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Lactiplantibacillus plantarum-fermented shallot (*Allium cepa* L.) extract: A novel natural antibiotic alternative in drinking water for *Escherichia coli*-challenged broilers

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ABSTRACT

Background: The global poultry industry is under pressure to reduce its reliance on antibiotics, necessitating the search for effective alternatives. Pathogenic *Escherichia coli* infections remain a major challenge, leading to significant economic losses due to impaired growth performance and increased mortality.

Aim: This study aimed to evaluate the efficacy of shallot extract (*Allium cepa* L.) fermented by *Lactiplantibacillus plantarum* 1582 fermented shallot (FS) as a potential antibiotic alternative in experimentally challenged *E. coli* broiler chickens.

Methods: A total of 300 1-day-old J-Dabaco broilers were randomized to six treatments (n = 50; five replicates of 10): negative control (NC), (PC), antibiotic control (AB), and three challenged groups receiving FS at 1%, 2%, or 3% (FS1–FS3). Challenged birds were orally inoculated with *E. coli* ExPEC_A338 (3×10^8 CFU/ml) on days 7 and 8. Outcomes included growth performance, cecal microbiota, jejunal histomorphology, immune-organ indices, serum Immunoglobulin M (IgM), Immunoglobulin A (IgA), and Immunoglobulin G levels, and mRNA expression of tight-junction proteins (ZO-1, occludin, and claudin-2) and cytokines [IL-4, IL-1β, TNF-α, and Interferon-gamma (IFN-γ)].

Results: Supplementation with FS, especially the 3% dose (FS3), significantly improved body weight gain by 9.4% and restored survival to 100%, matching the NC and AB groups. FS3 enhanced gut health by reducing the densities of pathogenic *E. coli* and *Salmonella* spp. while simultaneously increasing the densities of beneficial *Lactobacillus* spp. ($p < 0.05$). Morphological analysis showed that the FS3 group had the highest villus height (VH) (1,049.86 µm) and VH/Crypt Depth ratio (13.05) ($p < 0.05$). Although immune-organ indices remained unchanged, dietary FS at 2%–3% significantly upregulated ZO-1, while IFN-γ increased at 1% FS (FS1) but remained comparable to the challenged control at 2%–3% ($p < 0.05$). Serum IgM increased at 1%–2%, and serum IgA decreased at 3% ($p < 0.05$).

Conclusion: Collectively, these findings demonstrate that *L. plantarum*-FS extract, particularly at a 3% inclusion level, represents a promising non-antibiotic strategy for broilers challenged with *E. coli*, primarily through enhanced growth performance, intestinal integrity, and a favorable gut microbial balance.

Keywords: Antibiotic alternative, Broiler, *Escherichia coli,* Fermentation, *Lactiplantibacillus plantarum*.

Introduction

Avian pathogenic *Escherichia coli* (APEC) is one of the most critical bacterial agents impacting the poultry industry, causing significant global economic losses through systemic lesions such as polyserositis and cellulitis (Mehat *et al.*, 2021). This challenge is further exacerbated by the zoonotic potential of contaminated poultry products (Elsharawy, 2022) and the rapid escalation of antimicrobial resistance, emphasizing the urgent need for effective alternatives to conventional antibiotics (Sebastian *et al.*, 2021).

Phytogenic substances, particularly those derived from the *Allium* genus, have garnered attention owing to their potent bioactive components, such as quercetin and allicin (Salehi *et al.*, 2019; Hai *et al.*, 2025a). The antimicrobial and immunomodulatory activities of these compounds have been documented (Hai and Hoa, 2020; Hai *et al.*, 2025b). However, their efficacy is often constrained by poor bioavailability, as many active components remain trapped within the plant cell wall matrix (Liu, 2013).

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Fermentation-targeted bioprocessing offers a rational strategy to disrupt plant cell architecture, liberate bound phytochemicals, and potentiate their biological activity (Gao *et al.*, 2023). Although spontaneous fermentation can yield inconsistent outcomes and raise safety concerns (Chen *et al*., 2018), controlled fermentation with lactic acid bacteria (LAB) provides a safer and reproducible platform. LAB, particularly *Lactiplantibacillus* spp., can competitively inhibit enteric pathogens, such as *E. coli*, and modulate host immune function (De Montijo-Prieto *et al.*, 2023). Nevertheless, probiotics alone have shown limited protection under severe APEC challenge, suggesting that single-modality interventions may be insufficient (Rahayu *et al.*, 2021). The putative synergy of LAB-fermented botanical extracts, which combine antimicrobial phytochemicals with probiotic and postbiotic effects, remains insufficiently characterized in *E. coli* challenge models.

