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RESEARCH ARTICLE

Luteolin Enhances Intestinal Homeostasis and Restores Growth Performance in Broilers

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ABSTRACT

Deoxynivalenol (DON)-induced inflammation, oxidative stress, and intestinal dysbiosis pose significant economic challenges to the poultry industry by impairing broiler health and productivity. Luteolin (LUT) and activated charcoal (AC) have been investigated for their potential to alleviate DON-induced intestinal injury and inflammation. A total of 800 one-day-old Arbor Acres broilers were randomly divided into five dietary treatments, with eight replicates of 20 birds each: a basal diet (BD) (control), BD supplemented with 10 mg/kg DON (DON), DON+350 mg/kg LUT (DON_LUT), DON+200 mg/kg AC (DON_AC), and DON+350 mg/kg LUT combined with 200 mg/kg AC (DON_LUT_AC) for 42 days. Growth performance was evaluated throughout the experiment. Serum lipopolysaccharide (LPS), reactive oxygen species (ROS) malondialdehyde (MDA), antioxidant enzyme activities, and inflammatory cytokines (IL-1β, TNF-α, IFN-γ, and IL-6) were determined using ELISA. Cecal microbiota composition was characterized to assess DON-induced microbial alterations and the effects of dietary interventions. DON significantly impaired growth performance ($P<0.05$) whereas LUT markedly alleviated these adverse effects. In contrast with the DON group, both LUT and AC substantially reduced LPS, ROS, and MDA concentrations, enhanced antioxidant enzyme activities, and suppressed the production of pro-inflammatory cytokines (IL-1β, TNF-α, IFN-γ, and IL-6) ($P<0.05$). Cecal microbiota analysis demonstrated that LUT supplementation increased the proportion of non-pathogenic bacteria, including *Lactobacillus* and *Ruminococcus*, whereas DON- and AC-fed birds exhibited higher abundances of potentially detrimental genera, including *Lachnoclostridium* and *Alistipes* ($P<0.05$). Overall, LUT was the most effective dietary intervention for mitigating DON-induced inflammation, restoring intestinal homeostasis, improving antioxidant status, and promoting beneficial gut community in broilers.

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INTRODUCTION

Contamination of poultry feed with Fusarium-derived mycotoxins represents a significant global concern, with DON, a major trichothecene, recognized as one of the most prevalent and harmful toxins (Ochieng *et al.,* 2021). Deoxynivalenol contamination in poultry feed leads to substantial economic losses by impairing growth performance, nutrient digestibility, and gastrointestinal function, while inducing immunosuppression, oxidative stress, and both acute and chronic toxicosis (Sousa *et al.,* 2020). These detrimental effects emphasize the necessity of strategies to mitigate DON toxicity in poultry, as optimizing feed digestion, largely mediated by gut microbiota composition and function, is closely linked to enhanced growth performance in food-producing animals (Yadav and Jha, 2019). The intestinal microbiota critically regulates nutrient absorption, energy metabolism, and immune homeostasis, while its disruption, or dysbiosis, is increasingly associated with inflammatory responses and gastrointestinal pathologies (Hou *et al.,* 2022). These findings underscore the importance of maintaining a stable and diverse gut microbiota to support animal health and productivity. Due to the detrimental effects of mycotoxins

like DON, there is increasing interest in developing eco-friendly alternatives that function as growth promoters and non-toxic substitutes in animal production (Hossain *et al.,* 2012; Nisa *et al.,* 2023).

Various bioactive compounds, including flavonoids, organic acids, prebiotics, probiotics, essential oils, and phytochemicals, have been explored for their ability to improve production performance and attenuate mycotoxin-induced toxicity. Among these, luteolin (LUT), a naturally occurring flavonoid synthesized by plants, has attracted considerable attention due to its well-documented antimicrobial (Li *et al.,* 2021; Shaukat *et al.,* 2024), anti-inflammatory (Ziyan *et al.,* 2007), and antioxidant properties (Williams *et al.,* 2001; González-Ramos *et al.,* 2010). It is widely found in dietary sources, including celery, chamomile tea, parsley, perilla leaves, green pepper, and various fruits (Nabavi *et al.,* 2015; Hassan *et al.,* 2023), making it a promising functional feed additive for poultry nutrition.

Recent studies have demonstrated that DON induces oxidative stress in the intestine, leading to tissue damage and impaired gut integrity (Harris *et al.,* 2006; Hassan *et al.,* 2026). Luteolin (LUT) has been reported to preserve intestinal function, attenuate inflammation, and modulate gut microbiota, highlighting its potential as a dietary intervention to recover gut health and performance in poultry challenged with dietary mycotoxins (Li *et al.,* 2021).

Activated charcoal (AC) is widely recognized for its strong adsorptive capacity and is commonly employed to mitigate the toxic effects of various feed contaminants, including mycotoxins (Wang *et al.,* 2022). However, its potential roles in modulating inflammatory and oxidative stress responses in poultry production remain insufficiently explored (Burchacka *et al.,* 2019). LUT, a bioactive flavonoid with potent antioxidant and anti-inflammatory properties, has rarely been evaluated in combination with AC. The present study explored the combined effects of LUT and AC on growth performance, antioxidant capacity, immune response, and gut microbiota in broiler chickens as a natural strategy to mitigate feed-borne toxins.

MATERIALS AND METHODS

The experimental protocol was approved by the Animal Care Committee of the Institute of Animal Science, Guangdong Academy of Agricultural Sciences (GAASIAS-2024-012).

Reagents: Deoxynivalenol (DON, 98% purity) was produced by cultivating *Fusarium graminearum* spores (1×10^6 spores/g) on sterilized maize and rice substrates for four weeks at 30°C and 70% humidity. To facilitate uniform fungal development, the culture flasks were shaken at weekly intervals throughout the incubation period. After incubation, the contaminated substrate was collected, homogenized, oven-dried at 65°C, and analyzed to confirm the required DON concentration. Luteolin (LUT, ≥99% purity) and activated charcoal (AC) were procured from the Aladdin Biochemical Technology Co., Ltd. (Shanghai, P.R. China); DON concentrations (~10 mg/kg) were confirmed using HPLC (Agilent 1260, C18 column) and AgraQuant® DON ELISA test kit.

Birds' diet and management: A 42-day feeding trial was conducted with 800 one-day-old Arbor Acres broilers assigned to five dietary treatments. Birds received a corn–wheat–soybean basal diet supplemented with 10 mg/kg DON alone or combined with LUT (350 mg/kg) and/or activated AC (200 mg/kg) (Wang *et al.,* 2022; Xia *et al.,* 2024) (Fig. 1). Broilers were housed in battery cages under controlled conditions with *ad libitum* access to feed and water (Table 1). Temperature was gradually reduced from 32 ± 2°C to 23°C, while relative humidity was maintained at 65–75%.

