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RESEARCH ARTICLE

# The Influence of *Lactobacillus casei* FA23 as Probiotic and Feed Additive on Enhancing Growth, Health, Gut Integrity, Cecal Microbiota, and Meat Quality in Japanese Quails

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## ABSTRACT

The study investigated the potential of a novel probiotic candidate, *Lactobacillus casei* FA23, as an alternative to antibiotics in poultry nutrition. Out of 45 isolates, *L. casei* FA23 was selected based on its superior antimicrobial activity and was identified using MALDI-TOF MS. The selected isolate demonstrated strong probiotic properties, including high tolerance to simulated gastrointestinal conditions (89.6% viability at pH 2.5 and 0.3% bile salts), pronounced auto-aggregation (71.2%), and notable cell-surface hydrophobicity (58.3%) ($P<0.05$). *In vitro*, *L. casei* FA23 exhibited potent antioxidant capacity ($IC_{50}$ = 100µg/mL; $p<0.05$) and broad-spectrum antimicrobial activity against significant poultry pathogens, with MICs of 50µg/mL of cell-free extract against *Staphylococcus aureus* and *Candida albicans*. In a 42-day trial involving Japanese quail, dietary supplementation with *L. casei* FA23 (50–250mg/kg feed) substantially enhanced growth compared to control and antibiotic performance ($P<0.05$). At a dosage of 200mg/kg, responses were comparable to those of chlortetracycline, with a higher final body weight (250.3 vs. 235.2g), an improved feed conversion ratio (2.95 vs. 3.18), and reduced mortality ($P<0.05$). Supplementation improved serum biochemistry (organ function and lipid profile), humoral immunity (IgG, IgM), and systemic antioxidant status ($P<0.05$). At the molecular level, *L. casei* FA23 upregulated genes related to the intestinal barrier (*Occludin, MUC2*), innate defense (*AvBD6*, lysozyme), and nutrient transport (*SGLT1*), while downregulating IL 1β ($P<0.05$), indicating improved gut integrity and reduced inflammation. Meat quality was enhanced by higher protein content, lower fat content, better water-holding capacity and tenderness, and decreased TBARS and TVBN ($P<0.05$). Cecal microbiota shifted toward a healthier profile, with lower overall bacterial and fungal counts and increased lactic acid bacteria. Overall, *L. casei* FA23 is a potent, multifunctional probiotic and a promising natural alternative to in-feed antibiotics in quails.

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## INTRODUCTION

The Japanese quail has become a key species in poultry production, valued for its high-quality organic meat and eggs that greatly contribute to human nutrition (Zhang *et al.,* 2021). Improving poultry productivity requires a thorough understanding of how diet affects systemic physiology, which can be achieved by implementing advanced nutritional strategies (Korver, 2023). As there is increasing pressure to replace in-feed antibiotics with alternatives that provide similar economic benefits, developing sustainable, biologically derived feed additives has become essential for modern intensive poultry systems (Rafiq *et al.,* 2022). Within this context, a primary concern regarding poultry diets is how feed additives affect gut health and function (Chen *et al.,* 2022). Certain feed supplements, such as botanicals, electrolyte blends, prebiotics, probiotics, and organic acids, have positive effects on animal physiology and performance. Conversely, harmful agents, including mycotoxins and other foodborne contaminants, can damage intestinal tissues, weaken immune function, and reduce productivity and product safety (Stoev, 2025). Properly chosen feed additives can maximize feed utilization, promote growth, and improve health indicators in birds, while also reducing risks posed by harmful factors. In modern poultry nutrition, significant interest has been shown in feed additives such as prebiotics, probiotics, and synbiotics due to their strong potential to enhance production and decrease reliance on antibiotic growth promoters (Rafiq *et al.,* 2022). Probiotics, in particular, have garnered recognition as efficacious nutritional supplements capable of serving as alternatives to antibiotic growth promoters (Anggriawan *et al.,* 2024). To optimize intestinal health and nutrient utilization in poultry, functional feed formulations frequently incorporate specific probiotic strains, including *Lactobacillus* spp., *Bifidobacterium bifidum*, and *Enterococcus faecium*. These microorganisms beneficially influence the composition of the gut microbiota and help maintain the structural integrity of the intestinal mucosa. The combined use of probiotics with other bioactive substances forms the basis for developing advanced multifunctional feed additives to improve overall flock health and production efficiency (Kaźmierczak-Siedlecka *et al.,* 2021).

Among probiotic candidates, *Lactobacillus* species occupy a central position in poultry nutrition. They adhere to the intestinal epithelium, produce organic acids and bacteriocins that suppress pathogenic bacteria, and stimulate mucosal immune responses, thereby strengthening gut barrier function and resilience (Krysiak *et al.,* 2021). Dietary inclusion of *Lactobacillus* has been linked to increased villus height, a higher villus height-to-crypt depth ratio, improved feed conversion, and greater body weight gain, particularly under conditions in which antibiotic withdrawal has created gaps in disease control and performance consistency (Chai *et al.,* 2023).

Multiple studies have reported the favorable effects of *Lactobacillus* species on key poultry production traits. Supplementation with *L. paracasei* and *L. rhamnosus* improved body weight gain, feed intake, and feed conversion ratio in chickens (Xu *et al.,* 2019; Eglite *et al.,* 2023). Likewise, *L. reuteri* enhanced growth performance and intestinal morphology in *E. coli*–challenged broilers (Li *et al.,* 2025). In terms of blood biochemistry, *L. farciminis* and *L. rhamnosus* increased serum total protein, albumin, and globulin while lowering cholesterol and triglyceride levels (Eglite *et al.,* 2023).

On meat quality, *L. farciminis* and *L. rhamnosus* positively influenced fatty acid profiles (e.g., higher omega-3 and omega-9, lower saturated fats) and cholesterol content in broiler breast muscle (Eglite *et al.,* 2023). Additionally, *Lactobacillus* strains reshape the gut microbiota by boosting beneficial *Lactobacillus* populations and reducing pathogenic microbiota such as *E. coli* and *Salmonella* in the cecum and ileum (Jia *et al.,* 2025).

Despite these advances, significant research gaps remain regarding *Lactobacillus casei* FA23, particularly in Japanese quails. Prior studies have primarily focused on broilers and other chickens, employing strains such as *L. acidophilus*, *L. plantarum*, or *L. rhamnosus*, with limited data available for quails. To date, no reports have examined the quail-specific effects of *L. casei* FA23 on growth metrics, serum biochemical profiles, meat quality traits (including fatty acid composition and tenderness), or intestinal health parameters (such as villus architecture and microbiota diversity). This lack of information is notable because Japanese quails differ from chickens in growth rate, metabolic characteristics, and carcass composition, which may influence their response to probiotic supplementation. The current study fills this gap by evaluating the effectiveness of *L. casei* FA23 supplementation in growing quails and providing new insights into its effects on quail production, health, and product quality. Since probiotics have been proven to improve poultry health and productivity, it was hypothesized that including them in the diet would surpass control diets by enhancing key performance indicators such as growth rate, feed efficiency, immune response, and gastrointestinal health. Therefore, this research aimed to assess the impact of *Lactobacillus casei* FA23, isolated from poultry feces, on growth performance, carcass characteristics, blood biochemistry, intestinal health, and meat quality in Japanese quails, highlighting its potential as a targeted probiotic for this increasingly significant poultry species.

