

Research article

Whole plant black ginger in *Escherichia coli*-challenged yellow-feather broilers under cold-humid stress: a One Health model for reducing antimicrobial demandDoan T. H. Hanh¹, Tran V. H. Nguyen¹, Tran T. K. Ngan² and Phan V. Hai^{1*}¹Faculty of Animal Sciences and Veterinary Medicine, University of Agriculture and Forestry, Hue University, Hue City, Vietnam²School of Agriculture and Natural Resources, Vinh University, Vinh City, Vietnam*Corresponding author: Phan V. Hai, phanvuhai@hueuni.edu.vn

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Article information: Hanh DTH et al., 2026. *One Health Microbiol. Infect.* Vol. 2, No. 1: 122-131. <https://doi.org/10.66585/ohmi.2026.2.0023>**Abstract**

Antimicrobial resistance (AMR) in poultry production is driven by interactions among animal health, antimicrobial use, farm microenvironments, bacterial ecology, and food-chain exposure. This study evaluated whole plant black ginger (*Kaempferia parviflora* Wall. ex Baker) powder as a locally available phyto-genic intervention for yellow-feather Rilai broilers challenged with avian pathogenic *Escherichia coli* (APEC) under cold-humid stress. A total of 180 one-day-old male chicks were assigned to four treatments, with three replicate pens per treatment and 15 birds per pen: non-challenged control, *E. coli*-challenged control, antibiotic-treated challenged control, and black-ginger-supplemented challenged birds. Black ginger was provided continuously from day 1 to day 84 at 5 g/kg feed, before and after the day-35 APEC challenge. Compared with the challenged control, black ginger supplementation improved clinical response and feed conversion and reduced cecal *E. coli* and coliform counts. It also lowered malondialdehyde concentration and was associated with lower end-point abundance of selected AMR genes (*bla*TEM, *tetA*, *sul1*, *qnrS*, and *mcr-1*) than in the antibiotic-treated challenged group. However, no pre-treatment AMR baseline, phenotypic resistance testing, or whole resistome analysis was performed; therefore, AMR results should be interpreted as cecal gene-abundance indicators rather than proof of reduced antimicrobial resistance. These findings support black ginger as a promising preventive phyto-genic supplement that may improve host resilience under combined climatic and infectious stress, but not as a replacement for veterinary antimicrobial therapy when clinically required.

Keywords: Antimicrobial resistance, Avian pathogenic *Escherichia coli*, Black ginger, Cold-humid stress, One Health, Phyto-genic intervention, Poultry microbiology

Introduction

Antimicrobial resistance (AMR) is not only a therapeutic problem in individual animals; it is a One Health challenge linking antimicrobial use, bacterial ecology, food-animal production, environmental contamination, and human exposure [1, 2]. Global surveillance and joint One Health action plans emphasize that reducing unnecessary antimicrobial use in livestock requires interventions that protect animal health while lowering antimicrobial selection pressure in farm microbial communities [3, 4].

Cold-humid weather is a recurring production constraint in Central Vietnam. Low temperatures increase maintenance energy requirements and can reduce productivity, while high relative humidity and wet litter promote microbial proliferation, respiratory stress, footpad problems, and pressure from enteric pathogens. In colored Rilai broilers, a cold-humid model with four weeks of brooding followed by eight weeks of wet-litter exposure at approximately 13-16 °C and 68-75% relative humidity has reproduced winter-stress conditions [5]. Under these conditions, bacterial disease is best viewed as an environment-animal-microbiome problem rather than a pathogen-only event.

Avian pathogenic *Escherichia coli* (APEC) is a major bacterial threat in broiler production because it can reduce growth, increase mortality risk, and necessitate antimicrobial treatment. Repeated empirical therapy,

especially when not guided by microbiological diagnosis, may maintain AMR selection pressure in intestinal, litter-associated, and farm-environment bacteria. From a One Health perspective, an effective alternative should reduce pathogen burden and clinical disease while preserving beneficial microbiota and avoiding additional antimicrobial pressure [6, 7, 8].

Black ginger (*Kaempferia parviflora*) is a Zingiberaceae medicinal plant rich in methoxyflavones and phenolic compounds, including 5,7-dimethoxyflavone and 5,7,4'-trimethoxyflavone. Experimental studies have reported antioxidant, anti-inflammatory, and antimicrobial properties of black ginger or its methoxyflavone-rich fractions [9, 10, 11]. Because the chemical profile depends on harvest stage, plant part, drying, and extraction conditions, raw-material standardization is essential before black ginger can be interpreted as a reproducible animal-health intervention [12, 13].

In Central Vietnam, black ginger can be cultivated as a local medicinal resource. Using whole plants may improve biomass utilization and local circular-economy value, but it also increases the need for batch-level quality control because bioactive methoxyflavones are concentrated mainly in rhizomes. Previous studies from our group support the broader concept that Vietnamese phyto-genic and probiotic resources can suppress poultry

enteropathogens and contribute to antibiotic-reduced production systems [14, 15, 16]. The central hypothesis was that continuous supplementation with standardized whole plant black ginger would reduce clinical severity and pathogen pressure under cold-humid stress while maintaining beneficial microbial indicators and lowering post-challenge AMR-gene abundance compared with antibiotic-treated challenged birds.