Table 1: Ingredients and nutrient composition of basal diet on a dry matter basis

Item	Starter (1–21 d)	Grower (22–35 d)	Finisher (36–42 d)
Ingredients (%)			
Corn	54.50	58.20	62.00
Soybean meal (44% CP)	36.00	32.00	28.50
Soybean oil	4.50	5.00	5.50
Dicalcium phosphate	1.80	1.60	1.50
Limestone	1.20	1.10	1.00
DL-Methionine	0.30	0.25	0.20
L-Lysine HCl	0.15	0.15	0.15
Vitamin–mineral premix[1]	0.50	0.50	0.50
Salt (NaCl)	0.30	0.30	0.30
Total	100.00	100.00	100.00
Calculated nutrient composition			
Apparent Metabolizable Energy (kcal/kg)	3,050	3,150	3,200
Crude protein (%)	22.50	20.50	19.00
Lysine (%)	1.30	1.15	1.00
Methionine (%)	0.55	0.50	0.45
Methionine + cysteine (%)	0.90	0.82	0.75
Calcium (%)	1.00	0.90	0.85
Available phosphorus (%)	0.45	0.40	0.38
Sodium (%)	0.15	0.15	0.15

Sample collection and treatment: At the end of the 42-day trial, BW, ADG, ADFI, and FCR were recorded. After an 8-h feed withdrawal, birds were euthanized, and blood samples were collected for biochemical analyses. Cecal contents stored at −80°C for 16S rRNA sequencing. Jejunum samples were fixed in 10% paraformaldehyde for histomorphology or snap-frozen for mRNA expression analysis.

Analysis of intestinal morphological structure: Samples from jejunal 42-day-old broilers were preserved using 10% neutral buffered formalin and processed through standard histological methods, which included paraffin embedding, slicing, and applying H&E stain. To check out the intestinal structure and histopathological alterations, we examined the prepared sections with a bright-field light microscope (Zhang *et al.,* 2022). The histopathological scores were determined by ImageJ software.

Measurement of inflammatory cytokines: Serum levels of interleukin-6 (IL-6), interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ) were measured in eight birds per treatment.

Analyzing broiler chicken jejunum for oxidative stress indices: Jejunum samples were homogenized and stored at −80°C. Levels of LPS, ROS, MDA, GSH-Px, T-SOD, and CAT were determined using commercial assay kits (Nanjing Jiancheng Bioengineering Institute) in eight samples per group.

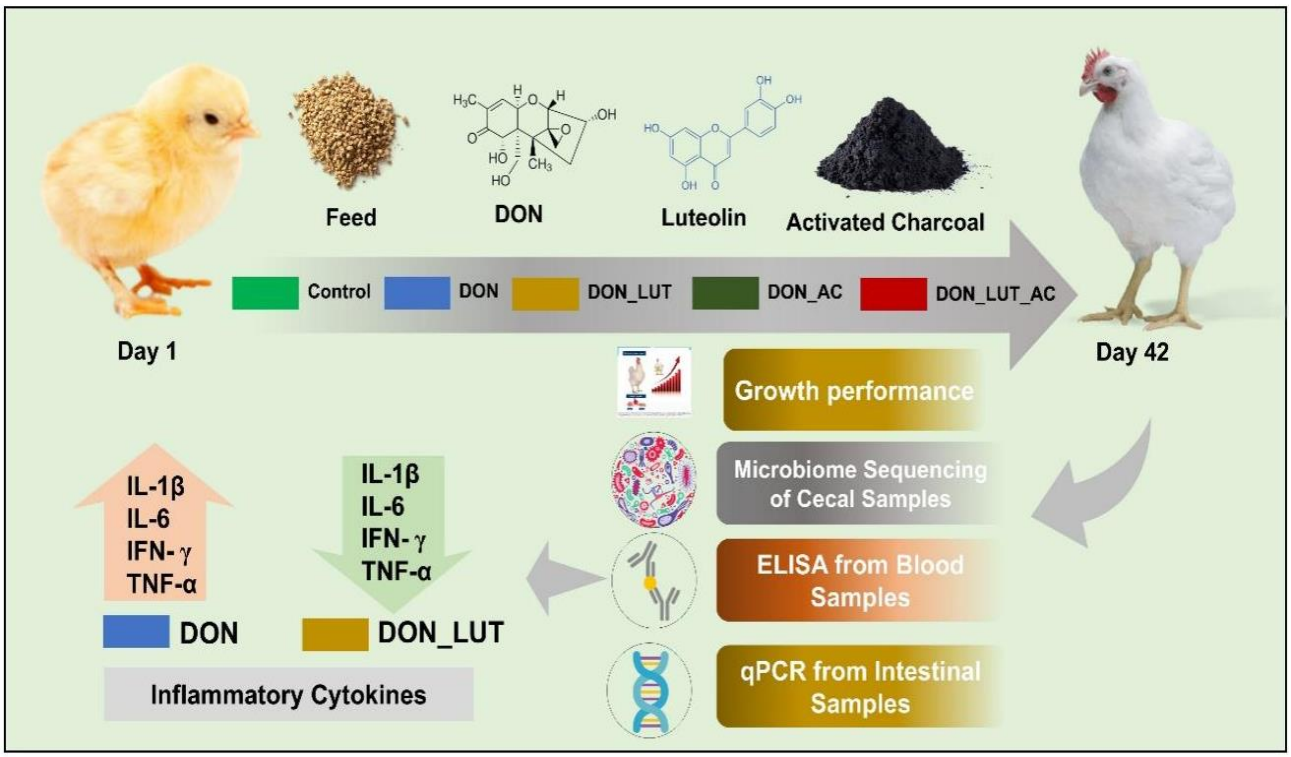


Fig. 1: Schematic overview of the experimental design evaluating the effects of LUT and AC supplementation on DON-induced toxicity, growth performance, gut microbiota, and gastrointestinal health in Arbor Acres broilers.

Determination of jejunum tissue immunoglobulin concentrations: Jejunal IgG, IgM, and IgA levels were determined using commercial ELISA kits according to the manufacturer's instructions, with absorbance measured at 450 nm (Farooq *et al.,* 2023).

RNA extraction and qPCR: Using quantitative real-time PCR (qPCR), we assessed how much mRNA of inflammatory cytokines was present in the jejunal tissues. From the jejunal samples, we isolated the total RNA and purified it, employing the RT Master Mix in combination with the gDNA Remover kit from Abclonal, based in Wuhan, China. We synthesized cDNA from purified RNA. This involved using 2× Universal SYBR Green Master Mix from Abclonal and specific primers for our genes, as shown in Table 2, with β-actin acting as our reference gene. Each of the samples underwent analysis in triplicate according to these thermal cycling parameters: first, an initial denaturation step at 95°C for 5 minutes, and then 40 cycles of amplification comprising 30 seconds at 95°C, 30 seconds at 60°C, and wrapping up with 20 seconds at 72°C. To determine how abundant the transcripts were, we applied the $2^{-\Delta\Delta Ct}$ approach (Livak *et al.,* 2001).