## MATERIALS AND METHODS

**Isolation, Screening, and Identification of Selected Isolates:** Fresh quail fecal samples were collected directly in sterile containers at the farm and transported to the microbiology laboratory under cooled conditions, where they were processed within 24 hours. Approximately 10 g of feces from each sample was blended with 90mL of peptone buffer to create an initial $10^{-1}$ suspension, which was then serially diluted tenfold up to $10^{-7}$. Aliquots from each dilution were plated on MRS agar and incubated at 37°C for 24 hours. Colonies were screened for antagonistic activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* with clear inhibition (Reda *et al.,* 2025).

The most promising isolate was initially characterized using classical criteria, including colony and cell morphology, Gram reaction, and a series of biochemical and physiological tests, in accordance with the taxonomic schemes outlined in Bergey's Manual of Systematic Bacteriology. For precise taxonomic assignment, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was used. Protein extracts from single colonies were prepared and spotted onto a Microflex LT/SH MALDI-TOF MS target (MALDI Biotyper, Bruker Daltonics, Bremen, Germany), overlaid with matrix solution, and analyzed. The resulting spectra were matched against the Bruker Biotyper reference database to obtain rapid, species-level identification (Fraser *et al*., 2016).

**Assessment of probiotic properties:** The probiotic characteristics of the *Lactobacillus casei* FA23 isolate were evaluated through a series of in vitro assays, including tolerance to acidic pH, bile salts, and NaCl, as well as autoaggregation, coaggregation, and cell-surface hydrophobicity. Acid tolerance was assessed by inoculating an overnight culture grown in MRS broth (1% v/v inoculum) into fresh MRS broth adjusted to pH 2.5 with 1 N HCl and incubating at 37°C for 3.0h. An identical culture grown in MRS broth at pH 6.5 served as a positive growth control, whereas sterile MRS broth without inoculum was used as a negative control. Viable counts before and after incubation were determined by serial dilution and plating on MRS agar, and survival was expressed as percentage (%) viability. Bile tolerance was evaluated using MRS broth supplemented with 0.3% (w/v) oxgall, with cultures incubated at 37°C for 3.0h and viable counts expressed as % survival. Cultures grown in MRS broth without bile salts were used as positive controls, and uninoculated bile-supplemented MRS served as the negative control. NaCl tolerance was determined by culturing the isolate in MRS broth containing 2.0–6.5% (w/v) NaCl, with growth monitored after 24.0h at 37°C by measuring optical density at 600nm; MRS broth without added NaCl served as the positive control, and uninoculated MRS broth as the negative control. Auto-aggregation and co-aggregation assays were performed using standard methods. These methods relied on the decrease in turbidity at 600nm after incubation for 4.0 hours at room temperature (25°C).

*Escherichia coli* ATCC 25922 was used as the model pathogen in coaggregation tests, and non-mixed monocultures of each strain served as negative controls. Cell surface hydrophobicity was determined using the microbial adhesion to hydrocarbons (MATH) method, in which bacterial suspensions prepared in phosphate-buffered saline (PBS; g/L: 8.0 NaCl, 0.2 KCl, 1.44 $Na_2HPO_4$, 0.24 $KH_2PO_4$; pH 7.2) were mixed with xylene in a 1:1 (v/v) ratio, vortexed, and allowed to separate. A bacterial suspension mixed with PBS without xylene was included as a positive control for maximal optical density, and PBS alone was used as the negative control.

## Biological Activities

**Antioxidant:** The radical scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) by *L. casei* FA23 was assessed in bacterial suspensions at concentrations of 50, 100, 150, 200, and 250µg/mL, in accordance with Abdel-Moneim *et al.* (2022). In summary, 0.5mL of ethanolic DPPH solution was combined with 1.0mL of *L. casei FA23* suspension, and the mixture was incubated in the dark at ambient temperature for 30 min. The absorbance was then measured at 517nm using a spectrophotometer (Shimadzu, Japan). The half-maximal inhibitory concentration ($IC_{50}$) is defined as the lowest concentration (µg/mL) needed to neutralize 50% of DPPH radicals (El-Saadony *et al.*, 2022).

**Antimicrobial:** Antimicrobial activity was evaluated using a disc diffusion assay adapted from standard methods for *Lactobacillus* postbiotics. Suspensions of *L. casei* FA23 were prepared at 50, 100, 150, 200, and 250µg/mL. Sterile 8 mm filter paper discs were submerged in each suspension for 30 minutes to achieve complete saturation, then placed on Mueller–Hinton agar plates inoculated with chicken pathogens (0.5 McFarland). *Escherichia coli, Salmonella Typhimurium, Campylobacter jejuni, Staphylococcus aureus*) and opportunistic fungi (*Aspergillus flavus, Candida albicans*). The agar plates were incubated for 24h at 37°C under appropriate aerobic or microaerophilic conditions, depending on the test organism. After incubation, the diameters of inhibition zones were measured in millimeters to quantify antimicrobial efficacy (Saad *et al*., 2021).

The minimum inhibitory concentration (MIC) was determined by a broth microdilution assay performed following standardized guidelines. Serial twofold dilutions of the antimicrobial preparation were prepared in suitable broth in 96-well microtiter plates, covering a range that included and extended beyond the concentrations used in the disc diffusion test. Wells were inoculated with standardized bacterial or fungal suspensions ($5 \times 10^5$ CFU/mL for bacteria), then incubated under appropriate conditions for 16–24h (Andrews, 2001).

**Experimental Design:** A total of 330 Japanese quails (10 days old, clinically healthy, and of similar body weight) were assigned to six dietary treatments in a completely randomized design. Each treatment had five replicates, with 11 birds per replicate (n = 55 per treatment), and the trial lasted 42 days.

All birds received a corn–soybean basal diet formulated to meet or exceed NRC (1994) Specifications for broilers, excluding antibiotic growth enhancers. The ingredient mix and the nutritional content of the baseline diet were previously detailed by Al-Quwaie (2023).

Dietary treatments were as follows: T1, control group receiving only the basal diet; T2, basal diet + virginiamycin 50mg/kg feed; T3, basal diet + *Lactobacillus casei* FA23 at 50mg/kg feed; T4, basal diet + *L. casei* FA23 at 100mg/kg feed; T5, basal diet + *L. casei* FA23 at 200mg/kg feed; T6, basal diet + *L. casei* FA23 at 250mg/kg feed.

The *L. casei* FA23 culture was grown in nutrient broth at 37°C for 24h, harvested by centrifugation, and resuspended to a final concentration of $1 \times 10^8$ CFU/mL. The bacterial suspension was lyophilized to give 2.5g, then incorporated into the feed to achieve the targeted inclusion levels (mg/kg), ensuring homogeneous distribution. Quails were housed in three-tier battery cages equipped with nipple drinkers and trough feeders, under standard management and environmental conditions appropriate for the species. Birds were monitored daily for health status and general behavior, and all management procedures were kept identical across treatments.

All animal-related procedures complied with institutional and national regulations for the care and use of laboratory animals. The experimental protocol was evaluated and approved by the Institutional Animal Care and Use Committee, in line with the ethical oversight typically described in comparable broiler probiotic research.

**Growth Performance:** The growth performance of the birds was evaluated during the trial period by measuring body weight (LBW), weight gain (WG), and feed intake (FI). Using these primary data sources, we calculated the feed conversion ratio (FCR) and feed efficiency was also determined. All calculations were executed according to Mohanta *et al*. (2022) and Saad *et al.* (2022).