To address this gap, this study evaluated the effects of a *Lactiplantibacillus plantarum*-fermented shallot (FS) (*Allium cepa* L.) extract administered via drinking water on growth performance, gut health, and immune responses in broiler chickens under experimental *E. coli* challenge. We hypothesized that the fermented extract would mitigate infection-associated performance losses by reinforcing intestinal barrier integrity, reshaping the gut microbiota toward beneficial taxa, and modulating systemic and mucosal immunity.

Materials and Methods

FS extract

Shallots (*A. cepa* L., GenBank ID: NC_057575.1) meeting the Vietnamese Good Agricultural Practices standards were purchased from Dien Mon, Thua Thien Hue, Vietnam. The raw material was pretreated (washed, peeled, and soaked in a 5% NaCl solution for 120 minutes) and then homogenized.

Microbial strain and fermentation process

The strain *L. plantarum* 1582 (GenBank ID: MT597487.1), isolated from indigenous chicken, was selected based on its resistance to *E. coli* and shallot extract (Phuong *et al.*, 2024). The strain was activated in de Man, Rogosa, and Sharpe (MRS) broth (Oxoid, UK) at 37°C for 24 hours.

The fermentation process followed the method described by Hai *et al.* (2025a), with modifications. The homogenized shallot was inoculated with a 1% inoculum ratio of *L. plantarum* 1582 (10^8 CFU/ml) in a medium supplemented with 4% NaCl and 3% glucose. The mixture was anaerobically incubated (Kuvings KGC-712CB) at 37°C at 60 rpm for 72 hours. The extract was filtered to yield the final product (~2.7–3 × 10^7 CFU/ml) and stored at 4°C. Table 1 presents the main bioactive components.

Pathogenic bacterial strains

The virulent *E. coli* strain ExPEC_A338 (GenBank ID: CP142559.1) (Hai *et al.*, 2015c) was activated in Luria–Bertani broth, centrifuged, washed, and diluted in phosphate-buffered saline (PBS) buffer (pH 7.2) to the required concentration.

Basal diet

Birds were fed a basal diet (Table 2) based on corn and soybean meal, which met the feeding standards for broilers set by the Ministry of Agriculture and Rural Development of Vietnam (TCN 661-2005) and was antibiotic-free.

Animals used and experimental design

A total of 300 1-day-old J-Dabaco colored feather male broilers were brooded communally (Days 1–7) and subsequently randomly allocated in a completely randomized design: 6 treatments × 5 replicate pens × 10 birds/pen: NC (negative control: no supplement, no challenge; isolated rearing), PC (positive control: challenged), FS1–FS3 (supplemented with 1%–3% FS in drinking water daily, challenged), and AB (antibiotic: Terra-Neocine - Mebipha JSC - Vietnam at 1 g/l for 5 days pre-challenge and 2 g/l for 5 days post-challenge). On days 7 and 8, the birds in treatments (2) through (6) were challenged via oral gavage with 0.5 ml of *E. coli* ExPEC_A338 suspension (3 × 10^8 CFU/ml). The NC group received 0.5-ml of PBS. Figure 1 summarizes the experimental layout and evaluated parameters.

Table 1. Bioactive constituents of fresh and *L. plantarum*-FS (*A. cepa* L.) extracts.

Ingredient	Fresh shallot	FS	Method of determination
Polyphenols (mg/g)	12–14	18–22	Folin- Ciocalteu
Quercetin (mg/g)	2–5	3–5	UV-Vis
Sulfur compounds			
Allicin (mg/kg)	1–2	1–2	GC-MS
Thiosulfate (mg/g)	5–7	3–4	GC
S-allyl cysteine (mg/g)	0.2–0.4	1–2	GC-MS
Organic acids			
Lactic acid (%)	-	1–2	HPLC
Acetic acid (%)	-	0.3–0.5	HPLC
Citric acid (%)	~0.3–0.5	~0.5–1	HPLC

All procedures for the care, housing, and slaughter of the experimental chickens complied with standards approved by the Animal Ethics Advisory Committee of Hue University, Vietnam.

Table 2. The nutritional composition of the basic diet.