Materials and methods

Black ginger cultivation, harvest, and whole plant preparation

Whole plants of black ginger (*Kaempferia parviflora* Wall. ex Baker) were harvested at 8-10 months after planting, when rhizomes were mature and expected to contain high concentrations of phenolic, flavonoid, and methoxyflavone compounds (Table 1). The three batches shown in Table 1 were independent analytical production batches prepared from pooled mature plants harvested at the same physiological stage; they were not plants

harvested at three different growth stages. Rhizomes, stems, and leaves were cleaned, sliced, dried at ≤45 °C, ground, and sieved to obtain a uniform whole plant powder. The same rhizome-to-aerial-part mixing procedure was used for all batches to reduce variation in bioactive concentrations.

Batch quality control included moisture, total phenolic content, total flavonoid content, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical-scavenging activity, total aerobic bacterial count, visible mold inspection, and marker methoxyflavones. Total phenolics were expressed as gallic acid equivalents (GAE) and total flavonoids as quercetin equivalents (QE). The values in Table 1 represent analytical concentrations per gram (g) dry weight (DW) of powder for each batch, the mean of the three batches, and the coefficient of variation (CV%). The proposed inclusion level was 5 g/kg feed, selected as a practical screening dose for a controlled first trial.

Table 1: Quality-control profile of whole plant black ginger powder.

Indicator	Unit	Batch 1	Batch 2	Batch 3	Mean
Moisture	%	8.2	8.0	8.4	8.2
Total phenolic content	mg GAE/g DW	31.4	30.7	32.1	31.4
Total flavonoid content	mg QE/g DW	17.8	17.4	18.2	17.8
DPPH radical-scavenging activity	% inhibition	72.6	70.9	73.8	72.4
ABTS radical-scavenging activity	% inhibition	68.9	67.6	70.2	68.9
5,7-dimethoxyflavone	mg/g DW	3.42	3.35	3.55	3.44
5,7,4'-trimethoxyflavone	mg/g DW	1.86	1.79	1.92	1.86
Total methoxyflavones	mg/g DW	8.95	8.72	9.20	8.96
Total aerobic bacteria	log ₁₀ CFU/g	3.1	3.2	3.0	3.1
Visible mold	Presence/Absence	Absent	Absent	Absent	Absent

GAE = gallic acid equivalents; QE = quercetin equivalents; DW = dry weight, CFU = colony forming unit, DPPH = 2,2-diphenyl-1-picrylhydrazyl, ABTS = 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid).

Experimental birds, treatments, and cold-humid stress model

A total of 180 one-day-old male Rilai yellow-feather broiler chicks were allocated to four treatments with three replicate pens per treatment and 15 birds per pen. Only male chicks were used to reduce variation associated with sex-related differences in growth rate, feed efficiency, and immune response in this first controlled challenge trial. The pen was the experimental unit for performance outcomes. Birds were brooded for the first four weeks under normal brooding management. The cold-humid stress phase was then applied during weeks 5-12, following a seasonal colored-broiler model under winter conditions [5].

During the cold-humid phase, birds were kept under naturally cool and humid winter conditions in an open-sided house with wet litter. Cold stress was not generated by artificial chilling; instead, the model used the natural

cold-humid season, and wet litter was maintained by periodic addition of clean water to keep the litter visibly moist but not flooded. Temperature and relative humidity were recorded at bird height using digital data loggers. The target profile was 12-18 °C with relative humidity ≥68%, with severe cold-humid windows defined as 12.6-15.5 °C and 71.2-72.9% relative humidity. This exposure was included because wet litter and cold stress can increase pathogen pressure and antimicrobial-treatment demand at farm level (Figure 1).

Basal diets and feeding management

Birds were fed phase-specific corn-soybean meal basal diets formulated for slow-growing yellow-feather broilers using starter (days 1-28), grower (days 29-56), and finisher (days 57-84) phases. Diets were formulated to meet or exceed poultry nutrient recommendations and practical requirements of colored broilers [17] (Table 2). No antimicrobial growth promoters or coccidiostats were

included in the basal diets. Feed and water were provided *ad libitum*. The black ginger (BG) group received the same basal diets with whole plant black ginger powder included at 5 g/kg feed by replacing an equal amount of corn. Black ginger was provided continuously from day 1 until day 84; therefore, it was evaluated as a preventive nutritional intervention rather than as a post-disease treatment. The antibiotic (AB) group received antibiotic therapy only after

E. coli challenge according to veterinary prescription and antimicrobial susceptibility testing.

For the BG diet, black ginger powder was included at 5 g/kg feed by replacing corn. The vitamin-mineral premix was included in all basal diets and supplied vitamins A, D₃, E, K, B-complex vitamins, and trace minerals according to farm specifications.