Weight gain (WG) = Final weight (FW) – Initial weight (IW)

**Biochemical Parameters:** Blood and tissue samples were collected at the end of the 42-day experimental period. From each treatment group, five birds (one bird per replicate; n = 3 per treatment) were selected at random and humanely euthanized using an R550 multi-output small-animal anesthesia system with isoflurane in oxygen. Induction was performed in an anesthetic chamber with a controlled fresh gas flow of 1.0–2.0 L/min until loss of posture and absence of reflexes were confirmed.

Immediately after deep anesthesia was confirmed, venous blood was collected from the hepatic portal vein using heparinized syringes and transferred to lithium-heparin tubes, which were kept on ice until processing. Blood samples were centrifuged at 5,000 × g for 15 min at 4°C to obtain plasma/serum for biochemical analysis. Functions of hepatic enzymes: alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and the AST/ALT ratio were determined using commercial diagnostic kits (Spinreact, Spain), following the manufacturer's instructions and the procedure described by Cheng *et al.* (2020), using an automated biochemical analyzer calibrated for poultry serum.

Liver samples were excised immediately after blood collection, rinsed in ice-cold 0.9% (w/v) physiological saline (4°C), blotted dry, and weighed. Subsamples were snap-frozen in liquid nitrogen and stored at −70 to −80°C for subsequent biochemical or molecular analyses, as rapid cooling and storage at ≤−70°C preserve enzyme and protein integrity.

Serum immunoglobulin A (IgA) and immunoglobulin M (IgM) concentrations were quantified using species-specific commercial chicken ELISA kits (Chicken IgA/IgM ELISA kits, Cloud-Clone, China). Assays were performed by sandwich ELISA in 96-well plates according to the manufacturer's protocols, and optical density (OD) was read at 450 nm using a microplate reader (BioTek ELx800, USA). Standards and samples were analyzed in duplicate, and intra-assay coefficients of variation were maintained below 10% as described by Merino-Guzmán *et al.* (2017).

**Gene Expression:** Total RNA was extracted from intestinal tissue using an RNase-free protocol, and the final RNA pellet was dissolved in diethylpyrocarbonate (DEPC)-treated water to prevent RNase-mediated degradation. RNA concentration and purity were assessed spectrophotometrically by measuring absorbance at 260 and 280 nm and calculating the A260/A280 ratio, as routinely used for RNA quality control in molecular studies. Only samples with A260/A280 ratios between ~1.8 and 2.1 were used for downstream analyses to ensure adequate purity for reverse transcription and qPCR (Stoikov *et al.* 2023; Zeng *et al.* 2024).

For first-strand cDNA synthesis, 3 µg of total RNA per sample were denatured and then immediately chilled on ice. The reverse-transcription (RT) mixture contained template RNA, 0.5µg oligo(dT)18 primers, 2µL of 10× RT buffer, 2µL of 10mM dNTP mix, 20–40 U RNase inhibitor, and 100 U of reverse transcriptase in a final volume of 20µL, following standard two-step RT-qPCR protocols. The reaction was incubated at 42–45°C for 30–60 min for cDNA synthesis, followed by enzyme inactivation at 70–85°C for 5–10 min. The cDNA was stored at −20°C until quantitative PCR analysis.

Each 10–20µL qPCR reaction contained 1–2µL of diluted cDNA, 200–400nM of each gene-specific forward and reverse primer (primer sequences listed in Table 1), 1× SYBR Green qPCR mix, and nuclease-free water. Thermal cycling was performed on a real-time PCR system with an initial denaturation at 95°C for 2–3 min, followed by 40 cycles of 95°C for 3–10s and 58–60°C for 20–30s for annealing/extension, with a final melt-curve analysis to verify amplification specificity. Relative mRNA expression levels were evaluated via $2^{-}\Delta\Delta Ct$ comparative technique with β-actin as the endogenous reference gene, following established procedures for qRT-PCR data analysis. Cycle threshold (Ct) values were used to derive ΔCt (target – actin) and ΔΔCt (treatment – control), enabling comparison of inter-sample expression levels using validated quantification approaches.

**Intestinal Microbial Quantification:** The intestinal digesta specimens were aseptically obtained, blended in sterile glass tubes, stored at 4°C, and processed within 4–6h to minimize alterations in microbial viability. Serial tenfold dilutions were prepared in sterile 0.85–0.9% saline or phosphate-buffered saline, and appropriate dilutions were plated for enumeration of different microbial groups following selective culture protocols adapted from (Abd El-Wahab *et al.* 2022; Wilson *et al.* 2024). Total aerobic bacterial count (TBC) was determined on Plate Count Agar (PCA; Oxoid or equivalent) incubated aerobically at 35–37°C for 24–48h. Total yeasts and molds (TYM) were enumerated on Sabouraud Dextrose Agar or Potato Dextrose Agar supplemented with chloramphenicol, incubated aerobically at 25–28°C for 3–5 days. Lactic acid bacteria (LAB) were quantified on de Man–Rogosa–Sharpe (MRS) agar (Difco, USA, or equivalent); plates were incubated under anaerobic or microaerophilic conditions (e.g., GasPak system) at 30–37°C for 48–72h. When necessary to suppress yeast growth, MRS agar was supplemented with cycloheximide, as commonly applied for LAB enumeration. After incubation, plates with 30–300 colonies were selected for counting. The numbers of colonies were multiplied by the reciprocal of the dilution factor to obtain colony-forming units per gram of digesta (CFU/g). For standardized data representation and statistical analysis, microbial counts for TBC, TYM, and LAB were transformed to log10CFU/g of digesta.

## Meat quality

**Sampling and preparation:** Quails were subjected to a fasting period of 6–8 hours, slaughtered in accordance with standard humane procedures, eviscerated, and subsequently chilled at 4°C for 24h prior to measurements (Dos Santos *et al.*, 2020). Breast and thigh muscles were excised from the left side of the carcass, trimmed of visible fat and connective tissue, and used for meat quality analyses (Rehman *et al.*, 2021).

**pH measurement:** The ultimate pH was measured 24 h postmortem in the breast and thigh tissues utilizing a calibrated penetration pH meter. The electrode was inserted at three distinct locations per sample, and the readings were subsequently averaged (Muraduzzaman *et al.*, 2023).

**Color measurement:** Meat surface color (*L*, a*, b**) was recorded on freshly cut breast and/or thigh surfaces after 20–30 min blooming at 4°C, using a portable colorimeter under D65 illumination and 10° standard observer (Gibson and Makir, 2019). Three readings were taken per sample at different points and averaged to obtain *L** (lightness), *a** (redness), and *b** (yellowness) values.

