



Lactic-fermented red onion (*Allium cepa* L.) extract in drinking water improves feed efficiency, selectively modulates cecal microbiota, and reduces ammonia emission in heat-stressed yellow-feather chickens

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Abstract

Heat stress is a major constraint to sustainable poultry production in tropical regions. This study evaluated the effects of *Lactobacillus plantarum* 1582-fermented red onion bulb extract (LFRO), supplied through drinking water, on growth performance, cecal microbiota, and excreta gas emissions in heat-stressed yellow-feather chickens. A total of 300 one-day-old male Rilai chicks were assigned to four treatments in a completely randomized design with five replicate pens of 15 birds each: unsupplemented control or LFRO at 25, 50, or 100 mg/L in drinking water. Birds were reared for 70 days under natural chronic tropical heat stress, with the temperature-humidity index predominantly within the severe-to-very severe range (28.9–31.1). Supplementation with 100 mg/L LFRO increased final body weight and body weight gain, reduced feed intake, and improved feed conversion ratio over the whole trial. The European Production Efficiency Index and Broiler Performance Efficiency Factor were also highest in the 100 mg/L group. Cecal 16 S rRNA sequencing showed no major changes in alpha- or beta-diversity, but several selected bacterial genera were modulated. LFRO at 50 and 100 mg/L reduced ammonia emission from excreta, whereas the other measured gases were not affected. This study shows LFRO at 100 mg/L is a natural drinking water supplement that improves feed efficiency and gut microbial balance in yellow-feathered chickens exposed to chronic tropical heat stress.

Keywords Ammonia emission · Cecal microbiota · Feed conversion ratio · Fermented red onion · Heat stress · Yellow-feather chicken

Introduction

Heat stress is one of the main environmental obstacles to poultry production in tropical and subtropical areas. Long exposure to high ambient temperatures and humidity causes many issues, including altered nutrient partitioning, more rapid growth degradation, less feed intake, disturbed intestinal barrier functionality, and an increase in both oxidative

and inflammatory pressure (Teyssier et al. 2022; Liu et al. 2023; Ncho 2025). The effect of heat stress on the gastrointestinal microbiota in chickens has been documented. Recent literature states that changes in the microbiota can be characterized by stress duration, age of the bird, diet, and other experimental constraints (Campos et al. 2025; Ncho 2025). These issues are especially reflected in the slow and medium growing local chicken genotypes raised in open sided houses, where environmental control is nearly absent.

With the global phase-out of antibiotic growth promoters, there is increasing interest in phytogetic, probiotic, prebiotic, and postbiotic additives that can support bird performance and gut health particularly, in the context of stress. Fermented plant extracts are particularly attractive because lactic fermentation can increase the release and bio-availability of plant bioactives, generate organic acids, and provide postbiotic metabolites that may improve nutrient

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utilization and resilience towards thermal stress (Adli et al. 2024; Alharthi et al. 2025; Waqas et al. 2026). Recent studies in poultry further support the use of probiotic and phyto-genic-based strategies to attenuate heat stress responses through the utilization of the antioxidant, immune response, intestinal barrier, and microbiota pathways in poultry (Barekatain et al. 2025; Al-Khalaifah et al. 2025).

Among *Allium* species, red onion (*Allium cepa* L.) is a promising candidate for tropical poultry production because it contains quercetin and compounds with antioxidant and antimicrobial effects such as polyphenols and organosulfur compounds (Kothari et al. 2019; Malematja et al. 2023). However, the use of these unfermented *Allium* extracts can be inconsistent because the stability and bioavailability of the active compounds vary with processing, plant material, and delivery route. Lactic fermentation with *Lactobacillus plantarum* has the ability to release bound flavonoids, increasing organic acid content, and enriching the final extract with microbial metabolites (Hai et al. 2024, 2025).

Although fermented *Allium* extracts have shown potential in broilers, the novelty of the present study is that it evaluates *L. plantarum*-fermented red onion extract (LFRO) as a drinking-water additive in yellow-feather chickens exposed to natural chronic tropical heat stress. In addition, an assessment of productive performance, cecal microbial ecology, and excreta gas emissions has rarely been conducted in such an experimental design under practical open-sided tropical housing conditions. This study was designed to investigate LFRO supplementation with the aim of improving feed efficiency, selectively modulate the cecal microbiota, and reduce ammonia emission from excreta.

Materials and methods

Ethics approval

All procedures involving animals were approved by the Animal Ethics Advisory Committee of Hue University, Vietnam (Approval No. HUVNO59, 31 March 2025), and animal handling complied with institutional requirements for the care and use of research animals.

Birds, housing, and thermal environment

A total of 300 one-day-old male Rilai yellow-feather chicks were used in a completely randomized design. Birds were assigned to four treatments with five replicate pens per treatment and 15 birds per pen. The feeding trial lasted 70 days. Chicks were reared in wire-floor battery cages in an open-sided house and had ad libitum access to feed and water throughout the experiment. A commercial anti-stress

solution was supplied in the drinking water during the first 3 days after placement.

