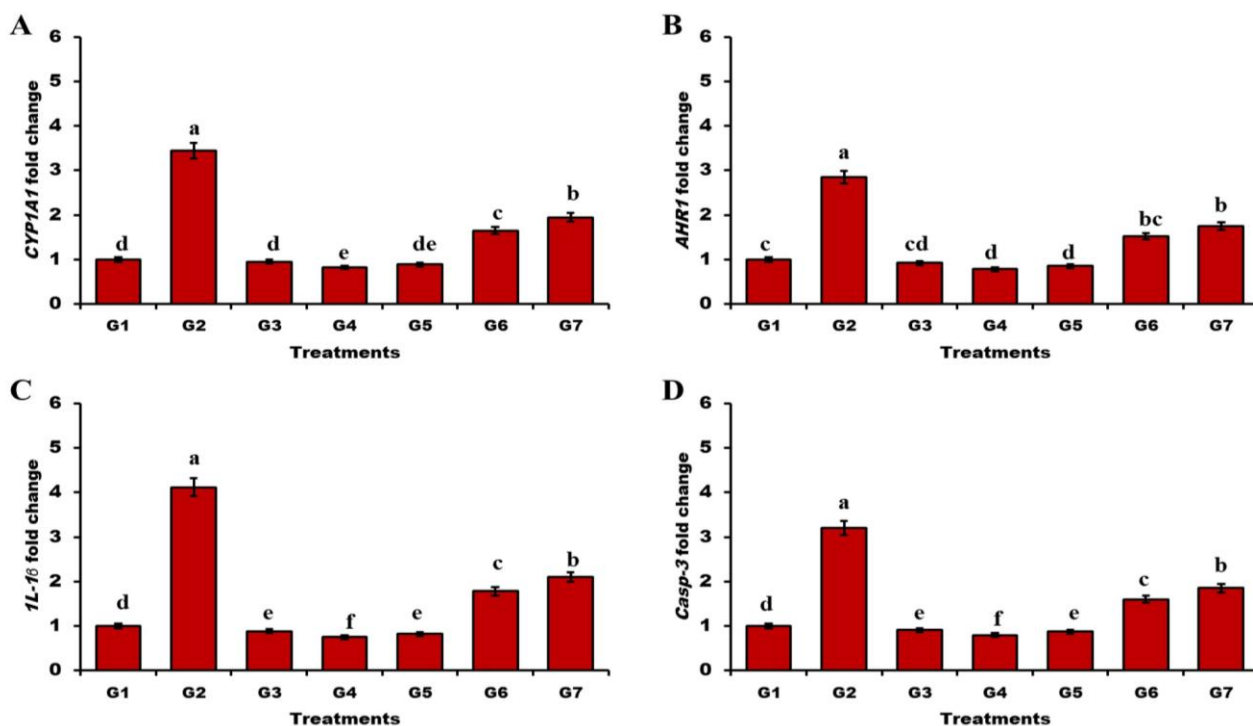


Fig. 2: Fold change in defence genes: (A) Cytochrome P450 Family I Subfamily A Member 1 (*CYP1A1*), (B) aryl hydrocarbon receptor 1 (*AHRI*), (C) Interleukin-1 Beta (*IL-1 β*), and (D) Caspase-3 (*Casp-3*) in response to aflatoxicosis in Japanese quails that were treated with *Lactobacillus plantarum* AB11 compared to commercial fungicide. Group 1: control diet without any additives; group 2: basal diet + 400 ppb aflatoxin (BAF 400); group 3: basal diet + 200 mg/kg feed AB11; group 4: basal diet + 400 mg/kg AB11; group 5: basal diet + 400 mg/kg feed Triton X100; group 6: AB11 + BAF 400; group 7: Triton X100 + BAF 400. Lowercase letters above columns indicate significant differences ($P<0.05$). All error bars represent the standard error of the mean (SEM) based on biological replicates ($n=3$ pens per treatment).