**Table 1:** Primer sequences and molecular specifications for studied genes

| Gene Symbol | Gene Name / Function | Primer Sequence (5' → 3') | Amplicon Size (bp) | Annealing Temp. (°C) | Accession No. (Source) |
|---|---|---|---|---|---|
| Reference Gene | | | | | |
| *GAPDH* | Glyceraldehyde-3-phosphate dehydrogenase | F: ATCACTGCCACCCAGAAGAC<br>R: CATCACGCCACAGCTTTCCA | 152 | 60 | XM_047698765.1 (*Coturnix japonica*) |
| Mucosal Integrity & Barrier | | | | | |
| *OCLN* | Occludin | F: TGTGCCCTACTTCACCTACGC<br>R: GCTGGAATAGTGGGTGACAGG | 118 | 60 | XM_015857184.2 (*C. japonica*) |
| *MUC2* | Mucin 2 | F: TGCTGACGAGTGTGATGAGC<br>R: AGCTCCAGGTCCAGTTGTTC | 145 | 59 | XM_015858486.2 (*C. japonica*) |
| Innate Immunity & Defense | | | | | |
| *AvBD6* | Avian Beta-Defensin 6 | F: CCTGCTCCTGCTGCTACTTC<br>R: GAGGTAGTCCTTGCCCGTTC | 102 | 60 | XM_047716943.1 (*C. japonica*) |
| *LYZ* | Lysozyme C | F: GGCTGGACACCATTGACTTC<br>R: CCAGCGTTGTAGGTGTTGGA | 96 | 59 | XM_015862802.2 (*C. japonica*) |
| Inflammatory Cytokines | | | | | |
| *IL-1β* | Interleukin-1 Beta | F: AGCTCCGACAGCACATTCAC<br>R: TCCACGGGCAAGACATAGAG | 131 | 60 | XM_015856715.2 (*C. japonica*) |
| *IL-10* | Interleukin-10 | F: CCTGCTCAGCACTGCTATGC<br>R: TTCTGGGGCATCTTCTCCAC | 108 | 60 | XM_047706382.1 (*C. japonica*) |
| Nutrient Transport | | | | | |
| *SGLT1* | Sodium/Glucose Cotransporter 1 | F: GGCTTCATCGTCATCGTGTC<br>R: CAGCATAGCCAGGTCATCCA | 124 | 60 | XM_015857090.2 (*C. japonica*) |

**Water-holding capacity (drip loss and cooking loss):** Drip loss: Approximately 20–30g of breast meat was weighed (W1), suspended in an airtight plastic bag without contact with the bag walls, stored at 4°C for 24h, gently blotted, and reweighed (W2); drip loss (%) was calculated as [(W1−W2)/W1×100] (Hussnain *et al.*, 2020).

Cooking loss: Standardized meat pieces were weighed, cooked in sealed polyethylene bags in a water bath at 75–80°C until reaching 72°C internal temperature, cooled to room temperature, blotted, and reweighed; cooking loss (%) was calculated as (Raw weight−Cooked weight)/Raw weight×100 (da Silva *et al.*, 2017).

**Tenderness (shear force):** Cooked samples (used for cooking loss) were cut into rectangular strips (1cm × 1cm cross-section, 2–3cm length) with fibers parallel to the long axis. Warner–Bratzler shear force was determined perpendicular to the fiber direction using a texture analyzer fitted with a V-shaped blade, and peak shear force (N) was recorded and averaged over 3–5 subsamples per bird (Rehman *et al.,* 2021).

**Proximate composition:** Moisture, crude protein, ether extract, and ash of breast and thigh meat were analyzed according to AOAC procedures (oven drying for moisture, Kjeldahl for nitrogen, Soxhlet for fat, muffle furnace for ash) and expressed on a percentage of fresh weight basis (Bostami *et al.*, 2021).

**Chemical freshness/oxidation indices:** Total volatile nitrogen (TVN, mg/100g) and thiobarbituric acid reactive substances (TBA, mg malondialdehyde/kg) were determined in minced quail meat to assess protein and lipid deterioration, respectively, following standard spectrophotometric methods (Edris *et al.*, 2014).

**Statistical Analysis:** Statistical analysis was conducted using SPSS (Version 23.0; IBM, United States of America). We reported the results as the mean ± standard deviation. The analysis of variance was used on the data in a one-way fashion. Using Fisher's least significant difference (LSD) test at the $P<0.05$ significance level, the group means were compared.

## RESULTS

**Isolation, screening, and identification of the selected isolate:** A total of 45 bacterial isolates were obtained from fecal samples and designated FA1–FA45. All isolates were screened for antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* using the agar diffusion method. Mean inhibition zone diameters differed significantly among isolates ($P<0.05$). Five isolates (FA9, FA11, FA23, FA31, and FA30) produced the largest inhibition zones, with mean values of 15, 25, 32, 23, and 20 mm, respectively ($P<0.05$ vs. the overall mean of the remaining isolates). Isolate FA23 showed the greatest inhibition zone (32mm), which was significantly larger than that of FA9, FA30, and the group of lower-activity isolates ($P<0.05$), and was therefore selected for further characterization.

Isolate FA23 displayed morphological and biochemical characteristics consistent with *Lactobacillus*. Cells were Gram-positive, non-spore-forming rods occurring singly or in short chains, catalase- and oxidase-negative, non-motile, and facultatively anaerobic. Growth in MRS broth resulted in lactic acid production, moderate acidification, and no gas formation. The isolate fermented glucose, lactose, maltose, and sucrose. Colonies on MRS agar were circular, convex, creamy white, and smooth. No growth was observed in media containing bile salts at the tested concentration. Based on these traits, FA23 was presumptively identified as *Lactobacillus casei*. MALDI-TOF MS confirmed this identification, with an identification score of 2.28 and the highest similarity to *L. casei* DSM 20011.

**Probiotic properties of FA23 isolate:** *Lactobacillus casei* FA23 maintained 89.6% survival after 3h exposure to pH 2.5 and 0.3% bile salts. The isolate grew in media containing up to 6.5% NaCl. Auto-aggregation and co-aggregation values were 71.2 and 64.5%, respectively. Cell-surface hydrophobicity was 58.3%.

## Biological activities

**Antioxidant activity:** *Lactobacillus casei* FA23 showed concentration-dependent DPPH radical scavenging activity with an $IC_{50}$ of 100µg/mL (Fig. 1).

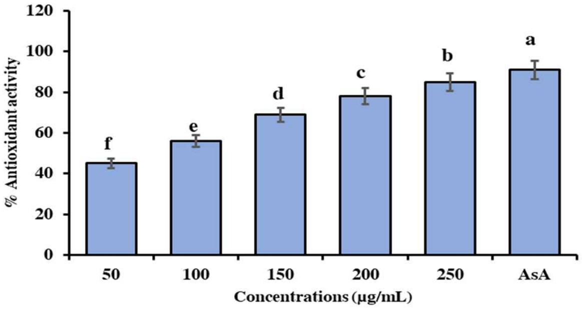

**Fig. 1:** Antioxidant activity of *Lactobacillus casei* FA23 at different concentrations against DPPH free radicals. IC50=100µg/mL.

**Antimicrobial activity:** The antimicrobial activity of *L. casei* FA23 increased with concentration (Table 2). Inhibition zones ranged from 8.2–12.5mm at 50µg/mL to 17.9–32.0mm at 250µg/mL. The inhibition zones significantly increased in a concentration-dependent manner (P<0.05). Inhibition zones were comparable to those obtained with gentamicin and nystatin (P=0.0395). Minimum inhibitory concentration (MIC) values differed significantly among the tested microorganisms (P<0.05). The lowest MIC (50µg/mL) was recorded for *Staphylococcus aureus* and *Candida albicans*, followed by *Salmonella Typhimurium* (100µg/mL). *Escherichia coli, Campylobacter jejuni,* and *Aspergillus flavus* exhibited intermediate susceptibility, with MIC values ranging from 150 to 200µg/mL. In contrast, *L. casei* FA23 exhibited the highest MIC statistically (250µg/mL) in comparison to all other tested strains.