Birds were vaccinated against infectious bronchitis (H120, Massachusetts serotype – GI-1) and Newcastle disease (LaSota, lentogenic, B1 type) on day 7 and against infectious bursal disease (Winterfield 2512, Intermediate Plus) on day 14.

Ambient temperature (T, °C) and relative humidity (RH, %) inside the house were recorded throughout the experiment, and the temperature-humidity index (THI) was calculated according to Habeeb et al. (2018) as $THI = (0.8 \times T) + [(RH/100) \times (T - 14.4)]$. Following the same classification, THI values < 27.8 indicate no heat stress, 27.8–28.8 moderate heat stress, 28.9–29.9 severe heat stress, and > 30.0 very severe heat stress. As shown in Fig. 1, weekly THI values remained predominantly within the severe-to-very severe range (approximately 28.9–31.1), with the highest thermal load observed in week 8. Accordingly, the birds were considered to be exposed to natural chronic heat stress during most of the trial, providing a biologically relevant tropical stress model (Teyssier et al. 2022). Ambient temperature (T, °C) and relative humidity (RH, %) inside the house were recorded throughout the experiment, and the temperature-humidity index (THI) was calculated according to Habeeb et al. (2018) as $THI = (0.8 \times T) + [(RH/100) \times (T - 14.4)]$. Following the same classification, THI values < 27.8 indicate no heat stress, 27.8–28.8 moderate heat stress, 28.9–29.9 severe heat stress, and > 30.0 very severe heat stress. As shown in Fig. 1, weekly THI values remained predominantly within the severe-to-very severe range (approximately 28.9–31.1), with the highest thermal load observed in week 8. Accordingly, the birds were considered to be exposed to natural chronic heat stress during most of the trial, providing a biologically relevant tropical stress model (Teyssier et al. 2022).

Preparation of lactic-fermented red onion extract

Fresh red onion (*Allium cepa* L.) bulbs were cleaned, peeled, washed, and homogenized. Fermentation was performed following Hai et al. (2024), with minor modifications. Briefly, red onion mash was inoculated with *L. plantarum* strain 1582 at 1×10^8 CFU/mL in a medium containing 5% NaCl and 3% glucose. Fermentation proceeded at 37 °C for 72 h under anaerobic conditions with gentle agitation. The final suspension was filtered to obtain the aqueous fermented extract. The resulting product contained viable $3\text{--}4 \times 10^7$ *Lactobacillus* spp./mL and measurable concentrations of polyphenols, quercetin, organosulfur compounds, and organic acids (Table 1).

Fig. 1 Weekly ambient temperature, relative humidity, and temperature-humidity index (THI) recorded during the experiment

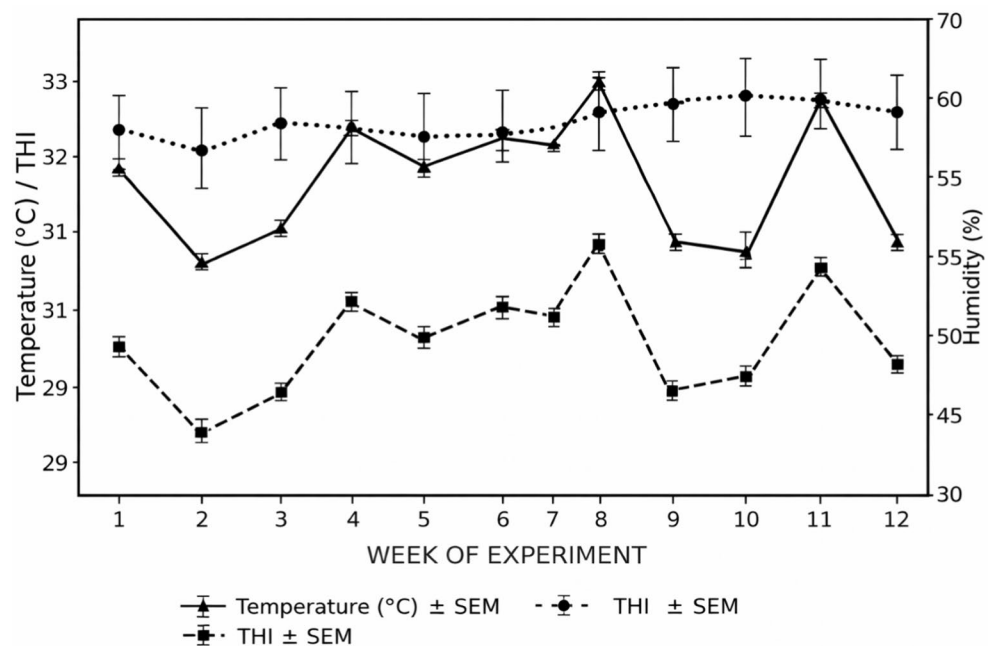


Table 1 Nutritional and bioactive composition of the lactic-fermented red onion bulb extract