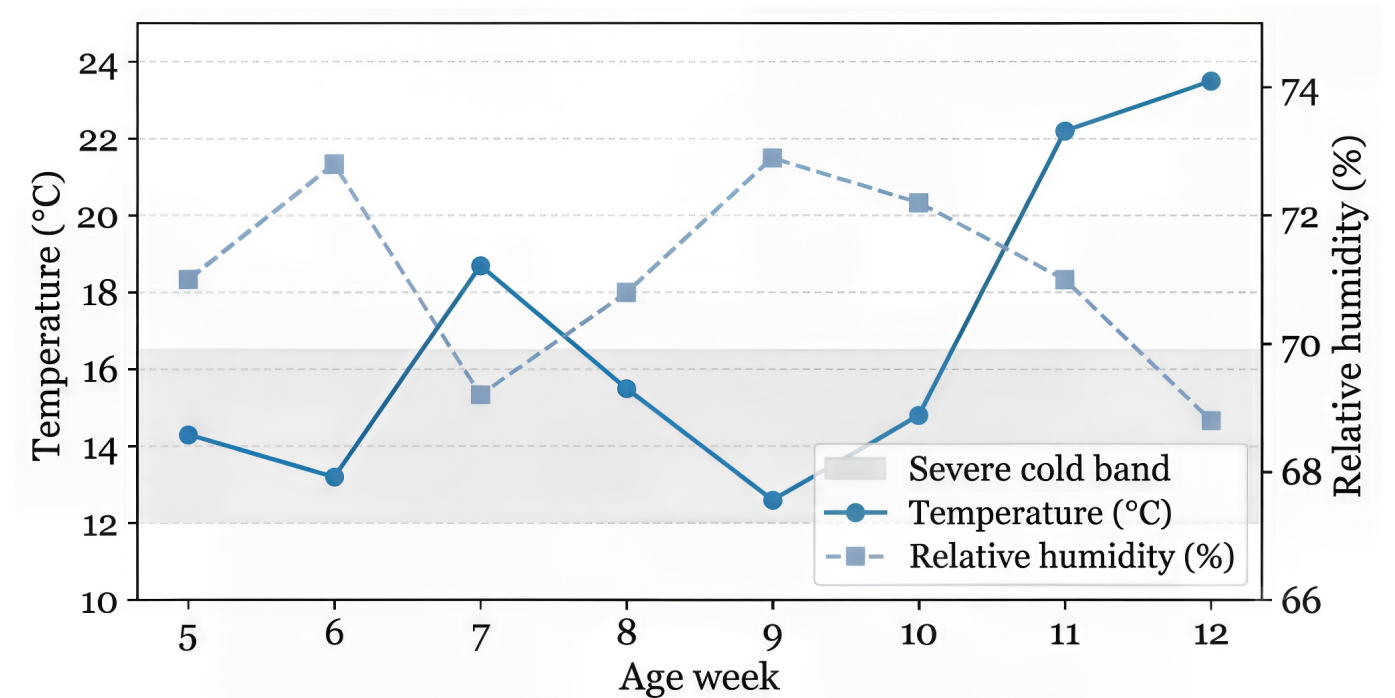


Figure 1: Daily temperature and relative humidity during the cold-humid exposure period. The shaded area indicates the severe cold band used to represent the cold-humid risk window under winter conditions.

Table 2: Ingredient and calculated nutrient composition of basal diets for yellow-feather broilers.

Ingredient/nutrient	Starter (days 1-28)	Grower (days 29-56)	Finisher (days 57-84)
Ingredient composition (%)			
Corn	52.80	55.10	58.20
Soybean meal	36.50	32.80	28.60
Rice bran	3.00	4.00	5.00
Vegetable oil	3.80	4.20	4.80
Fish meal	1.50	1.00	0.50
Dicalcium phosphate	1.35	1.30	1.25
Ground limestone	0.85	0.90	0.95
Salt	0.35	0.35	0.35
DL-methionine	0.18	0.16	0.14
L-lysine HCl	0.22	0.18	0.15
Vitamin-mineral premix	0.25	0.25	0.25
Total	100.0	100.0	100.0
Calculated nutrient composition			
Metabolizable energy (kcal/kg)	3000	3050	3100
Crude protein (%)	21.5	19.5	18.0
Lysine (%)	1.20	1.05	0.92
Methionine + cysteine (%)	0.85	0.78	0.72
Calcium (%)	0.95	0.90	0.85
Available phosphorus (%)	0.42	0.38	0.35

Escherichia coli challenge and sampling

At day 35, after birds had completed brooding and entered the cold-humid exposure phase, birds in the *E. coli*-challenged control, antibiotic control, and black ginger groups were orally challenged with 1 mL of inoculum containing approximately 1 × 10⁸ colony forming unit (CFU)/bird of an avian pathogenic *E. coli* field isolate designated APEC-HUAF-EC01. The isolate was obtained from colibacillosis-affected broilers, confirmed as *E. coli*

by culture, biochemical identification, and PCR, and stored at -80 °C until use. Non-challenged controls received sterile phosphate-buffered saline. The antibiotic group received enrofloxacin in drinking water at 10 mg/kg body weight per day for 5 consecutive days after challenge, as selected by antimicrobial susceptibility testing and veterinary prescription. Samples were collected at day 42, representing the acute post-challenge phase, and at day 84, representing the recovery phase (Figure 2).

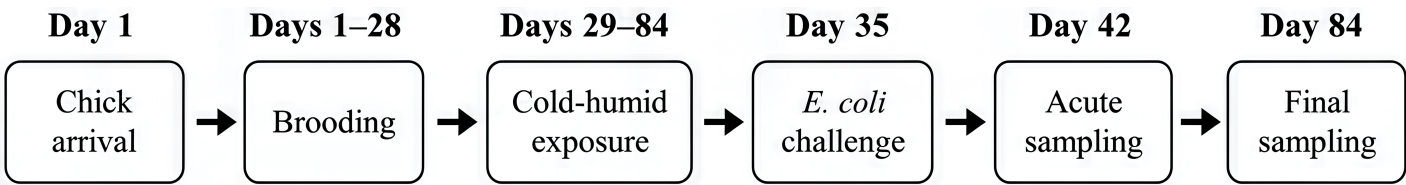


Figure 2: Experimental design and sampling timeline under cold-humid stress.