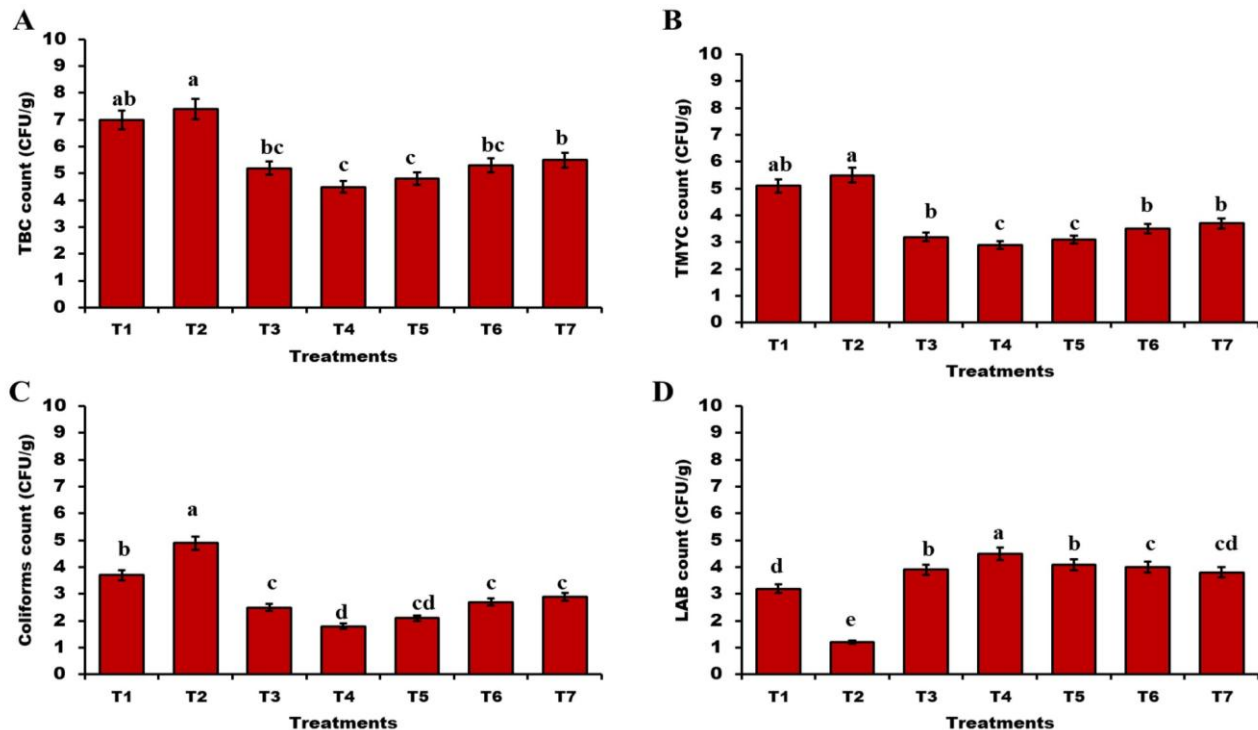


Fig. 3: Microbial counts A-D [Total bacteria count (TBC), total mold and yeast count (TMYC), coliforms, and lactic acid bacteria (LAB)] in quails' intestine affected by different concentrations of *Lactobacillus plantarum* AB11 on aflatoxin-challenged Japanese quails. Group 1: control diet without any additives; group 2: basal diet + 400 ppb aflatoxin (BAF 400); group 3: basal diet + 200 mg/kg feed AB11; group 4: basal diet + 400 mg/kg feed AB11; group 5: basal diet + 400 mg/kg feed Triton X100; group 6: AB11 + BAF 400; group 7: Triton X100 + BAF 400. Lowercase letters above columns indicate significant differences ($P < 0.05$). All error bars represent the standard error of the mean (SEM) based on biological replicates ($n = 3$ pens per treatment).