Component	Value (mean ± SE)	Method
Polyphenol (mg/g)	16.25 ± 0.22	Folin-Ciocalteu
Quercetin (mg/g)	4.67 ± 0.06	UV-Vis
Allicin (mg/kg)	1.25 ± 0.05	GC-MS
Thiosulfate (mg/g)	2.21 ± 0.08	GC
S-allyl cysteine (mg/g)	1.98 ± 0.05	GC-MS
Lactic acid (%)	1.32 ± 0.04	HPLC
Acetic acid (%)	0.46 ± 0.01	HPLC
Citric acid (%)	0.69 ± 0.02	HPLC

Table 2 Ingredient and nutrient composition of basal diets (as-fed basis)

Item	Starter phase (d 1–35)	Finisher phase (d 36–70)
Yellow corn (%)	48.1	57.4
Soybean meal (%)	43.1	34.7
Fish meal (%)	5.5	4.5
CaCO ₃ (%)	1.5	1.5
CaHPO ₄ (%)	1.0	1.0
Sodium chloride (%)	0.3	0.3
Choline chloride (50%)	0.02	0.02
DL-Methionine (%)	0.2	0.2
Vitamin premix (%)	0.1	0.1
Mineral premix (%)	0.2	0.2
Crude protein (%)	23.1	19.0
Crude fat (%)	4.28	5.38
Crude fiber (%)	4.15	4.15
Calcium (%)	1.10	1.00
Total phosphorus (%)	0.41	0.34
Lysine (%)	1.32	1.04
Methionine (%)	0.60	0.55
Methionine + cystine (%)	0.91	0.81
Metabolizable energy (kcal/kg)	3100	3150

Diets and experimental treatments

A corn-soybean meal starter diet was fed from day 1 to day 35 and a finisher diet from day 36 to day 70. Diets were formulated to meet Vietnamese feeding standards for broiler chickens (Table 2). The four treatments comprised an unsupplemented negative control (NC) and NC supplemented with 25, 50, or 100 mg/L fermented red onion extract in drinking water (T25, T50, and T100, respectively). The fermented extract was freshly diluted in drinking water each morning.

Day-old chicks were individually weighed upon placement to establish the initial average body weight. Thereafter, all birds were weighed at weekly intervals throughout the 70-day trial to determine weekly average body weight and body weight gain per bird. Daily feed intake was recorded on a per-bird basis and used to calculate the weekly feed conversion ratio (FCR). In addition, the European Production Efficiency Index (EPEI) and Broiler Performance Efficiency Factor (BPEF) were computed according to the equations of Lohakare and AbdelWareth (2022) as follows:

$$\text{EPEI} = [\text{Viability (\%)} \times \text{Live body weight (kg)} \times 100] / [\text{Age (days)} \times \text{FCR}]$$

$$\text{BPEF} = [\text{Live body weight (kg)} \times 100] / \text{FCR}$$

For the final calculation, live body weight was expressed in kilograms, viability was calculated as 100 - mortality (%), age was fixed at 70 days, and the overall 1–70 d FCR was used. These units explain the EPEI and BPEF values reported in Table 3.

Table 3 Effects of LFRO supplementation in drinking water on growth performance

Parameter	NC	T25	T50	T100	<i>P</i> -value
Initial body weight (g/bird)	44.5±0.81	45.1±1.04	45.0±0.92	44.7±0.87	0.741
Final body weight (g/bird)	1622.4±3.22 ^b	1639.6±55.53 ^b	1663.5±23.04 ^{ab}	1718.1±39.38 ^a	0.029
Body weight gain (g/bird)	1577.9±32.1 ^c	1594.6±38.4 ^{bc}	1618.5±29.8 ^{ab}	1673.5±41.2 ^a	0.022
Feed intake (g/bird, 1–70 d)	3125.2±68.5 ^a	2998±72.1 ^b	3005±45.3 ^b	2959±52.4 ^b	0.036
FCR (1–70 d)	1.98±0.04 ^a	1.88±0.03 ^b	1.86±0.02 ^b	1.77±0.02 ^c	0.029
Mortality (%)	2.67	1.33	1.33	4.00	0.783
European Production Efficiency Index (EPEI)	113.9±4.2 ^c	123.0±5.1 ^b	126.1±3.8 ^b	133.1±4.6 ^a	0.011
Broiler Performance Efficiency Factor (BPEF)	81.9±2.1 ^c	87.2±2.8 ^b	89.5±1.9 ^b	97.1±2.4 ^a	0.029

Data are presented as mean±SD; ^{a–c} different superscript letters within the same row indicate significant differences among treatment groups ($P < 0.05$). FCR: feed conversion ratio. EPEI and BPEF were calculated using final live body weight in kilograms and overall 1–70 d FCR

Cecal microbiota analysis

On day 70, one representative bird per replicate ($n=5$ per treatment) with body weight close to the pen mean was selected for cecal sampling. Cecal contents (250 mg) were aseptically collected, snap-frozen, and used for microbial DNA extraction with the E.Z.N.A. Soil DNA Kit (Omega Bio-Tek, Norcross, GA, USA). The V3-V4 region of the bacterial 16 S rRNA gene was amplified using primers 338 F and 806R. PCR products were purified with the Axy-Prep DNA Gel Extraction Kit and sequenced on an Illumina MiSeq platform (2×250 bp) by Tsingke Biotechnology Co., Ltd., following the protocol described by Lin et al. (2023). Operational taxonomic units (OTUs), alpha-diversity indices, beta-diversity patterns, and taxonomic composition were compared among treatments.