16S rRNA analysis: Fecal samples of 230 mg were used to extract microbial DNA by QIAGEN Mini Kit (Hilden, Germany) and stored at −20°C until analysis. The V3–V4 regions of the 16S rRNA gene were amplified using universal primers 341F (CCTAYGGGRBGCASCAG) and 806R (GGACTACNNGGTATCTAAT). Alpha diversity was evaluated based on OTU analysis using Shannon and Simpson indices (Schloss *et al.,* 2009), while species–sample associations and correlation networks were visualized using Circos and R (plotROC package), respectively.

Statistical analysis: Data was analyzed using one-way ANOVA in SPSS 20.0 (Chicago, IL, USA). Results are presented as mean ± SEM. Graphs were generated using GraphPad Prism (version 10.6.0), and Spearman correlation analysis was performed using OECloud Tools (https://cloud.oebiotech.cn) and TUTU CLOUD (https://www.cloudtutu.com). Statistical significance was set at $P<0.05$.

Table 2: qPCR Primers

Gene Name	Primer Sequence 5'-3'	Accession Number	TM
β-Actin	F:CCCGCAAATGCTCTAAACC R:CCAATCCTGTCTTGTTTTATGC	NM_205518.1	56
IL-1β	F:TCTGCCTGCAGAAGAAGCC R:CCTCACTTTCTGGCTGGAGG	NM_204524.1	
IL-6	F:GGTGATAAATCCCGATGAAGT R:TCTCCATAAACGAAGTAAAGTCTC	NM_204628	60
TNF-α	F:TGTGTATGTGCAGCAACCCGTAGT R:GGCATTGCAATTTGGACAGAAGT	AY765397.1	
IFN-γ	F:CACTGACAAGTCAAAGCCGC R:ACCTTCTTCACGCCATCAGG	NM_205149.1 87	56
SOD	F:CGTCATTCACTTCGAGCAGAAGG R:GTCTGAGACTCAGACCACATA	NM_205064	
CAT	F:CCACGTGGACCTCTTCTTGT R:AAACACTTTCGCCTTGCAGT	NM_001031215.1	50
GPx1	F:GACCAACCCGCAGTACATCA R:GAGGTGCGGGCTTTCCTTTA	NM_001277853.1	
GST	F:AGTCGAAGCCTGATGCACTT R:TCTAGGCGTGGTTTCCTTTG	L15386.1	60

RESULTS

Growth performance: As shown in table 3, DON considerably reduced broiler ADG and ADFI and increased FCR ($P<0.05$), whereas supplementation with LUT or AC, particularly LUT, markedly ameliorated these DON-induced growth impairments.

Table 3: Impact of LUT and AC supplementation on Arbor Acres broiler growth performance

Items	Control	DON	DON_LUT	DON_AC	DON_LUT_AC	SEM	P-value
1-21 days							
ADFI (g/d)	51.34	51.52	51.42	50.00	51.95	0.66	0.063
ADG (g/d)	32.47	31.73	32.34	31.05	31.15	0.87	0.363
F/G	1.58	1.63	1.59	1.61	1.67	0.03	0.078
22-42 days							
ADFI (g/d)	133.15^{b}	130.64^{b}	132.27^{b}	135.86^{a}	136.53^{a}	1.85	0.047
ADG (g/d)	68.00^{b}	65.15^{c}	72.45^{a}	71.38^{a}	71.92^{a}	1.95	0.012
F/G	1.95^{a}	2.00^{a}	1.83^{c}	1.90^{b}	1.90^{b}	0.04	0.021
1-42 days							
ADFI (g/d)	96.85^{ab}	92.57^{c}	95.94^{b}	97.87^{a}	96.69^{ab}	1.25	0.039
ADG (g/d)	52.48^{b}	49.06^{c}	55.86^{a}	53.40^{b}	54.15^{ab}	1.10	0.015
F/G	1.85^{ab}	1.89^{a}	1.72^{d}	1.83^{b}	1.79^{c}	0.04	0.032

The morphological structure of the intestine: The control group showed no pathological changes as evidenced by normal intestinal architecture seen on the H&E-stained sections, with well-defined and normal villi and crypts (Fig. 2A). The jejunal morphology of the DON group was in stark contrast to that of the other groups, with the villi being shortened, broadened, and damaged in places, with some upstanding and others fused side by side (Fig. 2B, blue and black arrows). The villus epithelium exhibited focal degeneration and loss of continuity in some regions (light blue arrow). The lamina propria was markedly expanded by diffuse infiltration of mononuclear inflammatory cells, suggesting active mucosal inflammation (red arrow). The crypts' hyperplasia was also observed (green arrow) (Fig. 2B). Broilers in the DON-LUT group displayed substantial preservation of intestinal morphology with relatively intact villi and crypt architecture resembling that of the control group (Fig. 2C). The DON-AC group exhibited partial recovery of intestinal morphology compared with the DON group. The villous architecture was substantially improved with reduced villus blunting, less villus fusion, and better preservation of epithelial continuity (Fig. 2D, black arrow). Mild inflammatory cell infiltration persisted within the lamina propria (Fig. 2D, red arrow), accompanied by slight crypt hyperplasia (green arrow). The DON_LUT_AC group demonstrated further restoration of the intestinal architecture, with improved villus integrity, better preservation of the epithelial lining, and more organized crypt structure compared with the DON group (Fig. 2E).

Effects of LUT and AC on oxidative stress induced by DON: It is known that DON-triggered oxidative stress causes systemic inflammation. Herein, birds challenged with DON exhibited significantly higher concentrations of LPS and ROS in jejunal tissues. Exposure to DON leads to a significant ($P<0.05$) increase in serum levels of inflammatory cytokines (IL-1β, TNF-α, IFN-γ, and IL-6) in birds compared to control, confirming that DON-induced oxidative stress may trigger systemic inflammation. DON-LUT treatment significantly reduced inflammatory cytokine levels compared to DON_AC and DON_LUT_AC groups, and qPCR analysis confirmed that DON markedly upregulated ($P<0.05$) IL-1β, TNF-α, IFN-γ, and IL-6 expression in jejunal tissues relative to control group (Fig. 3). DON_LUT treatment effectively mitigated DON-induced toxicity and more strongly suppressed inflammatory cytokines compared to AC or DON with LUT and AC, while DON_AC and DON_LUT_AC had no significant effect on IL-6 and IL-1β mRNA but substantially decreased IFN-γ and TNF-α mRNA expression (Fig. 3).