Measurements

Body weight, feed intake, mortality, and clinical scores were recorded. Clinical scores were recorded daily from day 1 to day 7 after challenge using a 0-3 scale: 0 = normal; 1 = mild ruffled feathers or transient depression; 2 = moderate depression, reduced activity, diarrhea, or respiratory signs; and 3 = severe depression, recumbency, marked respiratory signs, or moribund condition. Dead birds were necropsied, and mortality was attributed to colibacillosis only when typical lesions, such as airsacculitis, pericarditis, perihepatitis, or septicemic lesions, were accompanied by *E. coli* recovery from internal organs. Cecal contents, cloacal swabs, and organ samples, where appropriate, were analyzed for *E. coli*, total coliforms, lactic acid bacteria (LAB), and total viable counts (TVC) using standard microbiological methods [18].

Serum malondialdehyde (MDA) was used as a lipid-peroxidation marker, and antioxidant indicators included superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT), and total antioxidant capacity

(T-AOC) [19]. Selected AMR genes in bulk cecal DNA, including *bla*TEM, *tetA*, *sul1*, *qnrS*, and *mcr-1*, were quantified by quantitative PCR (qPCR) and expressed as log₁₀ gene copies/g cecal DNA using relative quantification principles, as previously described [20].

For qPCR, DNA was extracted from cecal contents using the DNeasy PowerSoil Pro Kit (QIAGEN, Hilden, Germany) following the manufacturer's protocol. Reactions were performed on a CFX96 real-time PCR system (Bio-Rad, Hercules, CA, USA) using SYBR Green chemistry in 20 µL reactions containing 10 µL of 2× master mix, 0.4 µM of each primer, 2 µL of template DNA, and nuclease-free water. The cycling profile was 95 °C for 3 min, followed by 40 cycles of 95 °C for 10 sec, 58-60 °C for 30 sec, and 72 °C for 30 sec, with a melt-curve step to confirm amplicon specificity. Primer sequences are provided in Table 3. Because AMR genes were quantified from bulk cecal DNA, the results represent gene-abundance indicators in the cecal microbial community and do not identify the bacterial host species or demonstrate phenotypic antimicrobial resistance.

Table 3: Primer sequences used for quantitative PCR detection of selected antimicrobial-resistance genes.

Target	Forward primer (5'-3')	Reverse primer (5'-3')	Annealing (°C)
<i>bla</i> TEM	ATGAGTATTCAACATTTCCGTG	TTACCAATGCTTAATCAGTGAG	58
<i>tetA</i>	GGTTCACTCGAACGACGTCA	CTGTCCGACAAGTTGCATGA	60
<i>sul1</i>	CGGCGTGGGCTACCTGAACG	GCCGATCGCGTGAAGTTCCG	60
<i>qnrS</i>	ACGACATTTCGTCAACTGCAA	TAAATTGGCACCCCTGTAGGC	58
<i>mcr-1</i>	CGGTCAGTCCGTTTGTTC	CTTGGTCGGTCTGTAGGG	58
16S rRNA	CCTACGGGNGGCWGCAG	GACTACHVGGGTATCTAATCC	58

Two exploratory indices were calculated to summarize the One Health oriented outcomes. The therapeutic antimicrobial-demand index was calculated as the percentage of birds in each group requiring therapeutic antimicrobial treatment or rescue intervention after

challenge, with the AB group fixed at 100% by design. The composite One Health risk score was calculated as a weighted normalized score including clinical score (25%), mortality (20%), cecal *E. coli* load (20%), mean normalized AMR-gene abundance (20%), and MDA

concentration (15%). Each component was scaled from 0 to 100 using the range observed in the study, with higher values indicating greater risk. These indices were used only as integrative descriptive indicators and were not intended to replace direct statistical analysis of the underlying outcomes.

Statistical analysis

Data were analyzed using mixed models with treatment as a fixed effect and pen as a random effect. For growth performance, pen was the experimental unit. For bird-level microbiological and serum data, bird was nested within pen. Tukey adjustment was used for pairwise comparisons. Differences were considered significant at $p < 0.05$. Multiple-testing correction for gene-abundance endpoints used the false discovery rate method [21].

Results

Growth performance and clinical response

Performance data indicated that *E. coli* challenge reduced final body weight and average daily gain and worsened feed conversion under cold-humid stress. Antibiotic treatment partially restored final body weight and average daily gain. Black ginger improved feed conversion ratio and clinical response compared with the challenged control, but final body weight and average daily gain

remained numerically lower than in the antibiotic group (Figures 3 and 4). Therefore, black ginger should be interpreted as improving resilience and clinical response under challenge, not as being equivalent to antibiotic treatment for all growth outcomes.

Cecal pathogen load, microbial indicators, and oxidative status

Microbiological data indicated that the challenged control group had the highest cecal *E. coli* and total coliform counts at the acute post-challenge phase (Figure 5A). Antibiotic treatment and black ginger both reduced cecal *E. coli* count relative to the challenged control. The LAB response did not support a broad claim that the antibiotic group had significantly depleted LAB relative to all other groups; therefore, we interpret LAB results cautiously. In the present data, black ginger maintained numerically high LAB counts while reducing *E. coli* and coliform counts. The challenge effect included higher MDA concentration in challenged controls than in non-challenged controls (Figure 5B). Black ginger reduced MDA relative to the challenged control, whereas differences among antioxidant enzyme activities were less consistent and are interpreted only as supportive antioxidant indicators rather than definitive mechanistic proof.