Excreta gas measurement

Fresh excreta samples were collected from five points within each pen, pooled, and incubated in sealed plastic boxes at 25 °C for 7 days. Before measurement, the surface crust was manually disrupted for approximately 30 s to homogenize the sample. A 100-mL headspace sample was then withdrawn about 2 cm above the excreta surface. Ammonia (NH₃), hydrogen sulfide (H₂S), carbon dioxide (CO₂), methyl mercaptan, and acetic acid were measured using a portable gas analyzer (MultiRAE Lite, model PGM-6208; RAE Systems, Denmark).

Statistical analysis

Pen was considered the experimental unit for performance and excreta gas emission data, whereas the individual sampled bird served as the experimental unit for cecal microbiota analyses. Data were analyzed by one-way analysis of variance using SPSS version 23. When a treatment effect

was detected, means were separated by Duncan's multiple range test. Differences were considered significant at $P < 0.05$. Results are presented as means±standard error (SE).

Results

Growth performance

Growth performance results are summarized in Table 3. Initial body weight was similar among treatments ($P = 0.741$). Over the entire 70-day trial, supplementation with lactic-fermented red onion extract (LFRO) significantly affected final body weight ($P = 0.029$), body weight gain ($P = 0.022$), feed intake ($P = 0.036$), and feed conversion ratio ($P = 0.029$). Birds receiving 100 mg/L LFRO (T100) exhibited the highest final body weight and body weight gain, which were significantly greater than those in the negative control group (NC). Feed intake was significantly lower in all LFRO-supplemented groups compared with NC. The lowest (best) FCR was recorded in T100. Consequently, both the European Production Efficiency Index (EPEI) and Broiler Performance Efficiency Factor (BPEF) were significantly improved by LFRO supplementation ($P = 0.011$ and $P = 0.029$, respectively), with the highest values observed in T100. Mortality rate remained low and was not affected by treatment ($P = 0.783$).

Cecal microbiota

A total of 651 OTUs were detected in cecal samples, of which 601 were shared by all treatment groups (Fig. 2A). At the phylum level, Bacteroidetes and Firmicutes dominated the community, whereas *Bacteroides* spp., *Lactobacillus* spp., and the Rikenellaceae RC9 gut group were the most abundant genera (Fig. 2B, C).