Table 5: Effect of dietary AB11 at 200 and 400 mg/kg on growth performance parameters of aflatoxin-challenged Japanese quails

Group	LBW (g)	FBW (g)	BWG (g)	FI (g)	FCR	GR	PI
G-1	45.5±0.2 ^{bc}	275.0±2.1 ^c	229.5±2.3 ^c	415.0±2.5 ^b	1.81±0.02 ^b	193.0±1.2 ^{bc}	135.9±0.5 ^c
G-2	42.1±0.1 ^c	235.0±2.0 ^d	192.9±2.1 ^d	415.0±3.0 ^b	2.15±0.01 ^a	186.0±1.8 ^d	110.2±0.2 ^d
G-3	46.2±0.3 ^b	282.0±1.5 ^b	235.8±2.2 ^b	419.0±3.2 ^a	1.78±0.03 ^c	194.0±1.3 ^{ab}	145.7±0.3 ^b
G-4	47.9±0.2 ^a	295.0±2.2 ^a	247.1±2.1 ^a	420.0±3.3 ^a	1.70±0.02 ^d	197.0±1.2 ^a	153.5±0.2 ^a
G-5	47.1±0.1 ^{ab}	290.0±2.3 ^a	242.9±2.0 ^a	419.5±3.2 ^a	1.73±0.04 ^{cd}	196.0±1.5 ^a	150.1±0.3 ^{ab}
G-6	46.5±0.2 ^b	285.0±2.2 ^{ab}	238.5±2.2 ^b	416.5±2.5 ^{ab}	1.75±0.02 ^c	193.0±1.2 ^b	143.6±0.5 ^b
G-7	45.7±0.3 ^{bc}	280.0±2.4 ^b	234.3±1.9 ^b	415.5±3.0 ^b	1.77±0.05 ^c	192.0±1.7 ^c	141.9±0.7 ^{bc}

Data are presented as mean ± SE. Different superscript letters within each row indicate significant differences between means ($P < 0.05$). Parameters include live body weight (LBW), final body weight (FBW), body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR), growth rate (GR), and performance index (PI). Group 1: control diet without any additives; group 2: basal diet + 400 ppb aflatoxin (BAF 400); group 3: basal diet + 200 mg/kg feed AB11; group 4: basal diet + 400 mg/kg feed AB11; group 5: basal diet + 400 mg/kg feed Triton X100; group 6: AB11 + BAF 400; group 7: Triton X100 + BAF 400.

Table 6: Effect of dietary AB11 on serum, liver function, oxidative stress, and immunity markers in Japanese quails

Group	AST (U/L)	ALT (U/L)	MDA (nmol/ml)	SOD (U/ml)	GSH (μmol/L)	CAT (U/ml)	IgG (mg/dl)	IgA (mg/dl)
G-1	185±3.5 ^d	2.4±0.2 ^{bc}	3.8±0.2 ^c	35.1±1.5 ^c	48.2±1.9 ^b	28.1±1.0 ^d	958±4.2 ^b	177±1.0 ^d
G-2	269±3.0 ^a	3.9±0.1 ^a	7.0±0.1 ^a	25.2±1.3 ^d	32.3±2.1 ^d	21.9±0.9 ^d	835±4.0 ^d	140±1.2 ^e
G-3	178±3.2 ^d	2.2±0.3 ^c	3.5±0.3 ^c	37.8±1.2 ^b	51.0±2.0 ^b	30.0±1.2 ^c	1012±4.7 ^b	185±1.2 ^c
G-4	165±2.9 ^f	1.9±0.2 ^d	2.8±0.5 ^d	42.2±1.1 ^a	58.3±2.3 ^a	34.5±1.1 ^a	1121±4.3 ^a	215±1.0 ^a
G-5	170±2.8 ^e	2.0±0.3 ^d	3.1±0.1 ^d	40.7±1.3 ^a	56.1±2.5 ^a	33.0±1.2 ^a	1091±4.3 ^a	205±1.3 ^b
G-6	195±2.5 ^c	2.7±0.4 ^b	4.5±0.3 ^b	32.6±1.0 ^c	45.6±2.3 ^c	26.7±1.0 ^d	990±4.2 ^c	180±1.4 ^c
G-7	205±3.1 ^b	2.8±0.1 ^b	4.9±0.2 ^b	30.9±1.2 ^{cd}	43.5±2.1 ^c	25.1±1.3 ^d	970±4.0 ^c	172±1.1 ^d

AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, MDA: Malondialdehyde, SOD: Superoxide dismutase, GSH: Glutathione, CAT: Catalase, IgG: Immunoglobulin G, IgA: Immunoglobulin A. Different superscript letters (^{a,b,c,d}) within a column indicate significant differences ($p < 0.05$). Values are presented as mean ± SD. Group 1: control diet without any additives; group 2: basal diet + 400 ppb aflatoxin (BAF 400); group 3: basal diet + 200 mg/kg feed AB11; group 4: basal diet + 400 mg/kg feed AB11; group 5: basal diet + 400 mg/kg feed Triton X100; group 6: AB11 + BAF 400; group 7: Triton X100 + BAF 400.

DISCUSSION

Toxic byproducts known as aflatoxins, produced by certain mold species, routinely contaminate a wide range of foods and animal rations (Arroyo-Manzanares *et al.*, 2019). Contamination by aflatoxins may occur at various stages of the food production continuum, including cultivation, storage, and feed processing activities. AFB1, the most toxic compound in the aflatoxin group, is associated with

cytotoxic, genotoxic, and immunosuppressive effects, as well as teratogenicity, mutagenicity, and carcinogenicity (Zou *et al.*, 2023). AFB1 in feeds destroys essential dietary components while reducing overall feed intake due to lowered feeding incentive and nutritional quality, triggering both acute and chronic toxicities in animals, and causing sudden death (Sikiru *et al.*, 2024), ultimately resulting in considerable economic loss. Poultry production is particularly susceptible to the adverse effects of AFB1

contamination, as highlighted in numerous reports; AFB1 markedly impairs growth performance and quality (Sarker *et al.*, 2023).

AFB1 exposure has been shown to impair growth and reduce various egg production parameters in hens, including total output, egg size, and quality. These harmful effects are primarily due to the toxin's action on the liver, kidneys, digestive tract, and immune organs. Furthermore, AFB1-induced immunosuppression can weaken the effectiveness of vaccination strategies, raise susceptibility to infectious diseases, and increase mortality rates in poultry populations (Kipkoech *et al.*, 2025).

In the current study, challenging quails fed with AFB1 (400 ppb) showed detrimental effects on growth performance; however, including *L. plantarum* in the diet enhanced FBW and reduced FCR, which is consistent with previous findings studies, Elrayess *et al.* (2023) demonstrated that AFB adversely affected the growth performance of quails, resulting in increased FCR and decreased BWG; however, Mirakzahi *et al.* (2024) showed that dietary supplementation strategies, especially probiotic-based combinations with curcumin or sodium bentonite (SB), effectively ameliorated the deleterious effects of AFB1-contaminated feed on egg production (EP), egg weight (EW), and feed conversion ratio (FCR), highlighting their potential as practical countermeasures in poultry production.

Oxidative stress is triggered by various mycotoxins, including AFB1, DON, ZEN and T-2 toxin, as evidenced by extensive research. Consistent with these findings, the current study demonstrated that AFB1 supplementation caused oxidative stress in a dose-dependent way. This was evidenced by a gradual decline in SOD and CAT activity, accompanied by a corresponding increase in MDA concentrations. These results suggest oxidative stress worsens as the AFB1 dose increases. Key antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx) are critical for scavenging reactive oxygen species (ROS) and maintaining intracellular redox equilibrium (Afzal *et al.*, 2023).