Ingredient	%
Corn	61.03
Soybean meal	30.80
Soybean oil	2.50
Fish meal	1.50
$CaHPO_4$	1.40
Limestone	1.40
NaCl	0.37
Premix[1]	1.00
Total	100.00
Calculation of nutrient levels	%
Crude protein	19.00
Calcium	0.90
Available phosphorus	0.35
Lysine	0.85
Methionine	0.40
Methionine + cysteine	0.65
Metabolizable energy (MJ kg^{-1})	12.40

[1] Premix provides the following per kg of ration: vitamin A, 9,500 IU; vitamin B1, 1.5 mg; vitamin B2, 9.0 mg; vitamin B6, 3.0 mg; vitamin B 12, 0.02 mg; vitamin D3 , 2,375 IU; vitamin E, 19 IU; vitamin K3, 1.40 mg; biotin, 0.95 mg; folic acid, 0.93 mg; D-pantothenic acid, 9.3 mg; Cu (as copper sulfate), 15 mg; Fe (as ferrous sulfate), 60 mg; Mn (as manganese sulfate), 100 mg; Zn (as zinc sulfate), 70 mg; I (as potassium iodide), 0.50 mg; Se (as sodium selenite), 0.59 mg.

Data collection and parameter assessment

Growth performance

Feed intake (FI) for each pen was recorded daily at 07:00 hours, and body weight (BW) was measured at the beginning and end of the experiment. Mortality was checked daily to determine the survival rate. From these raw data, body weight gain (BWG, end BW - initial BW) and feed conversion ration (FCR, total FI/BWG) were calculated. The production efficiency index (PEI) was calculated using the following formula: (BWG (kg) × Survival rate (%) \ × 100)/(FCR × Experiment days) (Martins *et al.*, 2016).

Sample collection

On day 35, 15 birds/pen were randomly selected, anesthetized, and sampled. Blood was centrifuged at 3,000 rpm for 10 minutes to obtain serum. Immune organs (bursa of fabricius, thymus, and spleen) were weighed to calculate relative organ weights (g/100 g BW). Cecal digesta samples were aseptically collected for microbial analysis. Ileal tissue (midsection) was collected for histological and gene expression analyses.

Immunoglobulin content

Serum was isolated from blood samples for 10 minutes and subsequently preserved at −20°C until analysis. Serum immunoglobulin levels, including IgA (MBS705241), IgM (MBS706158), and Immunoglobulin G (IgG) (MBS260043), were quantified using commercial enzyme-linked immunosorbent assay kits (MyBioSource, San Diego, CA). The absorbance was read at 450 nm, and the final concentrations were derived from the respective standard curves.

Gene expression analysis

Total RNA was extracted from the ileal mucosa (Trizol, Invitrogen), and cDNA was synthesized (FIRE Script RT cDNA, Solid Biodyne). Reverse transcription quantitative polymerase chain reaction (RT-qPCR) was performed on a QuantStudio™ 5 system (Thermo Fisher) to quantify the relative expression of genes related

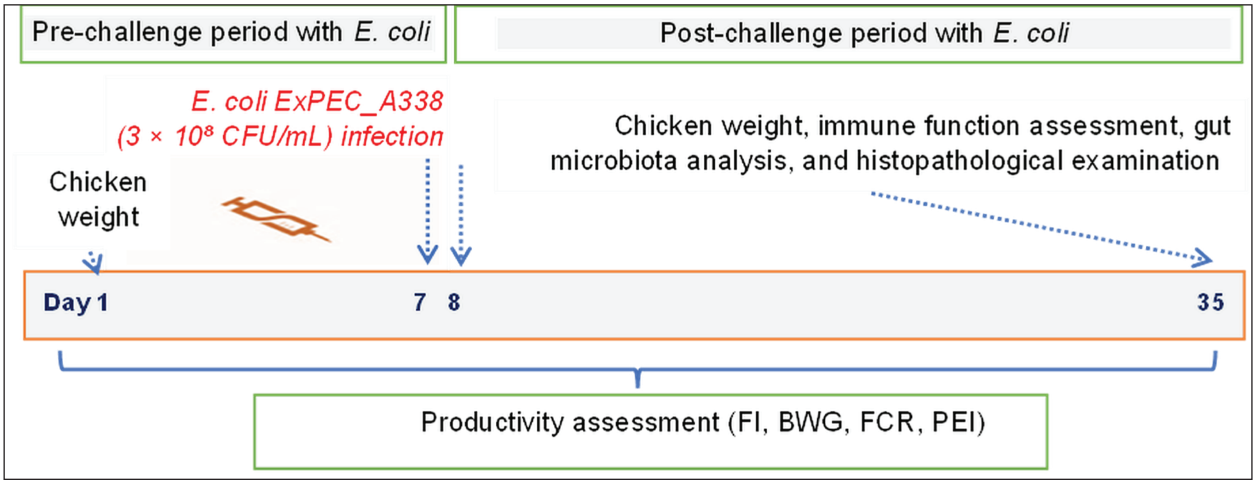

Fig. 1. Flowchart of the experimental design and parameters evaluated.

to immunity [IL-4, IL-1β, TNFα, Interferon-gamma (IFNγ)] and tight junction proteins (ZO-1, claudin-2, and occludin). Gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001) with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as the reference gene (Table 3).