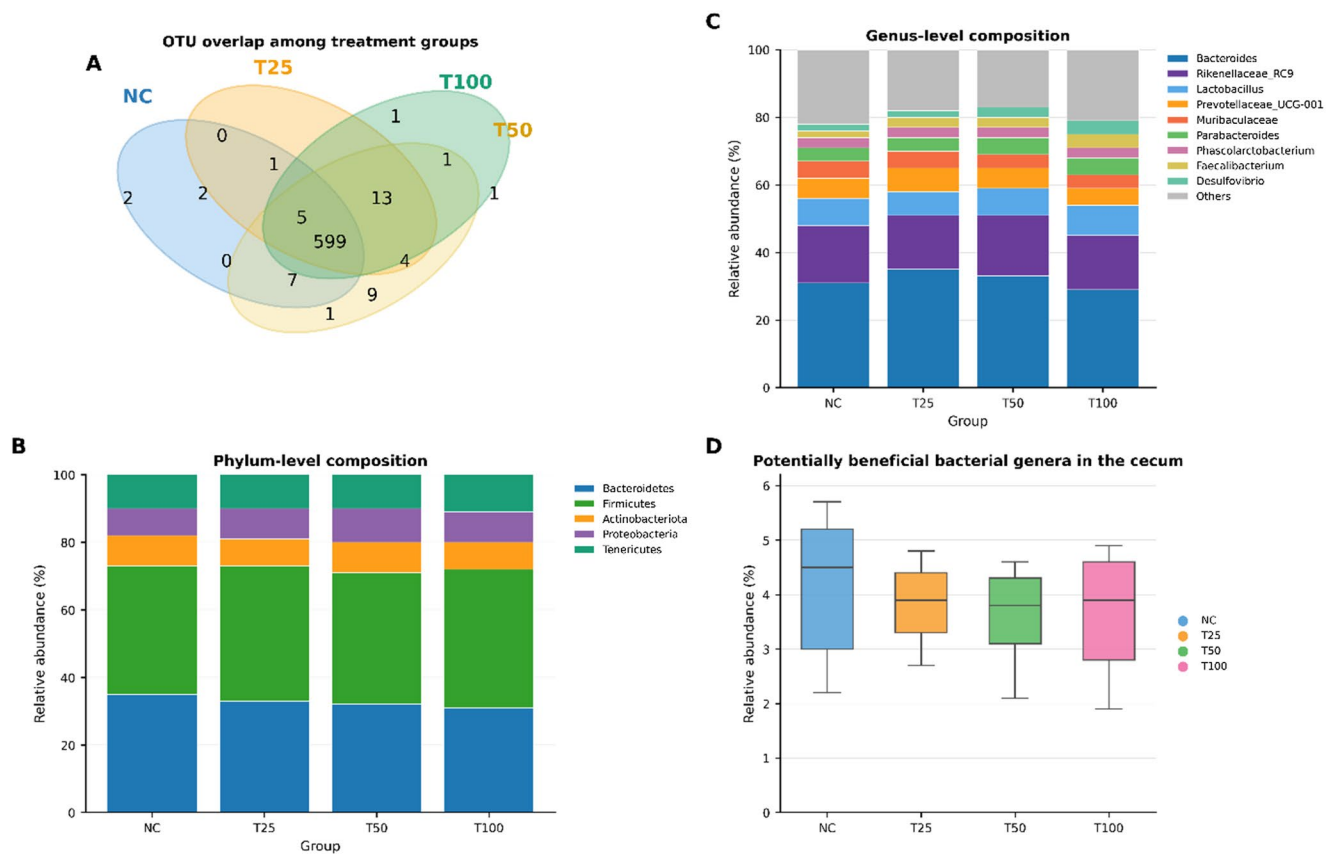


Fig. 2 Operational taxonomic unit overlap (A), taxonomic composition of the cecal microbiota at the phylum (B) and genus (C) levels, and selected bacterial genera (D-E)

LFRO supplementation did not significantly affect alpha-diversity indices ($P > 0.05$), including Shannon ($P = 0.298$), Simpson ($P = 0.322$), Chao1 ($P = 0.877$), and ACE ($P = 0.791$) indices (Fig. 3A-D). The PCoA plot did not show clear treatment separation (Fig. 3E), indicating that the major community structure was preserved. However, the relative abundance of selected bacterial genera previously associated with potentially unfavorable or beneficial gut functions differed among treatments ($P < 0.05$ for most selected genera; Fig. 4), suggesting selective rather than broad-scale microbial modulation.

Excreta gas emission

Effects of LFRO supplementation on excreta gas emission are shown in Table 4. Ammonia emission was reduced by LFRO supplementation ($P = 0.031$), with the lowest values observed in T50 and T100. In contrast, hydrogen sulfide, carbon dioxide, methyl mercaptan, and acetic acid were not affected by treatment ($P > 0.05$).

Discussion

The current investigation shows how drinking water with LFRO improves productivity for yellow-feathered chickens in stressful tropical environments with 100 mg/L posing the best result. The strongest response was observed at 100 mg/L, chickens obtained the highest weight gain despite lower feed intake, resulting in the best FCR and production efficiency indices. This suggests that the supplementation improved the chickens' feed utilization efficiency rather than their appetite. Under heat stress, reduced feed intake is a physiological strategy to limit metabolic heat production, but it often compromises growth. The T100 chickens were able to achieve greater growth despite the lower feed intake, which indicates greater nutrient utilization efficiency under heat stress compared to the other treatment groups.

It seems reasonable to assume that phytochemicals from fermented red onion combined with metabolites from fermentation could enhance productivity. Quercetin, polyphenols, and organosulfur compounds from *Allium* species can contribute antioxidant and anti-inflammatory activity, while lactic fermentation may increase the release of flavonoids and provide organic, and also enhance the balance of gut