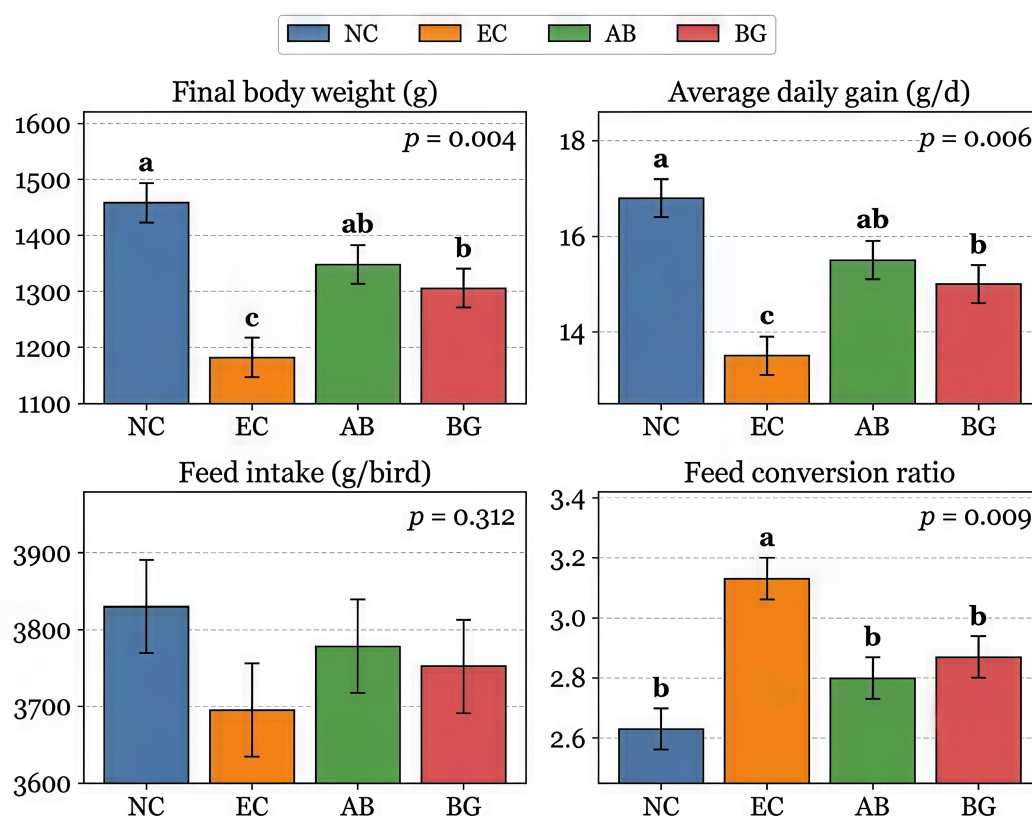


Figure 3: Growth performance of Rilai yellow-feather broilers under cold-humid stress. NC, non-challenged control; EC, *E. coli*-challenged control; AB, antibiotic-treated challenged control; BG, black-ginger-supplemented challenged group. Error bars represent SEM. Different letters above bars indicate significant differences among treatments within each panel ($p < 0.05$). The p value shown in each panel represents the overall treatment effect.

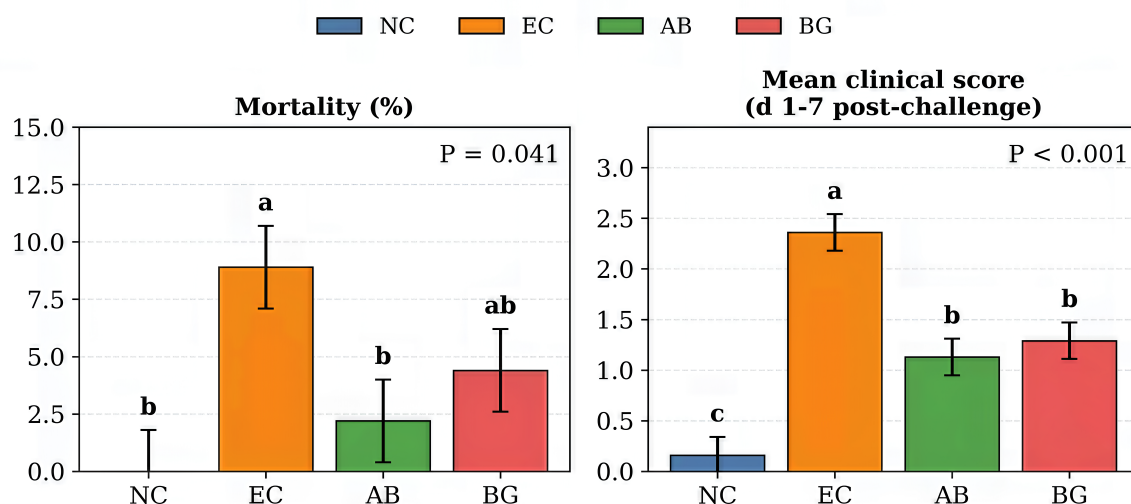


Figure 4: Mortality and clinical response of Rilai yellow-feather broilers under cold-humid stress. NC, non-challenged control; EC, *E. coli*-challenged control; AB, antibiotic-treated challenged control; BG, black-ginger-supplemented challenged group. Clinical score was recorded daily during days 1-7 post-challenge using a 0-3 scale and expressed as the mean value. Error bars represent SEM. Different letters above bars indicate significant differences among treatments within each panel ($p < 0.05$).