ELISA analysis of jejunal oxidant and antioxidant enzymes revealed that DON exposure significantly elevated MDA levels ($P<0.05$) compared to controls, while LUT and AC supplementation effectively reduced these oxidative markers. CAT, T-SOD, and GSH-Px activities were also significantly reduced in the DON treatment group ($P<0.05$), whereas LUT and AC administration restored these antioxidant enzyme levels (Table 5). RT-qPCR validation showed downregulated mRNA expression of SOD, GST, CAT, and GPx1 (Fig. 4) in DON-exposed birds ($P<0.05$), with LUT and AC treatments upregulating these antioxidant genes. Furthermore, immunoglobulin analysis demonstrated that DON-LUT and DON_LUT_AC dietary groups exhibited significantly higher jejunal IgA, IgG, and IgM concentrations ($P<0.05$) compared to DON and DON_AC groups, indicating enhanced immune response with LUT supplementation (Table 4).

Table: 4 Impacts of dietary supplementation with LUT and AC on serum were assessed by ELISA

Group	LPS (EU/mL)	ROS (U/mL)	IL-1β (pg/mL)	TNF-α (pg/mL)	IFN-γ(pg/mL)	IL-6 (pg/mL)	IgM (mg/mL)	IgA (mg/mL)	IgG (mg/mL)
CON	12.05±0.66^{d}	698.17±4.84^{c}	4.56±0.55^{cd}	20.32±0.77^{d}	17.13±0.55^{e}	10.62±0.62^{cd}	2.31±0.08^{a}	2.89±0.03^{a}	2.72±0.56^{a}
DON	216.76±0.69^{a}	786.9±3.32^{a}	8.22±0.79^{a}	39.02±2.80^{a}	29.08±1.78^{a}	19.08±1.26^{a}	1.77±0.05^{d}	1.65±0.07^{e}	1.65±0.06^{e}
DON_LUT	48.17±1.21^{c}	720.60±1.76^{b}	5.10±0.99^{c}	22.42±3.61^{c}	20.08±2.36^{d}	11.99±1.68^{c}	2.18±0.13^{b}	2.54±0.12^{b}	2.52±0.13^{b}
DON_AC	200.44±4.85^{a}	779.02±3.67^{a}	7.02±0.93^{b}	33.45±3.47^{b}	26.75±2.74^{b}	15.79±2.03^{b}	1.81±0.04^{c}	1.96±0.03^{d}	1.94±0.03^{d}
DON_LUT_AC	73.51±2.64^{b}	729.65±2.53^{b}	6.84±0.86^{c}	23.63±3.27^{c}	23.41±2.44^{c}	14.49±1.68^{b}	1.95±0.03^{c}	2.35±0.14^{c}	2.3±0.11^{bc}
P-value	<0.001	0.049	0.043	0.023	0.065	0.058	0.097	0.047	0.032

Significant differences ($P<0.05$) were found among a row of mean values labeled with various alphabet letters a–e. LPS: lipopolysaccharide, ROS: reactive oxygen species.

Table 5: The antioxidant parameters in chickens' livers fed a DON-contaminated diet were compared with LUT and AC

Group	MDA (nmol/mg prot)	CAT (U/mg prot)	T-SOD (U/mg prot)	GSH-Px (U/mg prot)
CON	1.95±0.02^{e}	69.76±1.69^{a}	72.15±0.60^{a}	47.81±0.99^{a}
DON	3.67±0.08^{a}	49.06±0.69^{e}	43.51±0.53^{e}	33.33±1.18^{e}
DON_LUT	2.02±0.02^{d}	64.03±1.33^{b}	54.45±1.12^{c}	45.51±1.05^{ab}
DON_AC	2.89±0.05^{b}	54.27±1.73^{cd}	64.45±0.58^{b}	37.8±0.36^{d}
DON_LUT_AC	2.25±0.06^{c}	57.77±1.42^{c}	49.12±0.02^{d}	42.15±0.28^{c}
P- value	0.048	0.033	0.043	0.034

MDA: malondialdehyde; CAT: catalase; T-SOD: total superoxide dismutase. GSH-Px: glutathione peroxidase.

Microbiota diversity in intestinal contents: Overall, eight samples from each group were collected. Bacterial DNA was positive in 102.30% (146.82/143.52) of the DON_LUT_AC samples and 95.76% (137.43/143.52) of the DON_AC samples and was negative in 82.45%

(118.33/143.52) of DON samples and 87.46% (125.53/143.52) of the DON_LUT samples in qPCR (Fig. 5H). Quality control assessments confirmed that blank samples from collection tubes and the experimental workspace were free of bacterial contamination, as no amplification signals were observed by qPCR (Fig. 5I). Similarly, reagent controls lacking DNA templates but containing all other qPCR components produced no detectable target products, confirming the specificity and reliability of the amplification results. Thus, exogenous contamination of bacteria can be ruled out. The configuration of cecal microbiota was investigated through 16S rRNA gene sequencing. The mean 16S rRNA concentration of DON_LUT_AC (146.82±43.41) samples was higher than that of control (143.52±27.55), DON_AC (137.43±28.25), DON (118.33±12.64), and DON_LUT (125.53±25) samples (Fig. 5). The rank-abundance curves for the OTUs described in Fig. 5 show that the abundance and evenness of the microbial community reduced in DON_LUT_AC samples. A total of 804 unique bacterial species out of 6901 at the OUT level were found among all five groups of chickens (Fig. 5).