**Growth performance:** Initial body weight and feed intake did not differ significantly among treatments (P<0.05) (Table 3). After 42 days, final body weight and body weight gain were higher (P<0.05) in the virginiamycin and 200mg/kg *L. casei* FA23 groups (final BW 252.1g and 250.3g; BWG 241.7g and 239.8g, respectively) compared with the control. Feed conversion ratio decreased from 3.18 in the control to 2.93–2.95 in supplemented groups (P<0.05). Mortality was reduced from 6.7% in the control to 3.3–5.0% in supplemented groups (P<0.05).

**Blood biochemical parameters:** *Lactobacillus casei* FA23 supplementation (200mg/kg) reduced serum AST (225 to 198U/L), ALP (450 to 395U/L), uric acid (6.8 to 5.7mg/dL), and creatinine (0.38 to 0.34mg/dL) verses control (*p*<0.05) (Table 4). Total protein (5.4 vs. control) and albumin (2.6 vs. control) were increased (P<0.05). Total cholesterol, triglycerides, and LDL were significantly decreased (155 to 138mg/dL, 85 to 72mg/dL, and 78 to 64mg/dL, respectively), while HDL increased to 76 mg/dL (P<0.05).

**Antioxidant and humoral status:** Dietary *L. casei* FA23 modulated serum antioxidant indices in a dose-dependent manner (P<0.05) (Table 4). At 200mg/kg, SOD increased from 85 to 110U/mL, CAT from 25 to 33U/mL, and GSH from 15.5 to 20.1µmol/L, whereas MDA decreased from 3.8 to 2.9nmol/mL (P<0.05). At the same dose, serum IgG and IgM also increased.

**Gene expression:** At 200mg/kg *L. casei* FA23, relative mRNA expression (fold change vs. control = 1.00) significantly increased for occludin (1.45-fold), MUC2 (1.52-fold), AvBD6 (1.67-fold), and lysozyme (1.50-fold) (P<0.05) (Table 5). Expression levels of these genes were equal to or greater than those in the antibiotic group. As shown in Fig. 2, IL-1β expression was decreased, while IL-10 and SGLT1 expression significantly increased compared with control (P<0.05).

**Meat quality:** Supplementation with 100–200mg/kg *L. casei* FA23 significantly affected breast meat quality traits (P<0.05) (Table 6). Meat pH decreased from 5.85 to 5.74. Drip loss and cooking loss decreased from 3.2 to 2.5% and from 18.5 to 16.2%, respectively. Shear force declined from 24.5 to 19.5 N (P<0.05). Crude protein content increased from 22.1 to 23.8%, and ether extract decreased from 2.5 to 2.0%. TVB-N decreased to 9.8mg/100g and TBARS to 0.41mg MDA/kg. Meat color parameters (*L*, a*, b**) were analysed as presented in Fig. 3.

**Table 2:** Antimicrobial activity and minimum inhibitory concentration (MIC) of *Lactobacillus casei* FA23 isolate against poultry pathogens

| Microorganism | Concentration’s µg/ml/inhibition zone diameters (mm) | | | | | Positive Control* | MIC (µg/ml) |
|---|---|---|---|---|---|---|---|
| | 50 | 100 | 150 | 200 | 250 | | |
| Bacteria | | | | | | | |
| *Escherichia coli* | 8.2±0.8e | 11.5±0.5d | 15.1±0.7c | 17.6±0.4b | 19.0±0.9b | 22.5±0.5a | 150 |
| *Salmonella Typhimurium* | 9.5±0.5e | 13.2±0.8d | 16.8±0.4c | 19.1±0.6bc | 20.8±0.7b | 24.0±0.3a | 100 |
| *Campylobacter jejuni* | - | 10.3±0.9d | 13.7±0.7c | 16.2±0.8bc | 17.9±0.5b | 21.0±0.4a | 200 |
| *Staphylococcus aureus* | 10.1±0.7e | 14.8±0.6d | 18.5±0.9c | 21.3±0.5bc | 22.9±0.8b | 26.2±0.6a | 50 |
| Fungi | | | | | | | |
| *Aspergillus flavus* | - | 9.1±0.7e | 14.3±0.9d | 20.5±0.8c | 25.7±1.1b | 28.0±0.6a | 200 |
| *Candida albicans* | 12.5±0.6f | 18.3±0.8e | 24.1±0.7d | 28.6±0.9c | 32.0±1.0a | 30.5±0.5b | 50 |
| Negative Control | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | -- | -- |

**Table 3:** Effect of dietary *Lactobacillus casei* FA23 supplementation on the growth performance of quail

| Parameter | Control (Basal Diet) | Antibiotic (CTC* 50 mg/kg) | FA23 (50 mg/kg feed) | FA23 (100 mg/kg feed) | FA23 (200 mg/kg feed) | FA23 (250 mg/kg feed) |
|---|---|---|---|---|---|---|
| Initial Body Weight (g/bird) | 10.5±0.5 | 10.4±0.4 | 10.6±0.6 | 10.5±0.5 | 10.5±0.4 | 10.6±0.5 |
| Final Body Weight (g/bird) | 235.2±8.7c | 252.1±7.5a | 241.8±6.9b | 248.6±7.1b | 250.3±6.3ab | 246.5±8.2 |
| Weight Gain (g/bird) | 224.7±8.5c | 241.7±7.3a | 231.2±6.8b | 238.1±7.0ab | 239.8±6.2ab | 235.9±8.0 |
| Feed Intake (g/bird) | 715.3±21.4a | 708.8±19.6c | 712.5±20.1ab | 710.2±18.9b | 706.4±17.5c | 719.8±22.3 |
| Feed Conversion Ratio (FCR) | 3.18±0.08a | 2.93±0.06b | 3.08±0.07ab | 2.98±0.05b | 2.95±0.06b | 3.05±0.08 |
| Mortality Rate (%) | 6.7±1.2a | 3.3±0.8d | 5.0±1.0b | 4.2±0.9c | 3.8±0.7d | 4.7±1.1 |