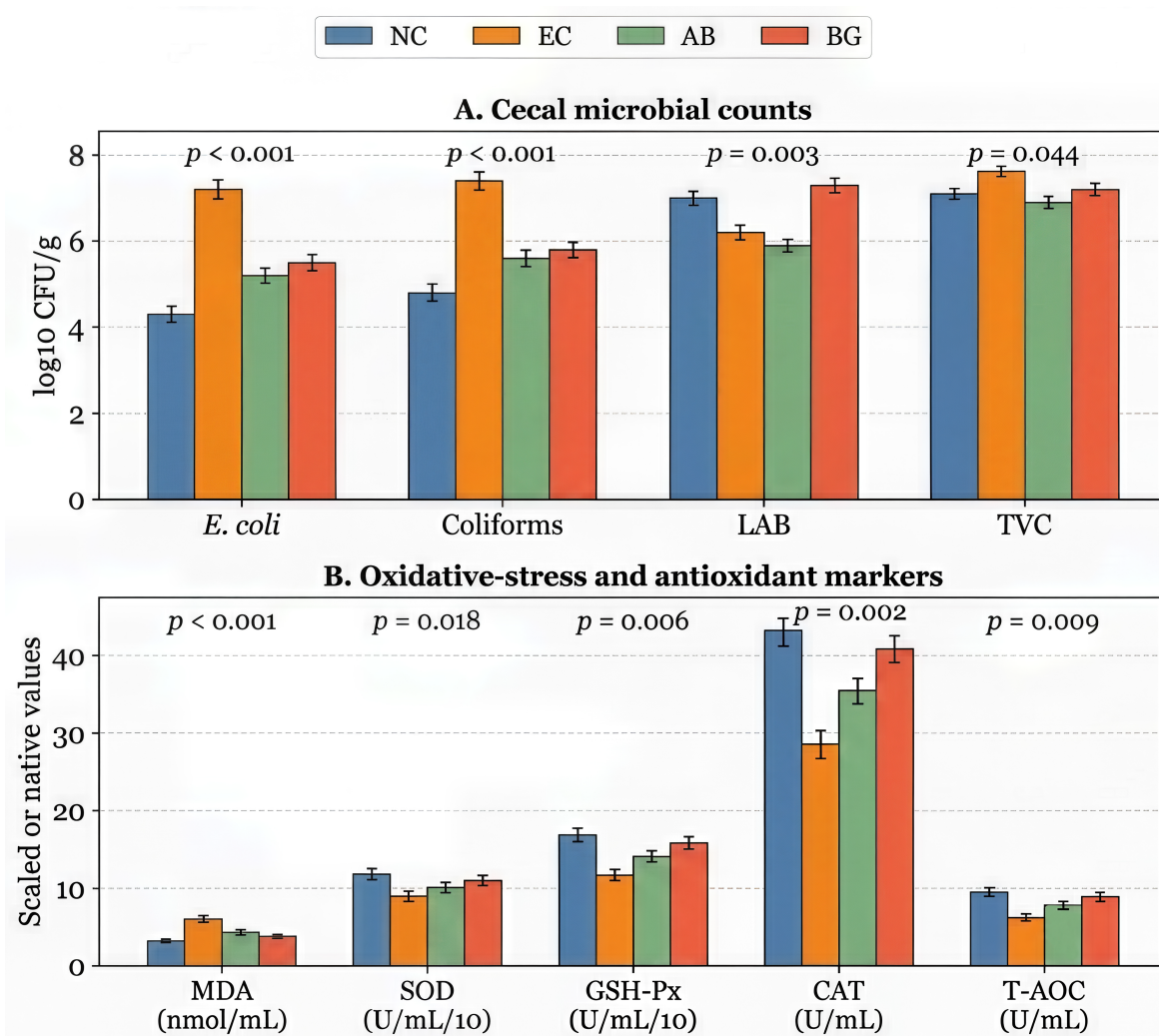


Figure 5: Cecal microbial counts and oxidative-stress markers at the acute post-challenge phase. NC, non-challenged control; EC, *E. coli*-challenged control; AB, antibiotic-treated challenged control; BG, black-ginger-supplemented challenged group; LAB, lactic acid bacteria; TVC, total viable counts; MDA, malondialdehyde; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; CAT, catalase; T-AOC, total antioxidant capacity; CFU, colony-forming unit. Error bars represent SEM. p values indicate overall treatment effects. Pairwise differences should be interpreted from the statistical grouping, not from bar height alone.

Antimicrobial-resistance gene indicators and integrated One Health indices

The qPCR values showed treatment effects for *bla*_{TEM} ($p < 0.001$), *tetA* ($p < 0.001$), *sul1* ($p = 0.002$), *qnrS* ($p = 0.006$), and *mcr-1* ($p = 0.018$), as shown in Figure 6A. Because no pre-treatment AMR baseline was available, these data cannot prove that black ginger reduced AMR genes from baseline. Instead, they show differences in end-point cecal AMR-gene abundance among treatment groups. The antibiotic group had the highest abundance

for several resistance markers, whereas the black ginger group showed lower end-point gene-abundance signals than the antibiotic-treated group while maintaining lower cecal *E. coli* counts than the challenged control. Integrated antimicrobial-demand and One Health risk indices also favored black ginger over the challenged and antibiotic groups (Figure 6B). These findings should be interpreted as microbiome-level AMR-gene indicators from bulk cecal DNA, not as evidence of phenotypic resistance, co-selection in a defined bacterial species, or complete resistome change.