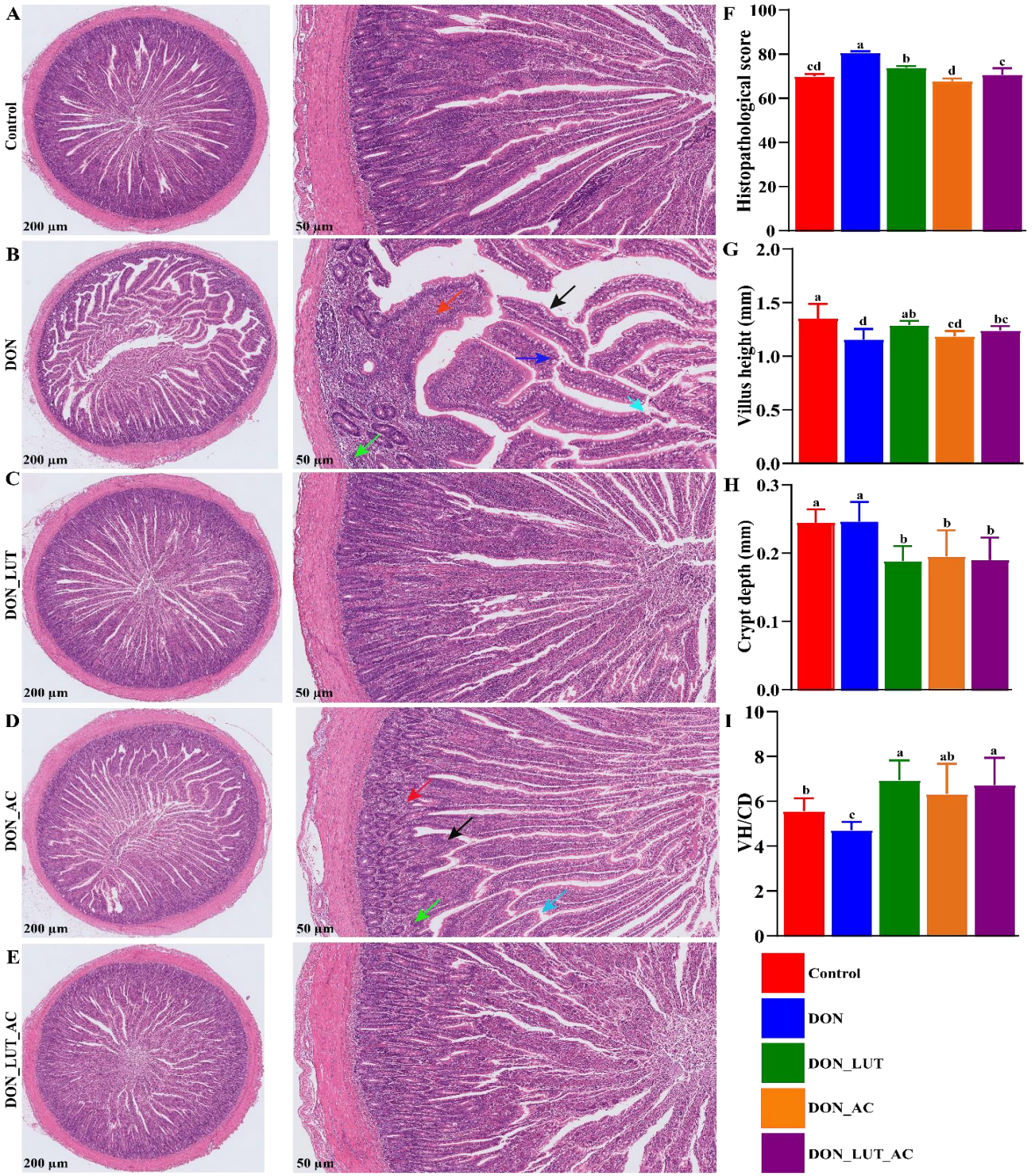


Fig. 2: The effects of LUT and AC supplementation on DON-induced jejunal inflammation in broilers were assessed by H&E staining. Villus height and crypt depth were measured from 10 representative villi per section using samples from eight birds per group. Data are presented as mean ± SEM, and different letters (a, b, and c) indicate significant differences among treatments (P<0.05). The scale bar indicates 50 μm.

A notable decrease was found in the α-diversity of DON group in contrast with control, DON_LUT, DON with AC, and DON with LUT and AC groups (Fig. 6). The correspondence between bacterial species at phylum level and the samples was shown through the Circos chart. It helps to understand the distribution ratio of bacterial species and their relative abundance among the groups. As shown in Fig. 6, Firmicutes were observed to be highly abundant in the DON_LUT_AC groups as compared with the DON_LUT and DON_AC groups. Similarly, Bacteroidota was highly prevalent in the DON_LUT group (Fig. 6). Irrespective of Firmicutes, Actinobacteriota and Desulfobacterota were highly abundant in DON and AC groups, respectively, in contrast with the other four groups (Fig. 6).

The abundance of pathogenic bacteria (Bacteroides) in the DON composite with AC and the DON composite with LUT and AC treatments was greater compared to other treatment groups (Fig. 6). The DON_LUT treatment had an abundance of gram-positive bacteria, Lactobacillus (16.52%) and Ruminococcus torques (6.30%), compared with the DON (Fig. 6). The DON_LUT group had a higher abundance of lactobacillus, which is distinguished by its capacity to produce lactic acid as a byproduct of glucose metabolism. Still, their level was lowered in the DON_AC (1.5%) and DON (3.01%) groups, respectively. In comparison with control and other treatments, DON_LUT and DON_LUT_AC had higher ($P<0.05$) norank_f__norank_o__Clostridia_UCG-014 (Fig. 6) and lesser ($P<0.05$) abundance of Alistipes.

LUT and AC treatments impede compositional alterations in cecal microbiota by attenuating DON-induced intestinal epithelium oxidative damage: 16S rRNA gene sequencing of the bacterial V3–V4 region was used to investigate the microbial community in chicken ceca. To understand how the use of LUT and AC supplements helps to keep cecal microbial richness high by reducing oxidative damage to intestinal epithelial cells caused by DON, we used a single-factor correlation network analysis, which considers species correlation on a genus level and particular environmental factors. To depict the degree of association between species, we computed correlation coefficients like Spearman rank using data from the top 50 species in terms of overall total abundance at the taxonomic level (Fig. 7A).

The varieties' richness is depicted by the size of the figure nodes. A favorable link among the groups and microbiota is seen by the light blue line. The size of the correlation coefficient is shown by the diameter of the line. A greater correlation between species is represented by a larger line.

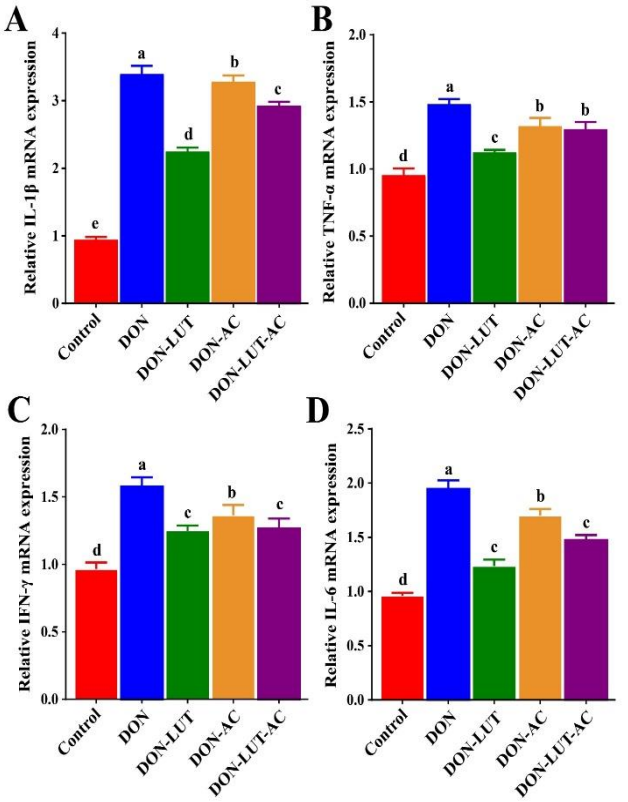


Fig. 3: Impact of LUT and AC dietary supplements on the jejunum's relative mRNA expression of inflammatory cytokines. (A) Jejunum tissue level of *IL-1β* mRNA. (B): *TNF-α* mRNA in tissue from the jejunum. (C) Jejunum tissue *IFN-γ* mRNA level. (D) The jejunum tissue *IL-6* mRNA level. Substantial differences ($P<0.05$) are indicated by distinct superscripts a, b, c, and d.