**Table 4:** Effect of dietary *Lactobacillus casei* FA23 on blood biochemical parameters in quail (42 days)

| Parameter | Control (Basal Diet) | Antibiotic (CTC 50 mg/kg) | FA23 (50 mg/kg feed) | FA23 (100 mg/kg feed) | FA23 (200 mg/kg feed) | FA23 (250 mg/kg feed) |
|---|---|---|---|---|---|---|
| Liver Function | | | | | | |
| ALT (U/L) | 42.5±3.2a | 40.1±2.9b | 41.8±3.1ab | 39.5±2.7c | 38.2±2.5c | 39.8±2.8c |
| AST (U/L) | 225±18a | 215±15bc | 220±17b | 208±14c | 198±12d | 212±16bc |
| ALP (U/L) | 450±35a | 420±30c | 435±32b | 410±28d | 395±25e | 415±30d |
| Total Protein (g/dL) | 4.8±0.3b | 5.1±0.3ab | 4.9±0.3b | 5.2±0.3ab | 5.4±0.4a | 5.1±0.3ab |
| Albumin (g/dL) | 2.2±0.2b | 2.4±0.2ab | 2.3±0.2b | 2.5±0.2a | 2.6±0.2a | 2.4±0.2ab |
| Kidney Function | | | | | | |
| Uric Acid (mg/dL) | 6.8±0.5a | 6.2±0.4b | 6.5±0.5ab | 6.0±0.4b | 5.7±0.4c | 6.1±0.4b |
| Creatinine (mg/dL) | 0.38±0.03 | 0.36±0.03 | 0.37±0.03 | 0.35±0.02 | 0.34±0.02 | 0.36±0.03 |
| Lipid Profile | | | | | | |
| Total Cholesterol (mg/dL) | 155±12a | 145±10b | 150±11ab | 142±9bc | 138±8c | 144±10b |
| Triglycerides (mg/dL) | 85±7a | 78±6b | 82±7ab | 76±5bc | 72±5c | 77±6bc |
| HDL-Cholesterol (mg/dL) | 65±5c | 70±5b | 67±5c | 72±5ab | 76±6a | 71±5b |
| LDL-Cholesterol (mg/dL) | 78±6a | 70±5b | 75±6a | 68±5c | 64±4d | 69±5c |
| Humoral Immunity | | | | | | |
| IgG (mg/dL) | 2.1±0.2c | 2.4±0.2b | 2.2±0.2c | 2.6±0.2a | 2.8±0.2a | 2.5±0.2ab |
| IgM (mg/dL) | 0.85±0.07d | 0.95±0.08c | 0.88±0.07d | 1.02±0.09b | 1.10±0.10a | 1.00±0.08b |
| Antioxidant Status | | | | | | |
| SOD (U/mL) | 85±7d | 95±8c | 90±8cd | 101±9b | 110±10a | 103±9b |
| CAT (U/mL) | 25±2d | 28±2c | 26±2cd | 30±3ab | 33±3a | 29±2b |
| Glutathione (GSH, µmol/L) | 15.5±1.2c | 17.2±1.3b | 16.0±1.2c | 18.5±1.4ab | 20.1±1.5a | 18.0±1.3ab |
| MDA (nmol/mL) | 3.8±0.3a | 3.4±0.3b | 3.6±0.3a | 3.2±0.2c | 2.9±0.2d | 3.3±0.3b |

**Table 5:** Effect of dietary *Lactobacillus casei* FA23 on gut health and immune-related gene expression in quail intestine

| Gene Category & Target | Control | Antibiotic (CTC 50 mg/kg) | FA23 (50 mg/kg feed) | FA23 (100 mg/kg feed) | FA23 (200 mg/kg feed) | FA23 (250 mg/kg feed) |
|---|---|---|---|---|---|---|
| Mucosal Integrity & Barrier | | | | | | |
| Occludin (OCLN) | 1.00±0.08f | 1.18±0.10d | 1.05±0.09e | 1.32±0.12b | 1.45±0.11a | 1.28±0.10c |
| *Mucin-2 (MUC2)* | 1.00±0.07f | 1.22±0.09d | 1.15±0.08e | 1.41±0.10b | 1.52±0.12a | 1.38±0.11c |
| Innate Immunity & Defense | | | | | | |
| *β-Defensin (AvBD6)* | 1.00±0.10f | 1.31±0.12d | 1.20±0.11e | 1.48±0.13b | 1.67±0.14a | 1.42±0.12c |
| Lysozyme (LYZ) | 1.00±0.09f | 1.25±0.11d | 1.10±0.10e | 1.39±0.12b | 1.50±0.13a | 1.35±0.11c |

**Table 6:** Effect of dietary *Lactobacillus casei* FA23 on meat quality parameters of quail

| Parameter | Control (Basal Diet) | Antibiotic (CTC 50 mg/kg) | FA23 (50 mg/kg feed) | FA23 (100 mg/kg feed) | FA23 (200 mg/kg feed) | FA23 (250 mg/kg feed) |
|---|---|---|---|---|---|---|
| pH Measurement | | | | | | |
| pH (24h) – Breast | 5.85±0.05 | 5.78±0.04 | 5.81±0.04 | 5.76±0.05 | 5.74±0.03 | 5.77±0.04 |
| pH (24h) – Thigh | 6.20±0.06 | 6.15±0.05 | 6.18±0.05 | 6.13±0.05 | 6.10±0.04 | 6.14±0.05 |
| Water-Holding Capacity | | | | | | |
| Drip Loss (%, 24h) | 3.2±0.3a | 2.8± 0.2b | 3.0±0.3ab | 2.7±0.2b | 2.5±0.2c | 2.8±0.2b |
| Cooking Loss (%) | 18.5±1.1a | 17.1±0.9b | 18.0±1.0ab | 16.8±0.8c | 16.2±0.7c | 17.0±0.9b |
| Tenderness | | | | | | |
| Shear Force (N) | 24.5±1.8a | 21.8±1.5b | 23.6±1.7ab | 21.0±1.4b | 19.5±1.2c | 21.5±1.6b |
| Proximate Composition – Breast Meat (%) | | | | | | |
| Moisture | 73.5±0.8 | 72.1±0.7 | 73.0±0.7 | 71.8±0.6 | 71.5±0.6 | 72.0±0.7 |
| Crude Protein | 22.1±0.6 | 23.0±0.5 | 22.4±0.5 | 23.3±0.6 | 23.8 ±0.5 | 23.1±0.6 |
| Ether Extract (Fat) | 2.5±0.3 | 2.2±0.2 | 2.4±0.3 | 2.1±0.2 | 2.0 ±0.2 | 2.2±0.2 |
| Ash | 1.25±0.08b | 1.28± 0.07b | 1.26±0.08b | 1.30±0.06a | 1.32 ±0.07a | 1.29±0.07b |
| Chemical Freshness / Oxidation | | | | | | |
| TVBN (mg/100g) | 12.8± 1.0a | 11.0± 0.8b | 12.0±0.9a | 10.5±0.7bc | 9.8±0.6c | 10.9±0.8bc |
| TBARS (mg MDA/kg) | 0.58± 0.05a | 0.49± 0.04b | 0.54±0.05ab | 0.45±0.04c | 0.41±0.03c | 0.48±0.04b |

Abbreviations: TVB-N: Total Volatile Basic Nitrogen; TBARS: Thiobarbituric Acid Reactive Substances; MDA: Malondialdehyde.

**Microbial count:** Cecal microbial counts are presented in Fig. 4. Dietary *L. casei* FA23 significantly reduced total aerobic bacteria, yeasts, and molds and increased lactic acid bacteria counts compared with the control group, with the largest changes observed at 200mg/kg ($p<0.05$).

## DISCUSSION

These findings indicate that *L. casei* FA23 possesses notable biological activity and exerts broad beneficial effects in quail. The strain shows moderate antioxidant capacity ($IC_{50}$ = 100µg/mL) and strong, dose-dependent antimicrobial activity, with inhibition zones and MIC values comparable to those of conventional antibiotics and antifungals. In *vivo*, dietary supplementation, particularly at 200mg/kg, improves growth performance and feed efficiency, reduces mortality, and favorably modulates blood biochemistry by enhancing liver and kidney function markers and improving lipid profile. The probiotic also strengthens the body's antioxidant defenses and immune system. It increases gene expression involved in the response to the innate immune system and the intestinal barrier, while also reducing inflammation. Additionally, it improves the microbial balance in the cecum by promoting beneficial lactic acid bacteria and reducing potentially harmful microbes. These combined effects lead to better meat quality, including higher protein levels, lower fat content, improved tenderness, and longer shelf life.