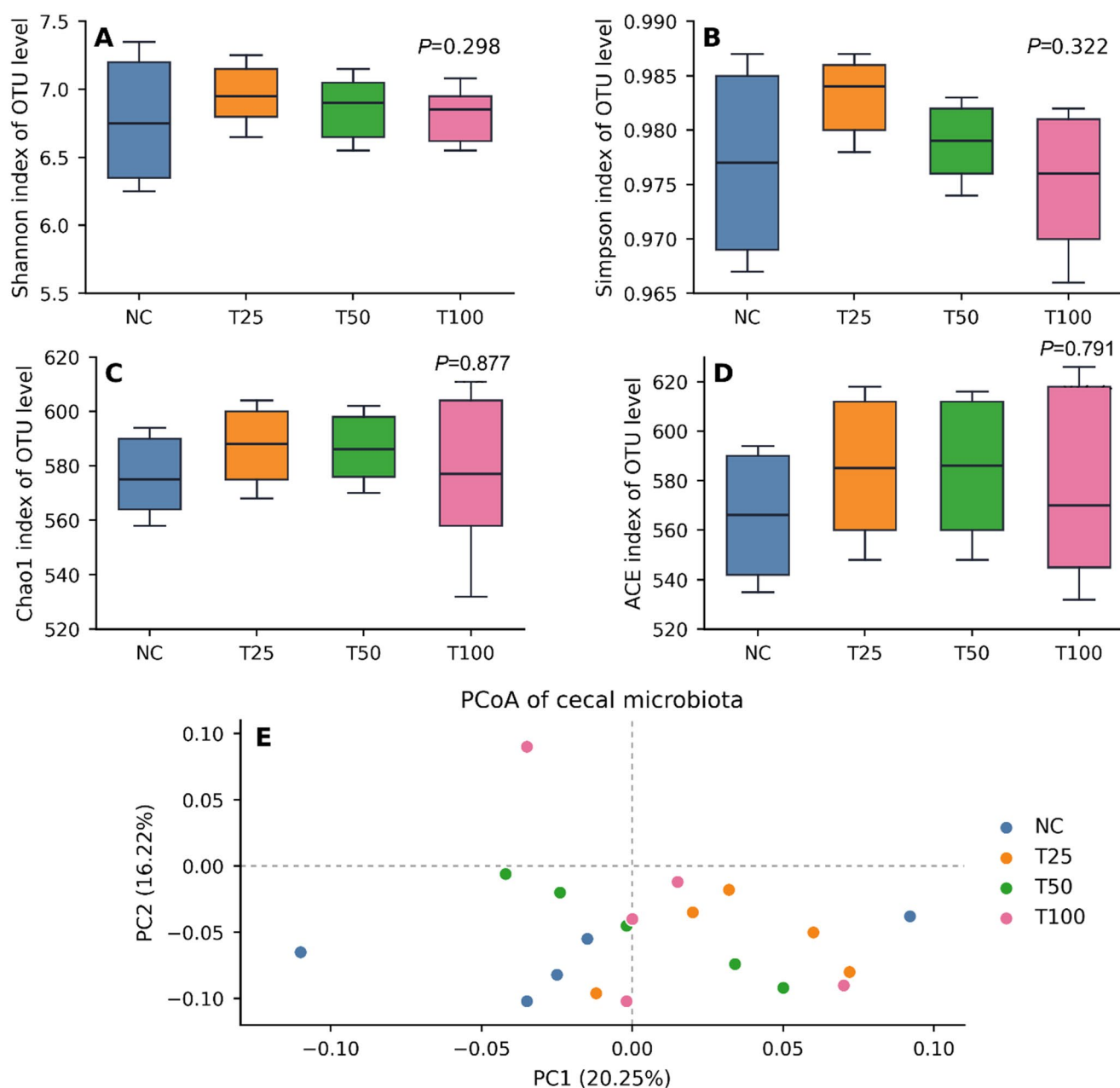
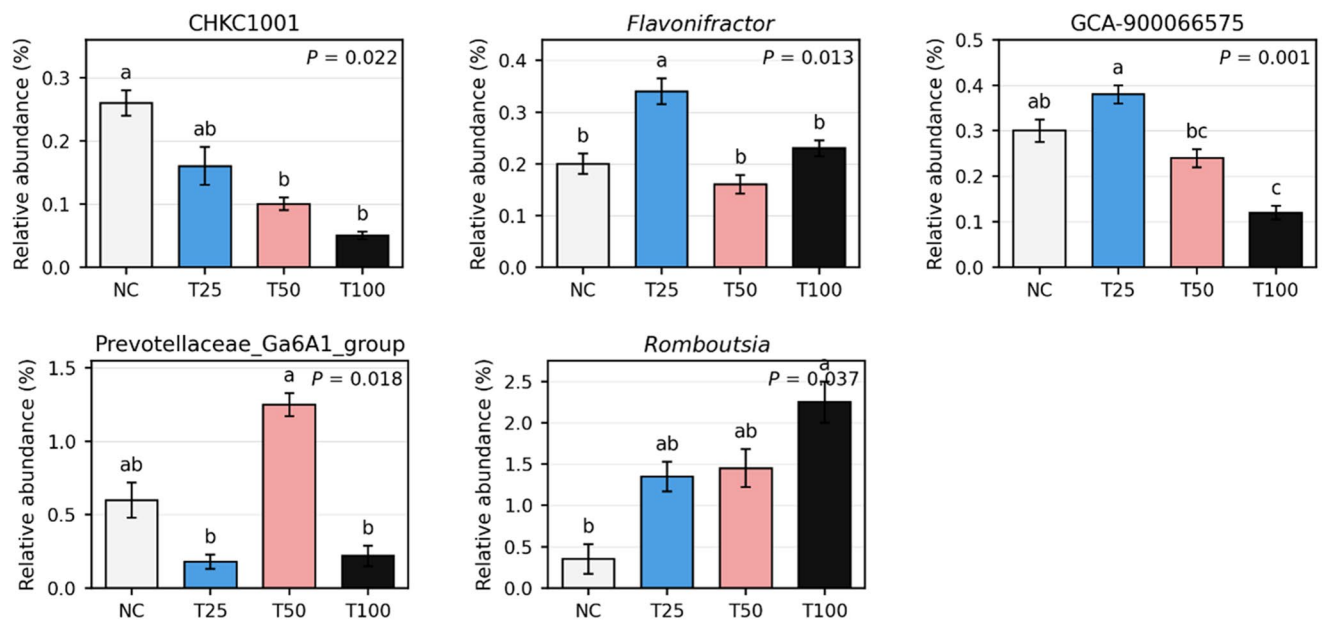