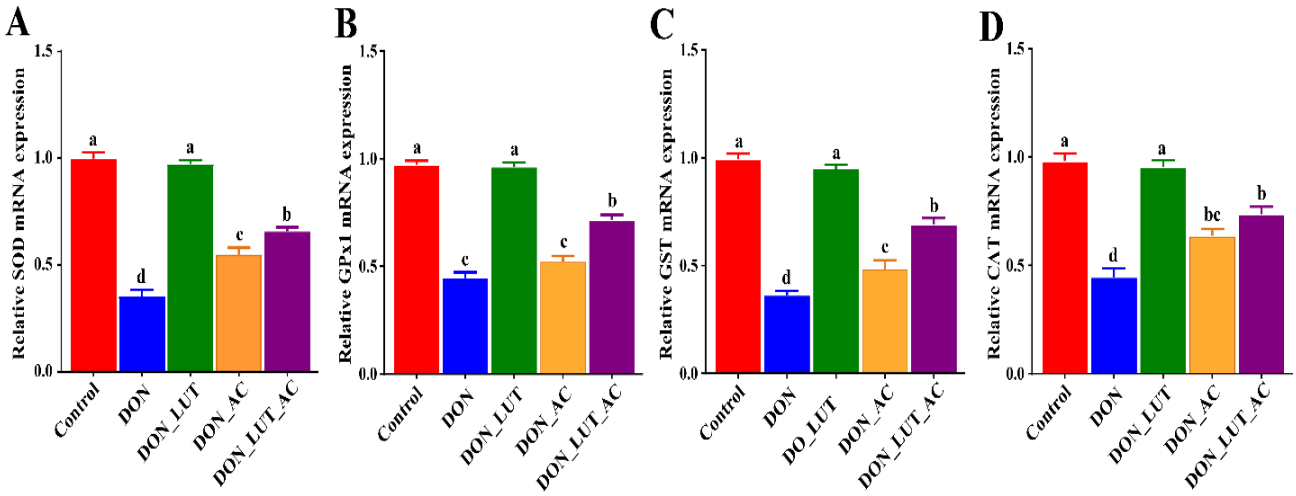


Fig. 4: The effects of LUT and AC supplementation on antioxidant gene expression in the jejunum of broilers exposed to deoxynivalenol were evaluated by measuring relative mRNA expression. The significant differences among groups were indicated by different superscripts (a, b, c, d; $P<0.05$).

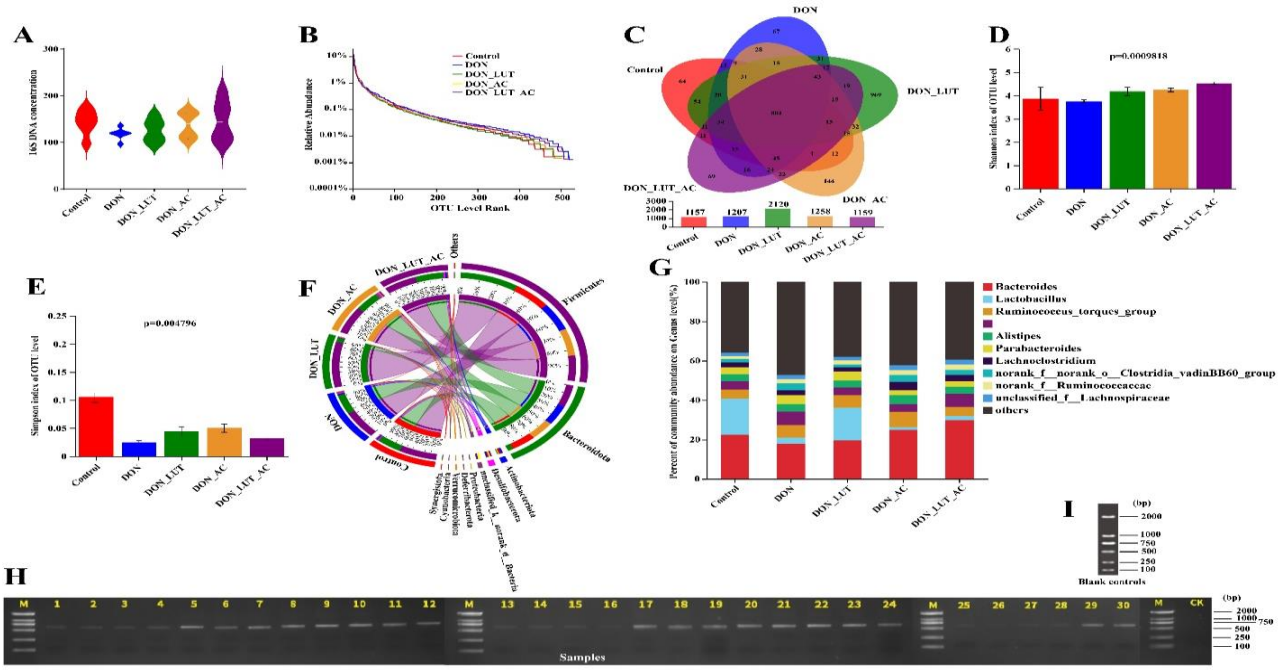


Fig. 5: Microbiota diversity in intestinal contents among five groups. (A) Bacterial DNA concentration in different samples. (B) Rank-abundance curves of OTUs across groups. (C) Venn diagram showing shared and unique OTUs. (D) Shannon diversity indices of OTUs. (E) Simpson diversity indices of OTUs. (F) Circos diagram depicting sample–species relationships at the phylum level. (G) Microbial community composition of cecal chyme at the genus level. (H) PCR products of cecal chyme with 750-bp target bands; M, marker (DL2000); CK, PCR reagent control (n = 8). (I) Blank control samples showing no target PCR products; M, marker (DL2000); CK, PCR reagent control. Abbreviations: PCR, polymerase chain reaction; OTUs, operational taxonomic units.