In the current study, dietary probiotics were associated with higher final body weight and body weight gain, as well as improved feed conversion efficiency, due to the antioxidant and antimicrobial properties of L casei FA23, which modulate the gut microbiota and oxidative stress. Similar growth-promoting effects of probiotics have been reported in broilers and ducks, where supplementation enhanced average daily gain, lowered feed conversion ratio, and in some cases matched or approached the effectiveness of antibiotic growth promoters (Sun *et al.*, 2022). For instance, Yang *et al.* (2024) observed increased average daily weight gain and a reduced feed–gain ratio in broilers fed *Lactiplantibacillus plantarum* HJLP (Yang *et al.,* 2024), while Pourakbari *et al.* (2016) showed that probiotic levels ≥0.01% improved body weight gain by 12% and lowered feed conversion by 5%. Increases in growth rate and feed efficiency have likewise been documented with *Bacillus*-based preparations and multistrain formulations in broilers and ducks (Younas *et al.*, 2025). These convergent findings support the notion that probiotics can serve as effective growth-promoting feed additives across poultry species.

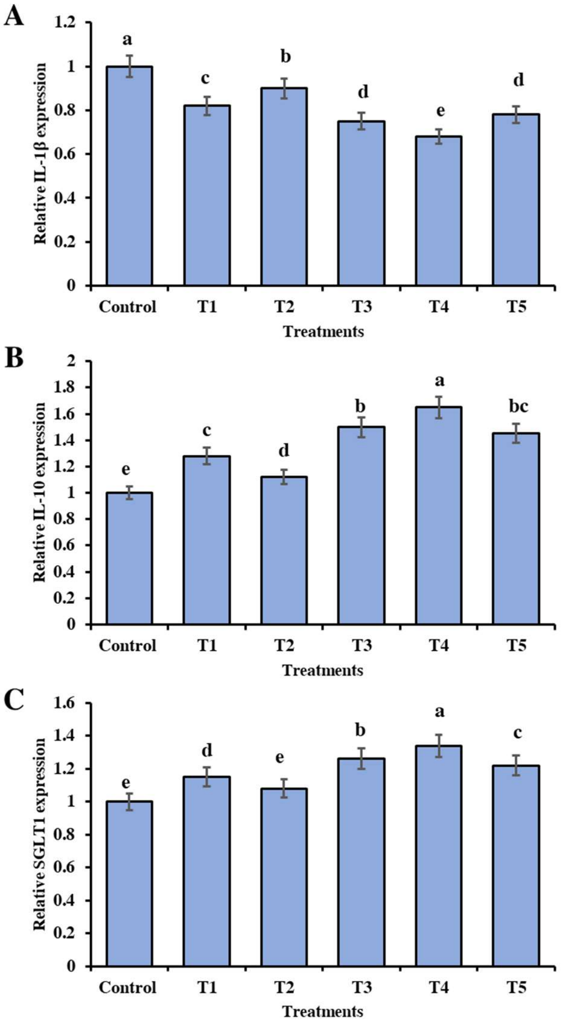

**Fig. 2:** The impact of dietary *Lactobacillus casei* FA23 isolate on the relative gene expression of Inflammatory Cytokines (A) IL-1β, (B) IL-10, and nutrient transport (C) SGLT1 genes in quails.

Probiotic supplementation also influenced carcass and immune organ development, as reflected by increased relative spleen weight in supplemented quails. Studies in broilers similarly show that probiotics and related functional additives can increase the relative weight of lymphoid organs (spleen, bursa of Fabricius, thymus), reflecting stimulation of systemic immune tissues (Soren *et al.*, 2024). Zhang *et al.* (2021) reported higher spleen and bursa indices and improved immune markers in broilers supplemented with drinking water probiotics, while Sun *et al.* (2022) documented enhanced immune indices and intestinal morphology in ducks receiving compound probiotics. These findings align with reports that probiotics modulate gut-associated lymphoid tissue and systemic immunity (Jha *et al.*, 2020; Ebeid *et al.*, 2021).

The current investigation further showed that probiotics positively influenced the serum protein profile, specifically through elevated total protein and albumin concentrations. Analogous increases in serum total protein, albumin, and globulin levels have been documented in poultry receiving functional supplements and probiotics, suggesting improvements in protein metabolism and humoral immune function (Soren *et al.*, 2024). Oleforuh-Okoleh *et al.* (2015) observed augmented serum protein fractions in birds supplemented with yeast or other functional additives, attributing these changes to improved amino acid absorption and protein synthesis. These findings align with existing literature indicating that probiotics can improve nutrient digestibility and nitrogen retention in poultry (He *et al.*, 2019; Sun *et al.*, 2022).

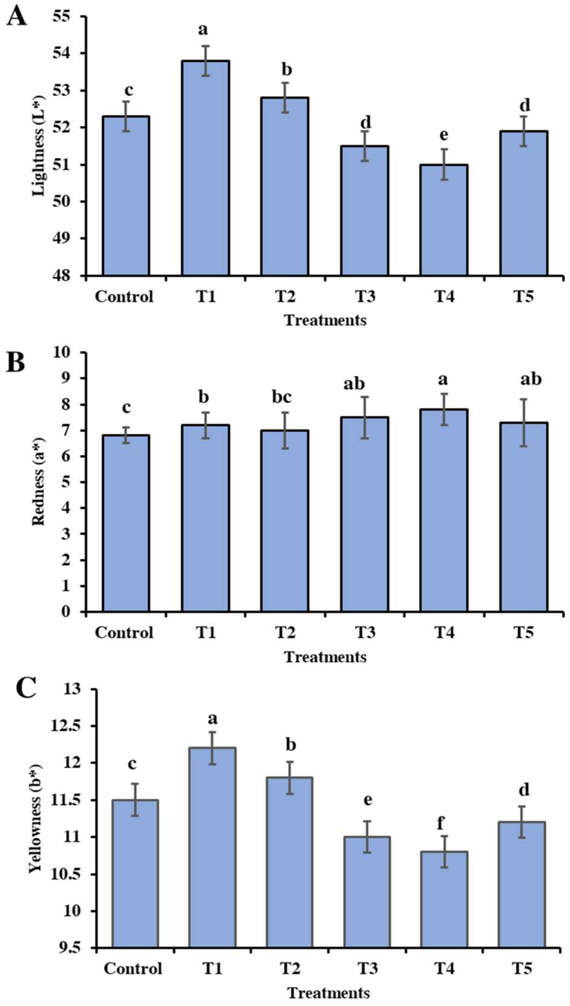

**Fig. 3:** The impact of dietary *Lactobacillus casei* FA23 isolate on the quails' meat color (A) lightness (L), (B) redness (a), and yellowness (b).

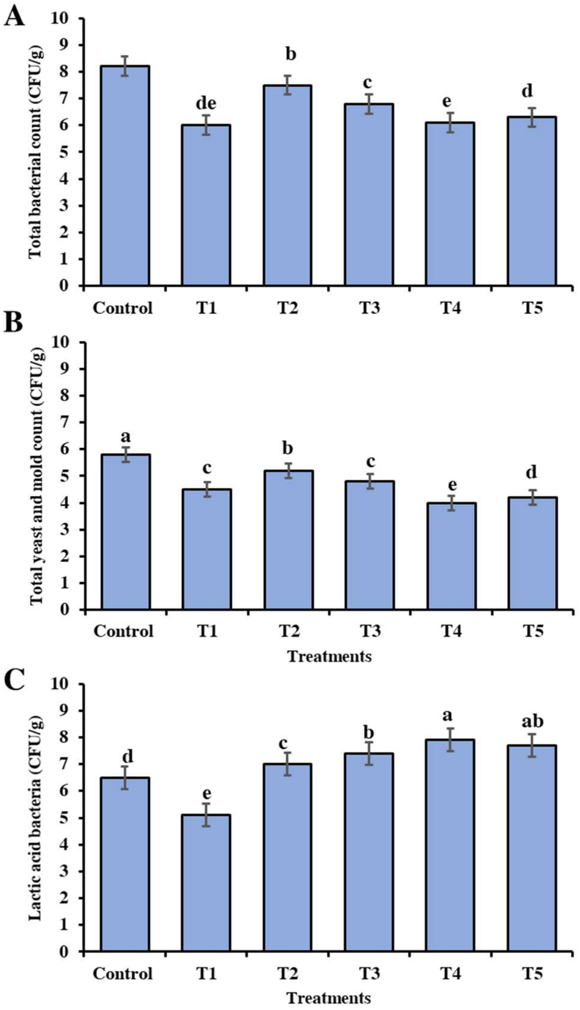

**Fig. 4:** The effect of dietary *Lactobacillus casei* FA23 isolate on regulating the gut microbiota count in quails (A) Total bacterial count (L), (B) Total yeast and molds count, and (C) lactic acid bacteria count.