Fig. 3 Alpha-diversity indices (A–D) and principal coordinates analysis of cecal microbiota among treatments (E). Alpha-diversity indices did not differ among treatments ($P>0.05$); exact P-values are shown in panels A–D

flora, nutrient absorption, and digestion (Kothari et al. 2019; Sugiharto 2020; Hai et al. 2024). These mechanisms are relevant during heat stress because oxidative injury, intestinal permeability, and inflammatory activation are central causes of impaired performance (Surai et al. 2019; Wan et al. 2021; Barekatain et al. 2025). The improved FCR in the present study is therefore consistent with a combined effect on oxidative balance, gut barrier stability, and nutrient utilization, although these mechanisms should be confirmed using direct biomarkers.

The current study complements recent research on the use of probiotics and phytochemicals as supplemental strategies to improve broilers' antioxidant status, immune response, and cecal microbial balance under heat stress (Al-Khalafah et al. 2025; Waqas et al. 2026). In the present study, the reduction in feed intake across LFRO groups, together with improved body weight gain at the highest dose, suggests that the additive did not merely increase consumption but improved the biological efficiency of consumed nutrients. This distinction is highly relevant for tropical poultry systems because additives that improve FCR without

Potentially harmful bacterial genera in the cecum



Potentially beneficial bacterial genera in the cecum

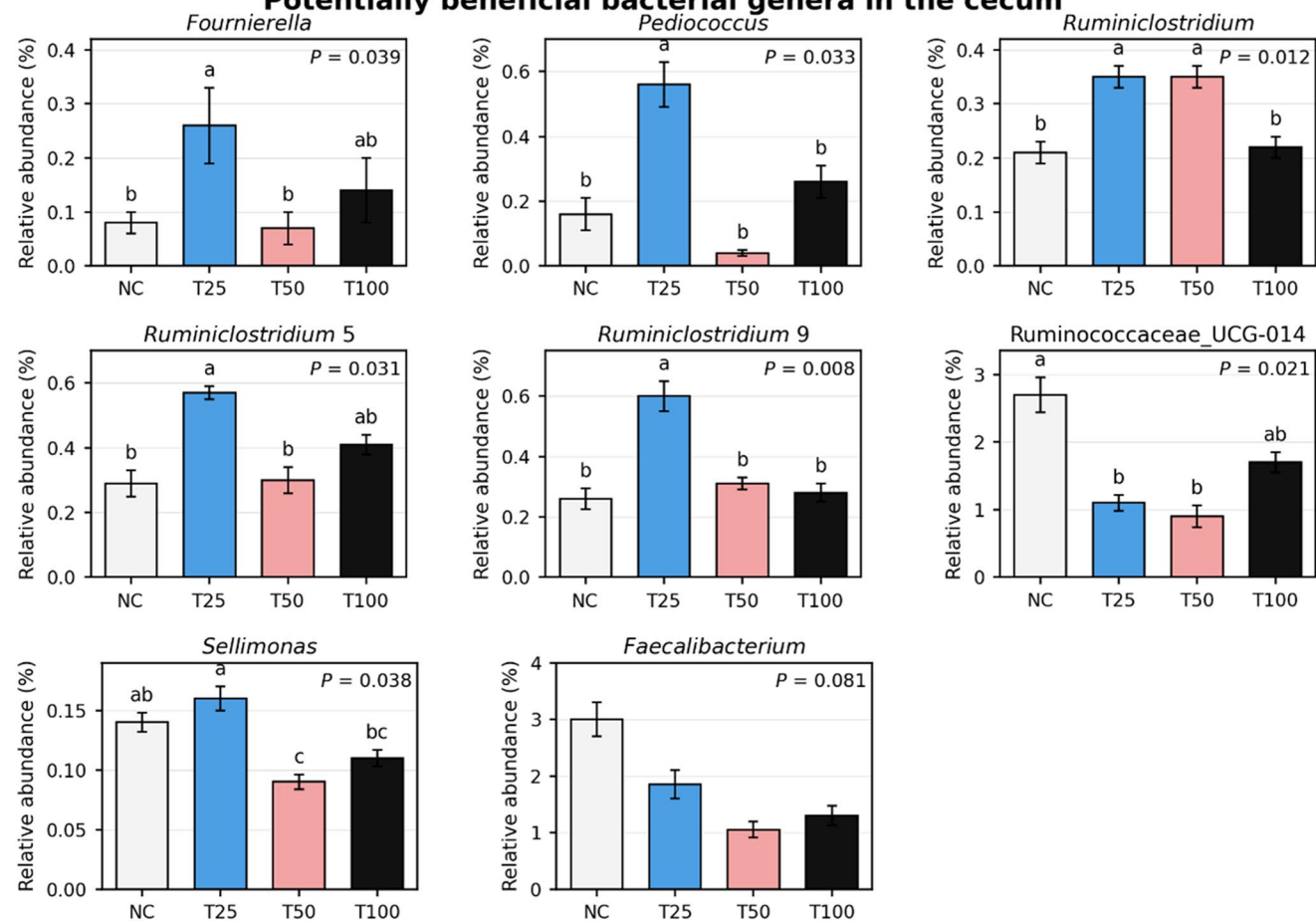


Fig. 4 Differentially abundant selected bacterial genera in the cecum of birds receiving fermented red onion extract. Different lowercase letters indicate significant differences among treatments ($P < 0.05$); panels without different letters indicate $P < 0.05$

Table 4 Effects of LFRO supplementation on noxious gas emissions from excreta

Parameter	NC	T25	T50	T100	SEM	<i>P</i> -value
NH ₃	11.75 ^a	10.38 ^{ab}	9.38 ^b	9.31 ^b	1.33	0.031
H ₂ S	1.73	1.58	1.33	1.35	0.23	0.612
CO ₂	1210.3	1190.1	1180.6	1150.2	113.2	0.567
Methyl mercaptan	5.63	4.50	3.75	3.65	0.32	0.205
Acetic acid	2.38	2.00	1.75	1.79	0.34	0.611

Within a row, means with different superscripts differ at $P < 0.05$

increasing feed intake may reduce metabolic heat burden and cost of production.

While LFRO did not significantly change alpha- or beta-diversity, indicating that the major cecal community structure was preserved, LFRO did alter some targeted genera, indicating a targeted rather than disruptive microbiota response. This interpretation is consistent with recent evidence that heat stress effects on chicken microbiota are context-dependent and may appear as changes in specific taxa or predicted functions rather than broad diversity shifts (Campos et al. 2025; Ncho 2025). Because 16S rRNA sequencing is primarily a taxonomic tool and does not offer functional resolution, we have been careful to describe these genus-level changes as targeted modulation of the microbiota rather than as evidence of beneficial or harmful function.