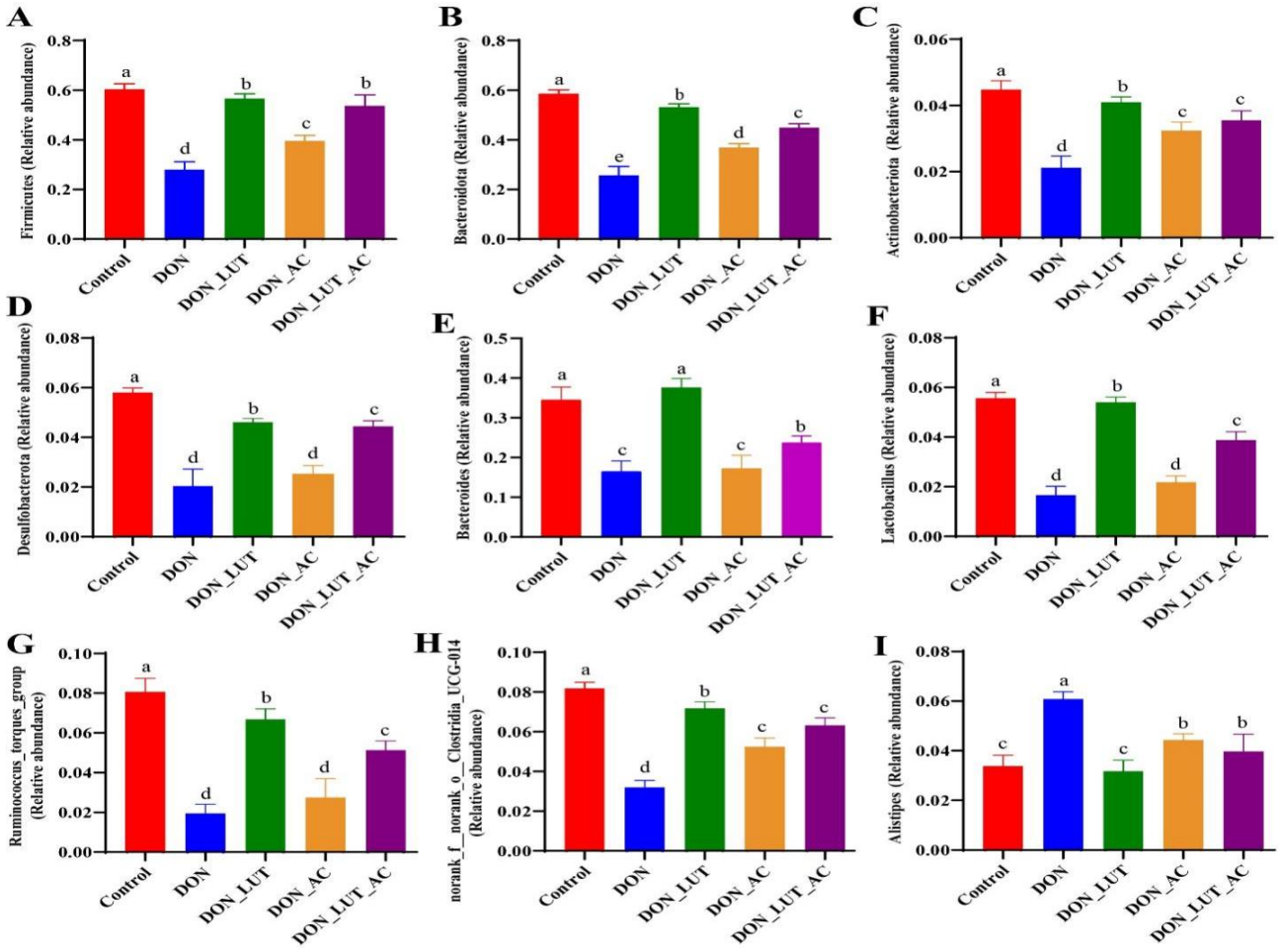


Fig. 6: Comparative analysis of cecum bacterial communities in the broiler chickens treated with LUT and AC supplementation against DON-induced toxicity by 16S rRNA gene amplicon sequencing. (A) The relative abundance of *Firmicutes*, (B) *Bacteriodota*, (C) *Actinobacteriota*, and (D) *Desulfobacterota* at the phylum level. (E) The relative abundance of *Bacteroides*, (F) *Lactobacillus*, (G) *Ruminococcus*, (H) *Clostridia*, and (I) *Alistipes* at the genus level.

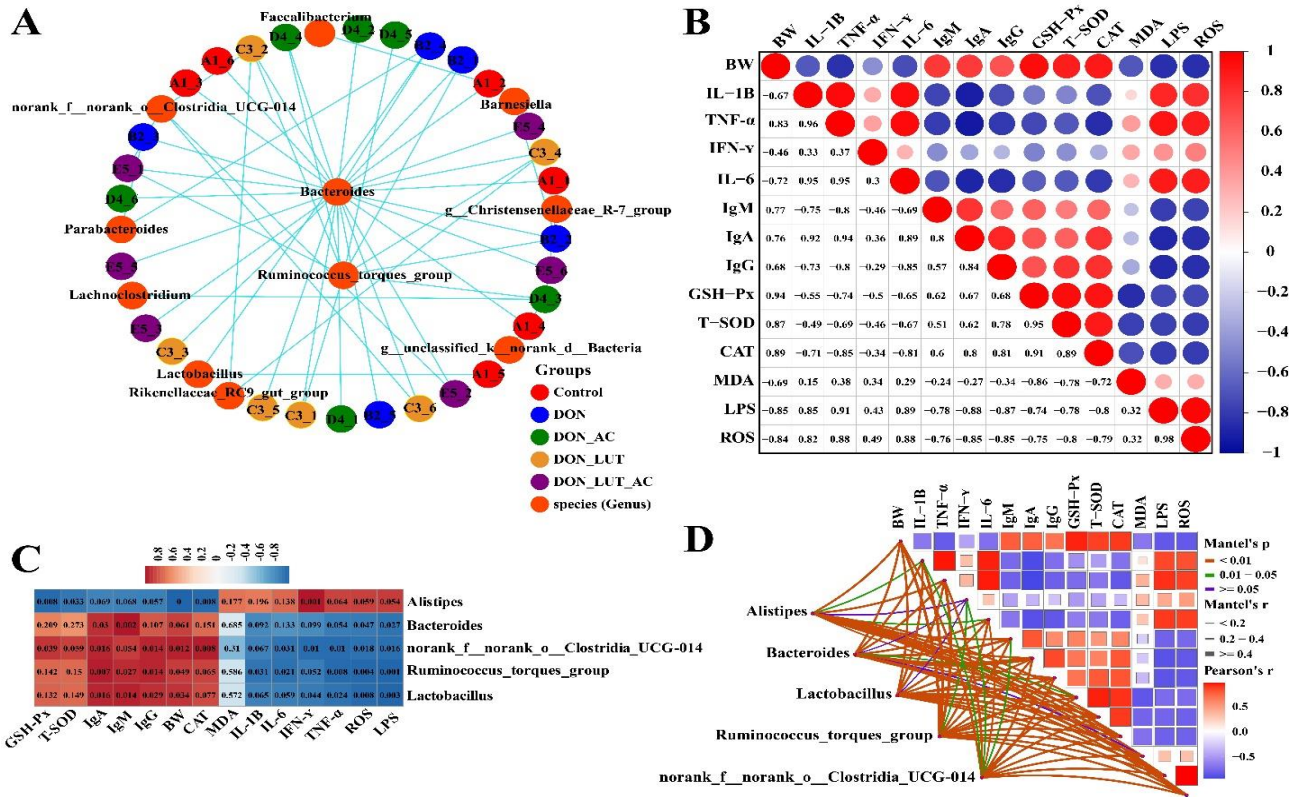


Fig. 7: Effect of LUT and AC supplementation on DON-induced intestinal injury by preserving gut microbial architecture, shown via (A) species-level co-occurrence network (Spearman |R|>0.50; light blue thin line, 0.5<R<0.70; thick line, R>0.70, P<0.05; colored circles indicate genera). (B) Pearson correlation heatmap, (C) Spearman correlation between gut microflora and host markers, and (D) Mantel test correlations between gut microflora and host markers (red, positive; blue, negative).