Gut health

The population of *E. coli*, *Salmonella spp.*, and *Lactiplantibacillus spp.* in cecal digesta was quantified by plate counting on selective agar media: Eosin Methylene Blue Agar supplemented with 1% CaCO3, *Salmonella Shigella* agar, and MRS agar. The corresponding reference standards were ISO 13349/2001, ISO 6579/2003, ISO 7937/2004, and ISO/Dis 11290/1994. The results were transformed into log10 CFU/g.

Histological morphology

Ileal samples were fixed in 10% formalin, processed histologically (5 µm sections), and stained with Hematoxylin and Eosin (H&E) (Liu *et al.*, 2025). Villus height (VH), crypt depth (CD), and surface area were measured using Image-Pro Plus software (v. 6.0).

Statistical analyses

Data were analyzed using the SPSS 22.0 software. The pen was considered the experimental unit for growth performance (n = 5 pens per treatment). Three birds per pen were sampled for microbiology, histomorphology, immune-organ indices, serum immunoglobulins, and gene expression data (n = 15 birds per treatment). Treatment effects were evaluated using the general linear model with treatment as a fixed effect. Mean separation was performed using one-way analysis of variance with Bonferroni post hoc test. The chi-square test (χ^2) was used for the frequency data (survival rate). Differences were considered statistically significant at $p < 0.05$. Data are presented as Mean ± Standard Error of the mean.

Ethical approval

All procedures concerning the care, housing, and slaughter of the experimental chickens were carried out in accordance with standards approved by the Animal Ethics Advisory Committee, Hue University, Vietnam (March 20, 2024).

Results

Effects of FS extract on broiler performance

The average water intake was monitored daily; the mean intake per bird was 135–185 ml/day (days 8–35). Based on the viable count of the FS (~2.7–3.0 × 10^7 CFU/ml), the estimated daily intakes of viable *L. plantarum* 1582 were approximately 3.6–5.6 × 10^7, 7.2–11.1 × 10^7, and 1.1–1.7 × 10^8 CFU/bird/day for the 1%, 2%, and 3% supplementation levels, respectively. During the pre-challenge period (days 1–7), BWG, FI, FCR, and survival did not differ among treatments (p = 0.26), indicating comparable baseline performance (Table 4). Following *E. coli* challenge (days 3–5), the challenged control (PC) exhibited marked performance depression, with BWG, FI, and survival reduced to 593.57 g, 1,030.10 g, and 76.67%, respectively. Moreover, characteristic APEC lesions were observed in the deceased birds (Fig. 2). Supplementation with FS extract mitigated these losses. Relative to PC, BWG increased by +3.05%, +8.15%, and +8.54% in FS1, FS2, and FS3, respectively, and survival was restored to 93.33%–100%, comparable to the NC and AB (Table 4).

Effects of FS extract on immune function

Table 5 shows the effects of treatments on immune organ indices and serum antibodies. No significant differences were observed in the relative weights of the spleen, bursa of Fabricius, or thymus (p > 0.05) or serum IgG concentration (p > 0.05). However, dietary treatment significantly modulated serum immunoglobulins: IgA differed among groups (p = 0.019), being lower in FS3 and AB than in NC, PC, and FS1; IgM also varied (p = 0.028), with higher concentrations in FS1 and FS2 than in NC.

Tight-junction gene expression was modulated by the dose of FS. ZO-1 mRNA abundance was significantly

Table 3. Primer sequences used for RT-qPCR.

Gene	Primer sequence (5′ to 3′)			GenBank ID
	Forward primer	Reverse primer	Size (bp)	
Tight-binding protein				
ZO-1	CTTCAGGTGTTTCTCTTCCTCCTC	CTGTGGTTTCATGGCTGGAT	121	XM_413773.4
Occludin	GCAGATGTCCAGCGGCCCCCC	CGAAGAAGCAGATGAGGCAGAG	89	NM_205128.1
Claudin-2	CATCCTCCTGGGTCTGGTTGGT	GACAGCCATCCGCATCTTCT	198	NM_001013611.2
Pre-inflammatory cytokines				
IL-4	GTGCCCACGCTGTGCTCTCTC	AGGAAACCTCT CCCTGGATGTC	82	GU119892.1
IL-1β	GCCCGAGCC AACCCCTGC	AGCAACGGGAC GGTAATGAA	204	NM_204524.1
TNF-α	CTCAGGACAGC CFCTGCCAACA	CCACCACACGA CAGCCAAGT	177	XM_015294125.2
IFN-γ	CCTCGCAACCT TCACCTCAC	CGCTGFC1ATCG TTG TCTTGGAG	76	FJ977575.1
GAPDH	AACTTTGGCAT TGTGGAGGG	ACGCTGGGATG ATGTTCTGG	130	NM_204305.1