As a byproduct of lipid peroxidation, MDA is a commonly used marker of oxidative stress and lipid membrane damage (Mohideen *et al.*, 2023). Overall, these findings indicate that AFB1 causes oxidative damage mainly through increased lipid peroxidation and excess oxygen radicals, combined with decreased SOD and GPX activity and lower glutathione peroxidase 1 (GPx-1) gene expression. However, Li *et al.* (2014) observed that although AFB1 raised lipid peroxidation in broilers at 74 µg/kg, it did not significantly alter antioxidant enzyme activities. This difference suggests that long-term exposure to low doses of AFB1 may impair antioxidant defenses in different ways, especially in layer chicks, where enzyme systems might be more vulnerable.

Numerous studies have confirmed that mycotoxin contamination impairs avian humoral immunity, characterized by depressed serum immunoglobulin levels (IgA, IgG, IgM) and compromised vaccine efficacy, as evidenced by significantly reduced antibody titers against infectious bursal disease virus (IBDV-Ab) and Newcastle disease virus (NDV-Ab) (Hou *et al.*, 2022). Consistent with prior findings, AFB1-infected broilers disrupted the immune responses in the current study. Various mycotoxins

have been reported to reduce vaccine-induced antibody titers (Mubarik *et al.*, 2025) and immunoglobulin levels.

However, other studies have shown that some mycotoxins have no significant effect on immunoglobulin concentrations and therefore do not impair humoral immunity following immunization or sensitization. Thus, the effect of AFB1 on antibody titers and immunoglobulin production appears to vary with the dose administered.

Cytokines are essential regulators of inflammation and immune response (Khaiboullina, 2026). In the fact, mycotoxins exert immunostimulatory or suppressive effects on key immune cells such as macrophages, T cells and B cells (Mróz *et al.*, 2022). Earlier research indicated that mycotoxin exposure can either increase or decrease the expression of inflammatory cytokine genes (Wang *et al.*, 2022). AFB1 reduced IL-1β, casp-3, CYP1A1, and AHR1 protein levels while increasing their mRNA expression, consistent with previous findings of reduced IL-2/IL-4 and increased IL-1α/IL-6/IFN-γ/TNF-α gene expression (Sikiru *et al.*, 2024).

AFB1 exposure (40–1000 µg/kg) in poultry triggered upregulation of intestinal/liver pro-inflammatory markers (TLR2, NOD1, NF-κB p65, iNOS, TNF-α, IL-6, IL-8) while suppressing tight junction proteins (occludin, claudin-1), elevating hepatic MDA, and impairing antioxidant enzyme function (Lai *et al.*, 2022). Studies in ducks have consistently shown that AFB1 exposure impairs hepatic antioxidant capacity by reducing the activities of superoxide dismutase (T-SOD), glutathione peroxidase (GPx), glutathione S-transferase (GST), and catalase (CAT), while also depressing serum total protein (TP) and albumin (ALB) levels and promoting the accumulation of AFB1 residues in liver tissue findings that parallel the oxidative stress profile observed in the present quail study (El-Sheshtawy *et al.*, 2021).

The cytokine response pattern observed likely reflects dose- and time-dependent modulation by AFB1, influenced by tissue susceptibility, host species, and other experimental conditions. Therefore, the increased mRNA expression of pro-inflammatory cytokines in this case may represent a compensatory response due to AFB1-mediated suppression of cytokine protein synthesis.

It is suggested that an imbalance may exist between beneficial and harmful bacterial populations in the intestines of broilers not subjected to stress (Almatary *et al.*, 2026). Also, the optimal performance in broilers is associated with maintaining a healthy microbial balance (Hossain *et al.*, 2026). However, stressors such as exposure to aflatoxins tend to reduce the numbers of beneficial bacteria while allowing harmful bacteria to proliferate and this microbial disruption can result in overt clinical signs like diarrhea or more subtle issues such as reduced growth performance and impaired digestion.