In the next step, Pearson's correlation heat map (correlation coefficient) was used to show the relationships between LPS, ROS, inflammatory cytokines, antioxidants and oxidant enzymes, and immunoglobulins in hens. Among them, IL-6 (P = 0.000, R = 0.894), IL-1β (P = 0.000, R = 0.849), IFN-γ (P = 0.000, R = 0.429), TNF-α (P = 0.000, R = 0.915), MDA (P = 0.000, R = 0.317), and ROS (P = 0.000, R = 0.976) were positively correlated with LPS. BW (P = 0.000, R = -0.846), antioxidant enzymes such as GSH-Px (P<0.005, R = -0.744), T-SOD (P<0.05, R = -0.776), and CAT (P = 0.0003, R = -0.798), and immunoglobulins such as IgM (P = 0.0001, R = -0.783), IgG (P = 0.000, R = -0.869), and IgA (P = 0.000, R = -0.878) were negatively correlated with LPS. The intestinal microbiota plays a key role in maintaining the health of chickens through patrolling antioxidants, oxidant enzymes, immunoglobulins, and inflammatory markers. For this, we created a correlation analysis between the microbiota and the host markers and presented them as a heatmap and mental test correlation graph (Fig. 7C). There was a positive correlation between Alistipes and LPS, ROS, TNF-α, IFN-γ, IL-6, IL-1β, and MDA in the DON group, as shown in the graph. Immunoglobulins (IgA, IgG, and IgM) and antioxidant enzymes (GSH-Px, T-SOD, and CAT) were positively linked with Bacteroides, norank_Clostridia_UCG-014, Ruminococcus torques group, and Lactobacillus in the DON_LUT_AC group.

DISCUSSION

Dietary DON exposure significantly impaired growth performance, as evidenced by reduced ADG and ADFI and increased FCR (Table 3). The results are similar to those reported previously which showed that DON negatively affects nutrient utilization and growth in broiler chickens (Riahi *et al.,* 2020, 2021; Li *et al.,* 2021; Marquis *et al.,* 2025). Dietary LUT supplementation markedly improved these performance indices in DON-challenged broilers, indicating its protective effect against DON-induced growth depression, which agrees with previous observations (Salemi *et al.,* 2022). The intestinal barrier is a key defense system controlling bacterial, antigen, and toxin translocation, and its disruption by dietary toxins induces oxidative stress, elevates pro-inflammatory cytokines, promotes epithelial apoptosis, and drives systemic gastrointestinal inflammation in chickens (Deng *et al.,* 2022; Shaukat *et al.,* 2025).

DON challenge markedly increased the jejunal expression of pro-inflammatory cytokines (IL-6, IL-1β, IFN-γ, and TNF-α), whereas LUT supplementation significantly suppressed these inflammatory responses (Fig. 3). These results demonstrated that LUT effectively alleviates DON-induced intestinal inflammation (Khatoon *et al.,* 2018). Among the detoxification strategies evaluated, the DON_LUT treatment showed greater protective efficacy than AC (Lawtae and Tangsathitkulchai, 2021). Although AC can adsorb DON, its lower protective effect may be associated with the nonspecific adsorption of lipids and other nutrients, thereby reducing nutrient availability (Xu *et al.,* 2021). DON exposure also induced oxidative stress (Ge *et al.,* 2020), as reflected by increased LPS, ROS, and MDA levels together with decreased CAT, GSH-Px, and T-SOD activities. LUT supplementation significantly reversed these alterations,

indicating enhanced antioxidant capacity in DON-exposed broilers (Osselaere *et al.,* 2013; Zhang *et al.,* 2022). Furthermore, LUT increased GST mRNA expression in the jejunum (Fig. 4), supporting its role in improving antioxidant defense. These findings are consistent with previous studies reporting the antioxidant effects of LUT (Frankič *et al.,* 2008; Khatoon *et al.,* 2018; Kulcs *et al.,* 2023). Microbiota analysis further demonstrated that LUT supplementation restored DON-induced microbial dysbiosis by increasing the abundance of beneficial bacteria, including *Bacteroides*, *Lactobacillus*, and *Ruminococcus*, while reducing the abundance of *Alistipes*. These microbial changes were accompanied *by* improvements in growth performance, antioxidant status, and inflammatory responses. The beneficial roles of *Lactobacillus*, *Bacteroides*, and *Ruminococcus* in maintaining intestinal homeostasis and immune function, including the regulation of inflammation, have been previously reported (Yang *et al.,* 2017; Chen and Zhang, 2019; Khan *et al.,* 2020; Ali *et al.,* 2022; Akhtar *et al.,* 2022). In agreement with earlier studies, LUT has also been shown to promote *Lactobacillus* enrichment (El-Sharkawy *et al.,* 2020; Ge *et al.,* 2020). Correlation analysis further supported these observations by showing that *Lactobacillus*, *Bacteroidetes*, and the *Ruminococcus torques* were positively associated with body weight, antioxidant enzyme activities, and immunoglobulin levels but negatively associated with LPS, ROS, MDA, and pro-inflammatory cytokines (Fig. 5). Conversely, *Alistipes* showed the opposite trend consistent with its reported association with gut inflammation (Wu *et al.,* 2019; Yang *et al.,* 2020). Collectively, these findings indicate that LUT alleviated DON-induced intestinal injury by restoring microbial homeostasis, enhancing antioxidant defenses, suppressing inflammatory responses, and improving growth performance in broiler chickens.

Conclusions: Luteolin combined with activated charcoal mitigates DON-induced growth impairment, inflammation, and gut dysbiosis in broilers by enhancing antioxidant defenses, modulating inflammatory responses, and restoring beneficial microbiota, highlighting its potential as a protective strategy against DON-related intestinal damage.

Conflict of interest statement: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this manuscript.

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Authors contributions: YBW and SQJ: conceptualization, methodology, and designed the study as well as visualization and supervision of the study. MH, QA and SX: performed the experiments, software and validation, writing original draft, review and editing, QF and JY: analyzed the data.

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