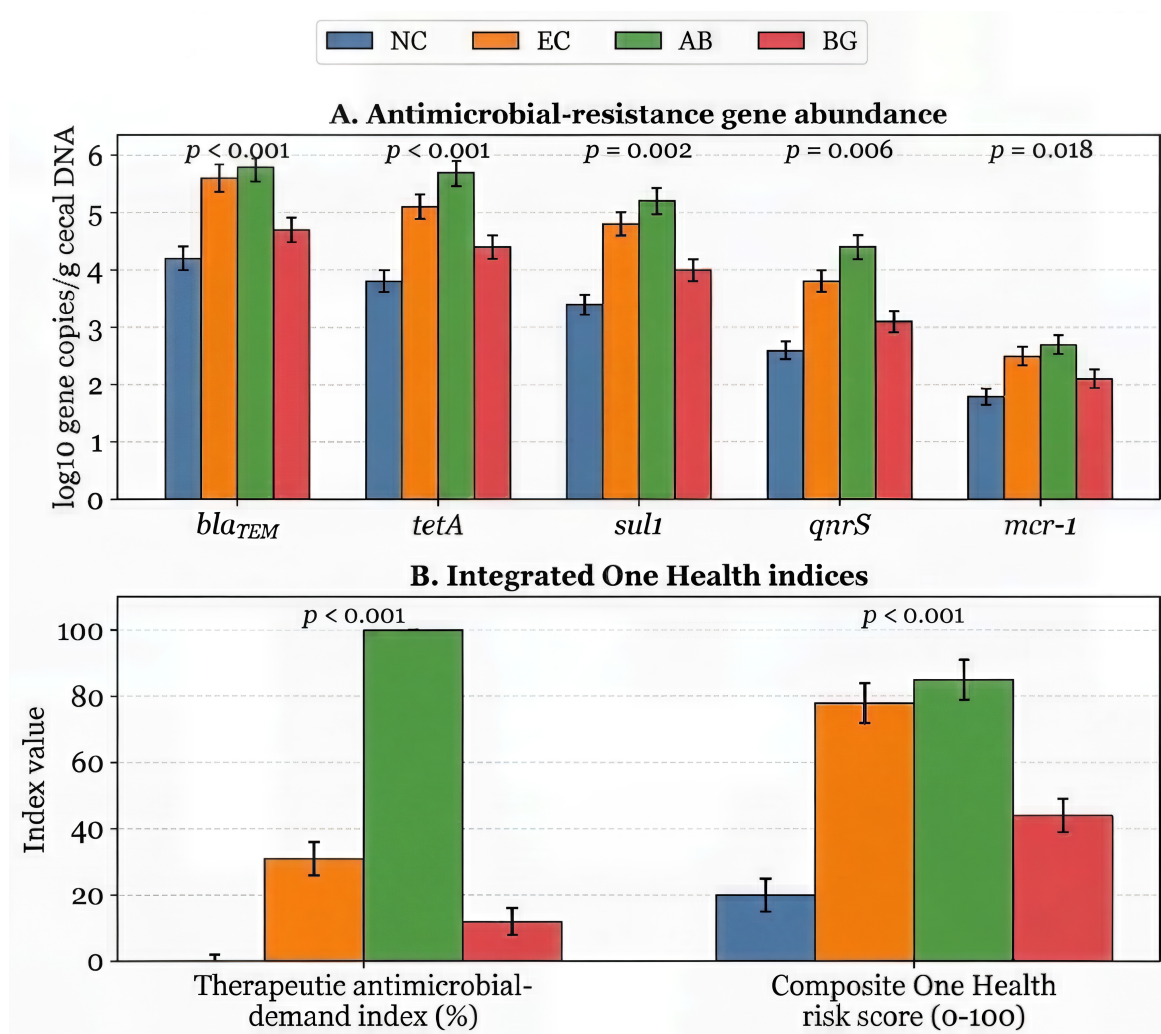


Figure 6: Antimicrobial-resistance gene abundance and integrated One Health antimicrobial-demand/selection-pressure indices. NC, non-challenged control; EC, *E. coli*-challenged control; AB, antibiotic-treated challenged control; BG, black-ginger-supplemented challenged group. Error bars represent SEM. p values indicate overall treatment effects. Gene-abundance data were obtained from bulk cecal DNA and represent end-point AMR-gene indicators rather than phenotypic resistance or host-specific gene carriage.

Discussion

This study presents a One Health intervention model rather than a conventional poultry feed-additive study. The experimental logic links animal health, farm environmental stress, bacterial pathogen pressure, gut microbial indicators, oxidative status, and AMR-gene abundance. This framing is consistent with the view that

antimicrobial demand in livestock is often driven by management and environmental risk factors rather than pathogen exposure alone [22, 23, 24]. However, the AMR component should be interpreted cautiously because the study measured selected genes in bulk cecal DNA at the post-challenge endpoint and did not include baseline

resistome measurements or phenotypic susceptibility testing of recovered isolates.

Cold-humid stress is central to the model. Low temperature can increase maintenance energy requirements and divert nutrients away from growth, while wet litter and high humidity increase microbial challenge. Under these conditions, an *E. coli* challenge is more realistic than a pathogen-only model under thermoneutral conditions. The reduction in clinical score and improved feed conversion in the black ginger group suggest that phytogenic interventions may improve resilience to combined environmental and infectious stress. Nevertheless, black ginger should be considered a preventive supplement that supports resilience, not a stand-alone therapeutic replacement for antibiotics in clinically severe colibacillosis.