Although the gut harbors naturally protective microbial communities, their composition is susceptible to changes influenced by environmental conditions and dietary inputs (Lutful Kabir, 2009). Consequently, careful management of these external factors could help in reestablishing the population of advantageous protective bacteria (Almatary *et al.*, 2026).

In the current study, the inclusion of AB11, particularly at 400 mg/kg (G-4), significantly reduced TBC, TMYC, and coliform counts, surpassing the reductions

observed in the infected group (G-2) and the untreated group (G-1). Additionally, LAB levels increased notably in groups supplemented with AB11, particularly in G-4. Our finding in concur with Skalny *et al.* (2024) who found that probiotics have been shown to alleviate AFB1-induced toxicity by fortifying intestinal barrier function, rebalancing gut microbiota composition, and facilitating AFB1 biotransformation into non-toxic metabolites.

In a compelling translational study, 12-week administration of the multi-strain probiotic ProBiotic-4 to senescence-accelerated SAMP8 mice not only enhanced cognitive performance and mitigated neuronal degeneration but also favorably altered fecal and cerebral microbiota profiles. These neuroprotective effects were mediated through reduced permeability of both intestinal and blood-brain barriers, attenuated systemic/cerebral inflammation (IL-6, TNF- α , LPS), inhibition of the TLR4/MyD88/NF- κ B axis and microglial activation, and suppression of oxidative/nucleic acid damage markers, including prevention of RIG-I multimerization (Skalny *et al.*, 2024). In another study, it was suggested that probiotics may help combat age-related cognitive decline (Yang *et al.*, 2020).

Conclusions: This study has successfully identified *L. plantarum* AB11 as a potent probiotic strain with exceptional antifungal and antioxidant activities. The *in-vivo* results conclusively demonstrated that supplementation with *L. plantarum* AB11 effectively counteracted aflatoxicosis in quails by improving growth, enhancing liver function, reducing oxidative stress, boosting immunity, and modulating gut microbiota. Importantly, the downregulation of key genes (*CYP1A1*, *AHR1*, *IL-1 β* , *Casp-3*) confirmed that the protective effects of AB11 operate at the molecular level, mitigating the fundamental pathways of aflatoxin-induced toxicity. Translating the promising protective effects of AB11 to commercial poultry production requires rigorous field validation under naturally contaminated feed conditions, where multifactorial stressors more accurately reflect real-world challenges.

Authors contribution: Conceptualization, SAA, MFA, ANA, HA, NAB, RO, and MAAAN; Formal analysis, NA, SAA, SAA, NAA, MAR, and HAE; Investigation, SAA, MFA, ANA, HA, NAB, RO, and MAAAN; Data curation, NA, SAA, SAA, NAA, MAR, and HAE; Writing original draft preparation, SAA, MFA, ANA, HA, NAB, RO, and MAAAN; Writing final manuscript and editing, NA, SAA, SAA, NAA, MAR, and HAE; Visualization and methodology, SAA, MFA, ANA, HA, NAB, RO, MAAAN, NA, SAA, SAA, NAA, MAR, and HAE; All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest: The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Acknowledgements: The authors gratefully acknowledge Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2026R367), Princess Nourah bint Abdulrahman

University, Riyadh, Saudi Arabia. The authors extend their appreciation to the Deanship of Research and Graduate Studies at King Khalid University for funding this work through a Large Research Project under grant number RGP2/696/46. We appreciate the financial support from the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia (Grant No. KFU266041). The authors extend their appreciation to the Deanship of Scientific Research at Northern Border University, Arar, KSA for funding this research work through the project number "NBU-FPEJ-2026-1261-03".

Funding: This research was funded by Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2026R367), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia. The authors extend their appreciation to the Deanship of Research and Graduate Studies at King Khalid University for funding this work through a Large Research Project under grant number RGP2/696/46. This research was funded by Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia (Grant No. KFU266041). This research was funded by Deanship of Scientific Research at Northern Border University, Arar, KSA for funding this research work through the project number "NBU-FPEJ-2026-1261-03".

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