higher in FS2 (1.71) and FS3 (1.70) than in PC (0.72) ($p < 0.05$). No significant differences in ZO-1 were detected among the PC, NC, FS1, and AB groups ($p > 0.05$). IFN-γ expression also differed among treatments (Table 6): NC displayed the highest level (2.91), significantly exceeding all other groups, whereas PC had the lowest (1.83). FS1 (2.57) was significantly higher than PC ($p < 0.05$), whereas FS2 (2.13), FS3 (2.18), and AB (2.11) did not differ from PC but were lower than NC.

Table 4. Effect of FS supplementation on broiler chicken growth performance.

Treatment	Pre-challenge period with *E. coli* (1–7 days old)				After-challenge period with (8–35 days old)			
	BWG (g/head)	FI (g/head)	FCR	Survival rate (%)	BWG (g/head)	FI (g/head)	FCR	Survival rate (%)
NC	64.23	77.84	1.21	100.0	658.59^{a}	1150.3^{a}	1.75^{b}	100.0^{a}
PC	65.2	77.07	1.18	100.0	593.57^{b}	1030.1^{b}	1.74^{b}	76.67^{c}
FS1	65.33	77.42	1.19	100.0	612.23^{b}	1119.4^{a}	1.83 ^{a}	93.33^{ab}
FS2	65.3	76.23	1.17	100.0	646.25^{ab}	1155.2^{a}	1.79 ^{ab}	90.0^{ab}
FS3	66	76.44	1.16	100.0	649.06^{ab}	1181.2^{a}	1.82 ^{a}	100.0^{a}
AB	66.33	76.45	1.15	100.0	658.86^{a}	1150.1^{a}	1.75b	100.0^{a}
Pooled SEM	2.42	0.68	0.06	-	31.22	60.42	0.12	-
p -value	0.285	0.443	0.269	-	0.031	0.024	0.029	-

Note: Within a column, mean values with different superscripts (a,b) indicate a significant difference ($p < 0.05$).