The use of whole-plant black ginger has local One Health relevance. It connects animal health with sustainable use of locally cultivated medicinal plants and reduced dependence on imported or routine antimicrobial inputs. At the same time, whole-plant products are inherently more variable than purified extracts. For this reason, batch-level standardization using moisture, total phenolic content, total flavonoid content, antioxidant activity, microbial quality, and methoxyflavone markers is necessary for reproducibility and translation into farm practice [25, 26].

Black ginger may act through both antibacterial and antioxidant pathways, but the present study cannot completely separate these mechanisms. The reduction in cecal *E. coli* and coliform counts supports an *in vivo* antibacterial-associated effect, whereas the lower MDA concentration supports an antioxidant-related effect. Because a separate *in vitro* minimum inhibitory concentration or time-kill assay against the challenge isolate was not performed, the antibacterial interpretation should remain limited to the *in vivo* microbiological outcomes observed in this challenge model. Future studies should combine *in vitro* antimicrobial testing, phytochemical fractionation, and *in vivo* challenge trials to determine whether the response is mainly due to direct antimicrobial activity, modulation of gut ecology, improved antioxidant status, or a combination of these mechanisms.

The cecal microbiological response provides a practical microbiology-based rationale for the intervention, but it should not be overinterpreted. Antibiotic treatment reduced *E. coli* counts, whereas black ginger reduced *E. coli* and coliform counts while maintaining numerically favorable LAB counts. This pattern is relevant in One Health microbiology because maintaining microbial balance may reduce future disease susceptibility. Nevertheless, culture-based indicator counts do not define full microbial community structure, and future work

should include 16S rRNA sequencing or metagenomics to clarify microbiome-level effects.

The AMR-gene findings are best interpreted as endpoint indicators of selection pressure, not as direct evidence of phenotypic resistance. Detection of *bla*TEM, *tetA*, *sul1*, *qnrS*, and *mcr-1* in bulk cecal DNA does not show which bacterial species carried the genes, whether the genes were located on mobile elements, or whether isolates were phenotypically resistant. This distinction is important because isolate-level comparison of antimicrobial-susceptibility phenotypes with resistance genotypes can reveal phenotype–genotype discordance and coresistance patterns that cannot be resolved by bulk-community qPCR alone, as demonstrated in multidrug-resistant *Salmonella enterica* serovar Choleraesuis recovered from diseased pigs [27]. The absence of pre-treatment AMR baselines further limits causal inference. Therefore, the conclusion has been revised from 'black ginger reduced AMR' to 'black ginger was associated with lower post-challenge abundance of selected AMR genes than the antibiotic group.'

From a field perspective, the most feasible recommendation is not to use black ginger as an emergency treatment after bacterial disease has already developed. Instead, standardized black ginger powder may be evaluated as a preventive feed supplement during high-risk cold-humid periods, together with litter management, brooding temperature control, hygiene, vaccination where appropriate, microbiological diagnosis, and responsible veterinary antimicrobial use. A related study evaluated *Lactiplantibacillus plantarum*-fermented shallot extract delivered through drinking water as a natural antibiotic alternative in *E. coli*-challenged broilers, illustrating a complementary phytogenic–probiotic intervention and an alternative route of administration [28]. Farmers should use only batches with verified moisture, mold absence, microbial safety, and methoxyflavone/phenolic profiles, because whole-plant phytogenic products can vary substantially in composition.

This study has several limitations. It used a single black ginger inclusion level, one challenge isolate, selected microbial indicators, and selected AMR genes. The sample size was appropriate for a controlled pilot challenge trial but was not large enough to define dose-response or field-level efficacy. *In vitro* antibacterial activity against the challenge isolate was not tested, and the AMR analysis did not include baseline sampling, phenotypic resistance testing, plasmid analysis, or whole-resistome sequencing. These limitations should be addressed before black ginger is recommended as a routine farm intervention.

Conclusions

This study supports whole-plant black ginger powder as a promising locally produced phytogenic supplement for *E.*

coli-challenged yellow-feather broilers under cold-humid stress. Continuous supplementation at 5 g/kg feed from day 1 to day 84 improved clinical response and feed conversion, reduced cecal *E. coli* and coliform counts, lowered MDA, and was associated with lower post-challenge abundance of selected AMR genes than antibiotic treatment. The main contribution is the integrated One Health logic: improving resilience during environmental stress may reduce therapeutic antimicrobial demand and limit additional antimicrobial selection pressure. However, the findings should be interpreted as pilot challenge-trial evidence. The study did not include pre-treatment AMR baselines, phenotypic resistance testing, whole-resistome analysis, or separate *in vitro* antibacterial testing. Before farm-level recommendation, black ginger should be validated in larger trials using standardized batches, clear quality-control criteria, environmental sampling, and economic assessment.

Declarations and supplementary information

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Author contributions: Conceptualization, DTHH and PVH; Methodology, DTHH and PVH; Formal Analysis, PVH; Investigation, DTHH and TVHN; Resources, PVH; Data Curation, DTHH and PVH; Writing - Original Draft Preparation, DTHH and PVH.; Writing - Review & Editing, PVH; Visualization, PVH; Supervision, PVH; Project Administration, TTKN. All authors approved the final version.

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Ethics statement: All procedures involving animals complied with Article 72 of the Vietnamese Law on Animal Husbandry (No. 32/2018/QH14). The research protocol was reviewed and approved by the institutional authority at Hue University, Vietnam.

Data availability: Not applicable.

Supplementary materials: Not applicable.

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