The control groups emitted higher levels of ammonia than the treatment groups T50 and T100 by about 20%, while the other measured gases (hydrogen sulfide, carbon dioxide, methyl mercaptan, acetic acid) showed no measurable differences among groups. This selective response suggests that LFRO mainly influenced nitrogen-related fermentation pathways rather than total gas production. A likely explanation is that improved feed efficiency reduced undigested nitrogen entering the hindgut, thereby lowering proteolytic fermentation and ammonia release. Organic acids and microbiota shifts may also reduce luminal pH or change nitrogen metabolism, but these possibilities remain inferential because nitrogen balance, excreta nitrogen, and urease activity were not measured.

Another strength of the study is the utilization of an open-sided tropical housing model with natural chronic heat stress, which increases relevance for yellow-feather chicken production in warm regions. The 70-day evaluation period, replicated pen design, and simultaneous measurement of growth performance, cecal microbiota, and excreta gas emission also provide an integrated assessment of animal productivity, gut ecology, and environmental output. The study also had some weaknesses, including the random sampling of cecal microbiota from only one bird per penned group and the use of only one male genotype across one season and one geographic location. The authors also noted the lack of measurement of oxidative stress, inflammatory cytokines, the assessment of intestinal morphology, and the evaluation of nutrient digestibility.

Subsequent studies should integrate LFRO dose-response assays with physiological biomarkers, gut histology, nitrogen balance, digestibility tests, gut microbiome metagenomics, and metabolomics. This would clarify whether the observed benefits are due to antioxidant shielding, gut barrier repair, microbial metabolites, nitrogen balance, and other mechanisms. LFRO will also gain credibility in diverse tropical production systems through multi-site research with different chicken genotypes and use of both sexes in the studies.

Conclusion

LFRO has proven to be an effective natural supplement to drinking water for enhanced productivity of yellow-feather chickens while managing the effects of prolonged tropical heat stress. The main benefit observed was an improvement in feed conversion efficiency, with an optimal concentration of 100 mg/L, resulting in reduced ammonia emissions in the range of 50–100 mg/L. LFRO also caused selective, genus-level modulation of the cecal microbiota with preservation of the overall microbiota. As an effective feed converter for tropical poultry and a potential means to offset nitrogen emissions, LFRO may be an effective non-antibiotic feed supplement for these systems.

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Author contributions Conceptualization: P.V.H.; Methodology: P.V.H., P.H.S.H.; Formal analysis: H.V.S.; Investigation: P.H.S.G., H.V.S.; Data curation: P.H.S.H.; Writing - original draft: P.V.H.; Writing- review & editing: P.H.S.H.; Supervision: P.V.H.; Funding acquisition: P.V.H.

Data availability The datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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