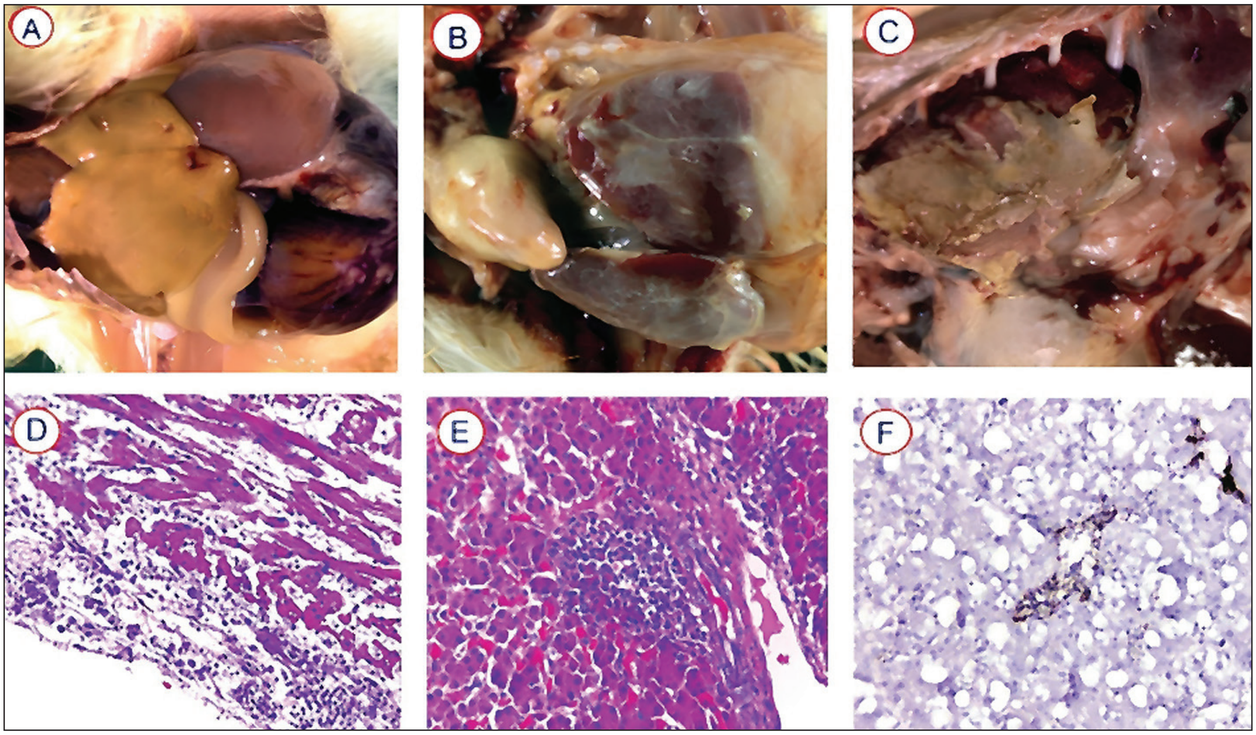

Fig. 2. Chickens, post-*E. coli* challenge, exhibited gross pathological lesions: (A) Distended, congested yolk sac with prominent blood vessels, containing abnormal brown fluid; (B) Polyserositis (comprising pericarditis, perihepatitis, peritonitis, and airsacculitis) resulting from the systemic spread of *E. coli*; (C) Pleuropneumonia and airsacculitis in broilers; and histopathological lesions observed under microscopy (H&E, 40×): (D) Liver with multiple areas of scattered fibrin deposition; (E) Pericardium characterized by an infiltration of intact or degenerated heterophils, macrophages, lymphocytes, and plasma cells, along with fibrin; (F) Fibrinoid necrosis was also evident in the follicular centers and blood vessels of the spleen, particularly the splenic arterioles.

Table 5. Immune organ indices and serum antibody concentrations of experimental chickens.

Treatment	Immune organ indices (g/kg BW)			Serum antibody concentrations (g/l)		
	Spleen	Fabricius	Thymus	IgA	IgM	IgG
NC	1.95	2.16	3.42	3.19^{a}	2.37^{b}	1.99
PC	1.83	2.05	3.82	3.26^{a}	2.85^{ab}	1.82
FS1	1.8	2.15	3.75	3.21^{a}	3.62^{a}	2.11
FS2	2.12	2.20	3.53	2.99^{ab}	3.39^{a}	1.85
FS3	2.23	2.45	3.91	2.65^{b}	2.81^{ab}	1.87
AB	1.93	2.21	3.52	2.55^{b}	3.12^{ab}	2.01
Pooled SEM	0.49	0.31	0.57	0.31	0.40	0.29
p -value	0.433	0.312	0.262	0.019	0.028	0.676

Note: Within a column, mean values with different superscripts (a,b) indicate a significant difference ($p < 0.05$).

Table 6. Effects of FS supplementation on the expression of immune genes.

Treatment	Immunogen expression						
	Tight junction protein expression			Pro-inflammatory cytokines			
	ZO-1	Occludin	Claudin-2	IL-4	IL-1β	TNF-α	IFN-γ
NC	0.71^{b}	1.25	1.28	2.56	1.46	1.70	2.91^{a}
PC	0.72^{b}	1.21	1.41	2.59	1.43	1.53	1.83^{c}
FS1	0.74^{b}	1.18	1.43	2.81	1.36	1.51	2.57^{b}
FS2	1.71^{a}	1.17	1.55	3.01	1.33	1.81	2.13^{bc}
FS3	1.70^{a}	1.18	1.41	2.89	1.44	1.66	2.18^{bc}
AB	0.70^{b}	1.18	1.35	2.87	1.33	1.68	2.11^{bc}
Pooled SEM	0.07	0.12	0.33	0.69	0.13	0.46	0.19
p -value	0.033	0.346	0.223	0.559	0.648	0.166	0.027

Note: Within a column, mean values with different superscripts (a,b) indicate a significant difference ($p < 0.05$).

Effects of FS extract on gastrointestinal health

FS altered the cecal microbiology and ileal histomorphology (Table 7). Representative images of ileal mucosal morphology are shown in Figure 3. Pathogen loads were greatest in PC (*Salmonella* spp., 6.67 log CFU/g; *E. coli*, 7.01 log CFU/g). Both FS and AB reduced these counts ($p < 0.05$), with FS3 exhibiting the lowest levels (*Salmonella*, 4.20 log CFU/g; *E. coli*, 5.20 log CFU/g), equal to or lower than AB (4.63 and 5.63 log CFU/g, respectively). The number of beneficial bacteria increased with FS, and FS3 achieved the highest *Lactobacillus* spp. density (7.21 log CFU/g), surpassing NC (4.83), PC (5.48), and AB (4.32) ($p < 0.05$).

Consistent with microbial shifts, ileal morphology improved in the FS group ($p < 0.05$). VH was greatest in FS3 (1,049.86 µm), exceeding NC (896.80 µm), PC (786.89 µm), and AB (807.07 µm); FS1 and FS2 were also higher than these three groups. CD in FS3 (81.33 µm) was similar to that in NC but significantly lower than that in PC, AB, FS1, and FS2 (92.57–106.43 µm). Consequently, the VH:CD ratio was the highest in FS3 (13.05), significantly exceeding PC, AB, and FS1 (7.42–9.22). Collectively, these results indicate the dose-responsive benefits of FS on performance, immune readouts, and gut health under *E. coli* challenge.