Supplementation with 200mg/kg of *L. casei* FA23 resulted in the improvement of liver and kidney function markers, specifically evidenced by reductions in AST, ALP, uric acid, and creatinine levels. Additionally, it contributed to an enhanced protein status, as indicated by increased total protein and albumin levels. The intervention also favorably modulated the lipid profile, demonstrated by decreases in total cholesterol, triglycerides, and LDL cholesterol, alongside an increase in HDL cholesterol, thereby indicating a beneficial effect on metabolic health. The observed decreases in nitrogenous waste markers align with existing literature, indicating that probiotics enhance nitrogen utilization while simultaneously diminishing catabolic byproducts. Reduced blood urea nitrogen levels, along with associated indicators, have been correlated with more effective dietary protein utilization and decreased proteolysis (Pourakbari *et al.,* 2016; Sun *et al.,* 2022). These findings support the previously discussed improvements in growth and feed efficiency and have been documented in broilers, where probiotic administration led to decreased ammonia and urea excretion (Yang *et al.,* 2024).

Modulation of serum lipid profile in probiotic-fed quails, characterized by lower triglycerides, total cholesterol, and LDL with increased HDL, is consistent with the hypolipidemic actions reported in broilers and ducks (Khochamit *et al.,* 2021; Shaufi *et al.,* 2024). Pourakbari *et al.* (2016) showed that probiotic supplementation reduced plasma triglycerides and total cholesterol in broilers, while multistrain and *Bacillus*-based preparations have repeatedly been associated with improved lipid indices and reduced blood lipids (Sun *et al.,* 2022; Younis *et al.,* 2025). Yalçın *et al.* (2013) also investigated the reductions in serum cholesterol and triglycerides in poultry fed yeast products. These converging data indicate that probiotic and yeast-based additives can beneficially modulate lipid metabolism in different poultry species. The increases in serum globulin and immunoglobulin fractions observed in the present work are consistent with the immunostimulatory effects of probiotics. Several studies in broilers have documented higher levels of IgG, IgA, or IgM, as well as enhanced cell-mediated responses, following probiotic administration (Yang *et al*., 2024; Younis *et al*., 2025). For instance, Yang *et al.* (2024) found increased serum IgM and IgY in broilers receiving *L. plantarum* HJLP 1, and He *et al.* (2019) reported elevated immunoglobulins in broilers receiving a multistrain probiotic mixture. Probiotic induced enhancement of immunoglobulin production and cytokine profiles has also been linked to increased vaccine responses and improved disease resistance (Qiu *et al*., 2021; Mazanko *et al*., 2022). These findings align with earlier observations by Khobondo *et al*. (2015) and Raheem *et al*. (2021) that probiotics increase antibody titers and enhance humoral immunity in poultry. *L. casei* FA23 improved systemic antioxidant defense in a dose-dependent manner (higher SOD, CAT, GSH; lower MDA) and enhanced humoral immunity (increased IgG and IgM), particularly at 200mg/kg. The oxidative status results observed in the present quail investigation, specifically the reduced malondialdehyde concentrations and elevated activities of key antioxidant enzymes, align with extensive research indicating that probiotics bolster antioxidant capacity in avian species. In broiler chickens, *Lactiplantibacillus plantarum*, *Bacillus subtilis*, and multistrain probiotic formulations have been documented to augment superoxide dismutase, catalase, and glutathione-related enzyme activities, alongside total antioxidant capacity, while simultaneously diminishing markers of lipid peroxidation, including MDA and reactive oxygen species (Yang *et al*., 2025). Furthermore, Bai *et al.* (2021) provided evidence that *B. subtilis* fmbJ enhanced hepatic and serum antioxidant enzyme activities, concurrently decreasing MDA and ROS levels, thereby improving both meat quality and oxidative equilibrium. Similar improvements under thermal or environmental stress conditions have been reported when birds received *Bacillus* and *Lactobacillus* based probiotics (Hashemitabar, Hosseinian, 2024). The antioxidant effects documented in the current work, therefore, fit within recognized probiotic mechanisms that mitigate oxidative stress and support tissue integrity.

At 200mg/kg, expression of tight junction (occludin), mucin (MUC2), antimicrobial peptides (AvBD6, lysozyme), and anti-inflammatory/transport genes (IL-10, SGLT1) increased, whereas pro-inflammatory IL-1β decreased. These changes were similar to or better than those of the antibiotic group, supporting improved gut barrier and immune status. The anti-inflammatory cytokine profile associated with probiotic treatment in this study is consistent with previous work showing that probiotics can shift immune responses toward an anti-inflammatory state. In broilers, *L. plantarum* and *Bacillus*-based probiotics have been reported to increase IL-10 and other regulatory cytokines while reducing IL-1β and related pro-inflammatory mediators (Chavan *et al*., 2025). Yang *et al*. (2025) showed that *L. plantarum* HJLP 1 decreased IL-1β and increased IL-10 in broiler serum, and Chavan *et al*. (2025) reported greater IL-10 expression and reduced inflammatory markers in birds receiving *Ligilactobacillus salivarius* and *Limosilactobacillus reuteri*. Such cytokine shifts are closely linked to improved gut barrier function, enhanced villus morphology, and improved performance indices (Qiu *et al*., 2021; Chavan *et al*., 2025). Taken together, the present quail data on growth performance, intestinal morphology, organ indices, serum biochemistry, antioxidant status, immune responses, and inflammatory mediators are in broad agreement with a growing body of evidence from broilers and ducks, indicating that well selected probiotic strains can safely enhance productivity and health through coordinated effects on gut structure, microbiota, metabolism, and immunity.

**Conclusions:** *Lactobacillus casei* FA23 is a powerful probiotic that acts as a versatile growth promoter and an alternative to antibiotics in quail. When used at an optimal dose of 200 mg/kg feed, it improves growth performance and feed efficiency by significantly enhancing gut health, strengthening the intestinal barrier, boosting innate immunity, modifying beneficial microbiota, and reducing inflammation. In addition to supporting growth, FA23 provides notable systemic benefits, including better metabolic profiles, increased antioxidant levels, and stronger humoral immunity. These benefits lead to higher meat quality, with more protein, less fat, increased tenderness, and longer shelf life. *Lactobacillus casei* FA23 is an all-in-one feed additive that promotes productivity by supporting overall health, offering a sustainable and effective strategy for antibiotic-free quail production.

**Authors contribution:** FSA: Conceptualization, visualization, methodology, writing the original draft, writing-review, and editing.

**Declaration of interests:** The author declares that she has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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