Discussion

Effects of FS extract on broiler performance

Growth improvements in FS birds are biologically plausible. Shallot contains antioxidant, anti-inflammatory, and antimicrobial polyphenols, flavonoids, and organosulfur compounds (Table 1). These compounds likely inhibited *E. coli* proliferation, improved intestinal homeostasis, and enhanced nutrient use. Fermentation increases efficacy by breaking plant cell walls, boosting bioavailability, and producing short-chain fatty acids that support enterocytes. Functionally, FS acts as a synbiotic. It combines prebiotic substrates from shallot with *L. plantarum*'s probiotic effects to lower luminal pH and suppresses pathogens.

These findings agree with those of previous studies. Fermented botanicals improve broiler performance (Hai *et al*., 2020; Adli *et al*., 2024), and lactic acid

Table 7. Effect of fermented supplement on intestinal health.

Treatment	Cecal microbiota			Ileal mucosal morphology (IMM)		
	Salmonella spp.	*E. coli*	*Lactiplantibacillus* spp.	VH (µm)	CD (µm)	VH/CD
NC	4.60^{b}	6.66^{ab}	4.83^{b}	896.80^{bc}	81.33^{bc}	11,13^{ab}
PC	6.67^{a}	7.0 1^{a}	5.48^{b}	786.89^{c}	106.43^{a}	7.42^{c}
FS1	4.62^{b}	6.23^{ab}	3.83^{bc}	928.19^{ab}	101.38^{a}	9.22^{bc}
FS2	4.47^{b}	5.57^{bc}	4.94^{b}	920.32^{ab}	92.57^{ab}	10.31^{ab}
FS3	4.20^{b}	5.20^{c}	7.21^{a}	1049.86^{a}	81.33^{bc}	13.05^{a}
AB	4.63^{b}	5.63^{bc}	4.72^{b}	807.07^{c}	105.04^{a}	7.73^{c}
Pooled SEM	0.34	0.48	0.79	86.8	21.09	0.71
p -value	0.031	0.022	0.013	0.011	0.027	0.009

Note: Villus height (VH), crypt depth (CD); within a column, mean values with different superscripts (a,b) indicate a significant difference ($p < 0.05$).

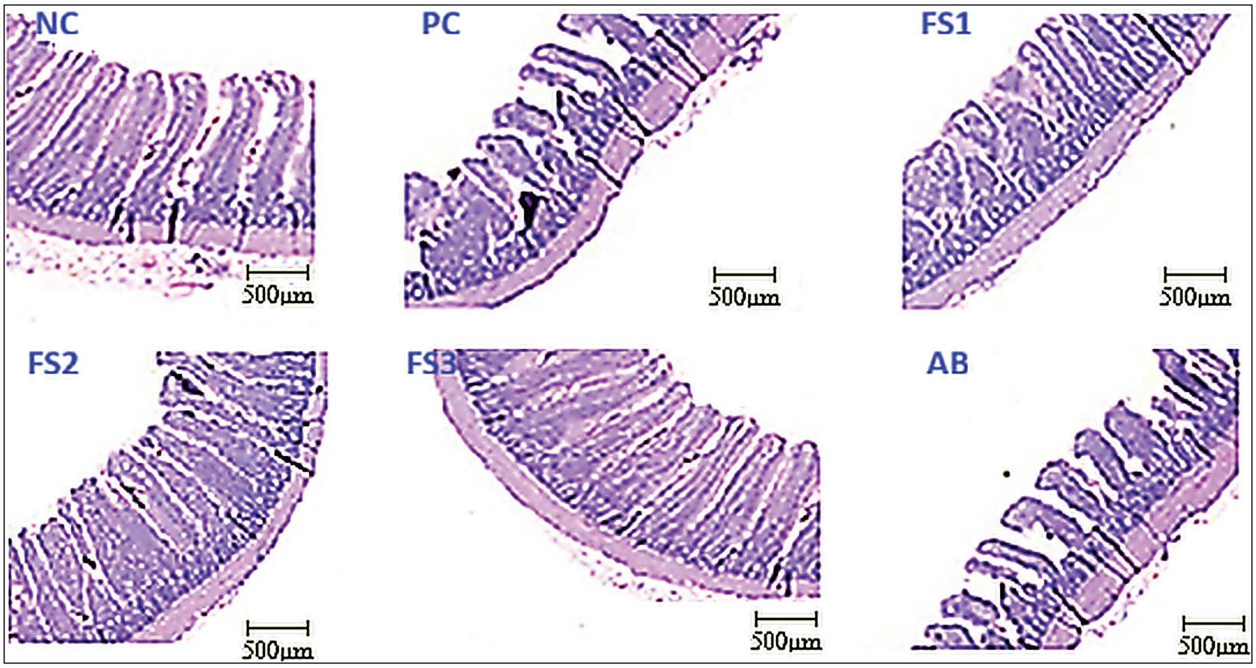

Fig. 3. Representative images of ileal mucosal lesions in *E. coli*-infected chickens from the positive control (PC) and antibiotic (AB) groups, which were ameliorated in the FS2 group and particularly in the FS3 group following FS supplementation.

bacteria enhance growth and feed efficiency (Liu *et al.*, 2025). Although the FCR was unchanged, the PEI increased in FS2 and FS3 vs the challenged control. This shows net production gains when morbidity and mortality are considered. FS offers a promising antibiotic-sparing strategy at 3% inclusion.

Effects of FS extract on immune function

No changes appeared in the spleen, bursa of Fabricius, or thymus indices. This indicates no overt lymphoid hypertrophy or atrophy. Serum immunoglobulins showed dose-dependent modulation. Elevated IgM at 1%–2% may reflect an early antibody response. Lower IgA at 3% (similar to the antibiotic group) likely indicates reduced antigenic load from a stronger barrier, although direct suppression cannot be ruled out (Ghareeb and K, 2012), but it may also reflect changes in mucosal immune activity. Therefore, we cautiously interpreted the IgA result and recommend future studies measuring mucosal secretory IgA and local immune-cell responses.

ZO-1 increased in FS2 and FS3, indicating tight-junction architecture reinforcement. ZO-1 is a central scaffolding protein that links transmembrane indicating tight junction components to the actin cytoskeleton (Gonzalez-Mariscal *et al.*, 2003). FS mainly strengthens the junctional scaffold (Awad *et al.*, 2009), matching the anti-inflammatory effects of fermented *Allium* compounds (Arreola *et al.*, 2015).

E. coli challenge lowered IFN-γ expression. FS at 1% partially restored it toward non-challenged levels. However, 2%–3% doses remained similar to the challenged control. This may reflect a *U*-shaped dose-response or lower antigenic stimulation (Marefati *et al.*, 2021). The absence of significant effects occurred on IL-1β or TNF-α. Overall, FS acts as a dose-dependent immunomodulator that concurrently strengthens epithelial defenses.

Effects of FS extract on gastrointestinal health

These results indicate that FS effectively modulates the intestinal microbiota. At 3% (FS3), it suppressed *Salmonella* spp. and *E. coli* to levels equal to or better than the antibiotic group (Mountzouris *et al.*, 2010; Gaggìa *et al.*, 2010). FS3 also markedly increased beneficial *Lactobacillus* spp. markedly—an effect absent in the antibiotic group. *Lactobacillus* spp. protect the mucosa by competing for adhesion sites and producing bacteriocins (Lee *et al*., 2011). Ileal VH increased with FS, especially at 3% (FS3) (Awad *et al.*, 2009), whereas CD decreased to nonchallenged levels in FS3 (Pelicano *et al.*, 2005). Thus, the VH:CD ratio was the highest in FS3, surpassing that in both the control and antibiotic groups.

Conclusion

Supplementation of drinking water with 3% *L. plantarum*- FSextract effectively mitigated *E. coli* challenge in broilers. The 3% FS group achieved BWG, PEI, and 100% survival, which were comparable to those of the nonchallenged and antibiotic controls and superior to those of the challenged control. Benefits aligned with improved gut health—suppressed cecal pathogens, enrichment of *Lactobacillus* spp., and optimized ileal histomorphology (higher VH, reduced CD, greater VH:CD) —supporting absorption and mucosal stability. FS displayed dose-dependent immunomodulation: 1%–2% increased serum IgM and 1% increased IFN-γ expression, whereas 3% decreased serum IgA and upregulated ZO-1. The absence of a non-FS extract control was a limitation; future studies should directly compare FS and non-fermented extract to confirm fermentation-specific effects.

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Conflict of interest

The authors declare no conflicts of interest.

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Authors' contributions

Experiment design: P.V.H., N.D.V., N.X.H., and H.T.A.P; Data analysis: P.V.H. and H.T.A.P.; Administrative and technical support: N.D.V. and V.T.M.T.; and manuscript drafting: P.V.H. and H.T.A.P.

Data availability

Data are stored according to the project records. The findings are provided by the corresponding author upon request.

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