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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 investigated the effects of *Lactobacillus plantarum* 1582-fermented red onion bulb extract (LFRO) supplied in drinking water on growth performance, cecal microbiota composition, and excreta gas emissions in heat-stressed yellow-feather chickens. A total of 300 one-day-old male Rilai chicks were allocated to four treatments in a completely randomized design (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 (temperature-humidity index predominantly 28.9–31.1). LFRO supplementation at 100 mg/L significantly increased final body weight and body weight gain while reducing feed intake and improving feed conversion ratio (FCR) over the entire trial. Both the European Production Efficiency Index (EPEI) and Broiler Performance Efficiency Factor (BPEF) were highest in the 100 mg/L group. Cecal 16S rRNA sequencing revealed no change in alpha- or beta-diversity but selective modulation of several bacterial genera. In addition, LFRO at 50 and 100 mg/L significantly reduced ammonia emission from excreta, while other gases remained unaffected. These results demonstrate that LFRO, particularly at 100 mg/L, is a promising natural drinking-water additive that can effectively alleviate the negative impacts of chronic heat stress on feed efficiency, gut microbiota balance, and environmental emissions in yellow-feather chicken production under tropical conditions.

1. Introduction

Heat stress remains one of the most important environmental challenges limiting poultry production in tropical and subtropical regions. Prolonged exposure to high ambient temperature and humidity depresses feed intake, impairs intestinal barrier function, induces oxidative stress, and ultimately reduces growth efficiency and feed conversion ratio (Brugaletta et al. 2022; Teyssier et al. 2022; Liu et al. 2023). These effects are particularly pronounced in slow- and medium-growing local chicken genotypes raised in open-sided houses, where environmental control is limited.

With the global phase-out of antibiotic growth promoters, there is increasing interest in natural phytogenic and postbiotic additives capable of supporting bird performance and gut health under thermal stress. Fermented plant extracts are especially attractive because lactic fermentation enhances the bioavailability of bioactive compounds, generates beneficial organic acids, and produces postbiotic metabolites that improve nutrient utilization and resilience to environmental stressors (Sugiharto 2020; Adli et al. 2024). Among *Allium* species, red onion (*Allium cepa* L.) is a promising candidate for tropical poultry diets due to its high content of quercetin and organosulfur compounds, which possess antioxidant, anti-inflammatory, and antimicrobial properties (Kothari et al. 2019; Malematja et al. 2023). However, the efficacy of raw extracts can be inconsistent owing to variable stability and bioavailability. Lactic fermentation with *Lactobacillus plantarum* has been shown to overcome these limitations by increasing the release of bioactive flavonoids and enriching the final product with organic acids and other functional metabolites (Huynh et al. 2014; Hai et al. 2024; Hai et al. 2025).

Although fermented *Allium* extracts have shown potential in broilers, limited information is available on their use as drinking-water additives for yellow-feather chickens under natural chronic heat stress, particularly when growth performance, cecal microbiota, and excreta gas emissions are evaluated simultaneously. Therefore, the objective of this study was to determine the effects of *L. plantarum*-fermented red onion bulb extract (LFRO) supplied in drinking water on productive performance, cecal microbial ecology, and noxious gas emission in yellow-feather chickens reared under tropical heat-stress conditions. We hypothesized that LFRO supplementation would improve feed efficiency, selectively modulate the cecal microbiota, and reduce ammonia emission from excreta.

2. Materials and methods

2.1. 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.

2.2. 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 and Newcastle disease on day 7 and against infectious bursal disease 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 × T) + [(RH/100) × (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 (Brugaletta et al. 2022; Teyssier et al. 2022).

2.3. 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 3–4 x 10^7 *Lactobacillus* cells/ml and measurable concentrations of polyphenols, quercetin, organosulfur compounds, and organic acids (Table 1).

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

2.4. 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.

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

Item	Starter phase (< 30 days)	Finisher phase (≥ 30 days)
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

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:

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

$$\mathrm{BPEF} = \frac{\text{Live weight (kg)} \times 100}{\mathrm{FCR}}$$

2.5. 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 16S rRNA gene was amplified using primers 338F and 806R. PCR products were purified with the AxyPrep 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.

2.6. 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 (NH_3), hydrogen sulfide (H_2S), carbon dioxide (CO_2), methyl mercaptan, and acetic acid were measured using a portable gas analyzer (MultiRAE Lite, model PGM-6208; RAE Systems, Denmark).

2.7. 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).

3. Results

3.1. 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).

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

Parameter	NC	T25	T50	T100	P 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 the treatment groups (P < 0.05). FCR: Feed conversion ratio.

3.2. 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).

LFRO supplementation did not significantly affect alpha-diversity indices or the overall beta-diversity pattern (Fig. 4), indicating that the major community structure was preserved. However, the relative abundance of several potentially harmful and potentially beneficial genera differed among treatments (Fig. 3), suggesting selective rather than broad-scale microbial modulation.

3.3. 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).

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

Parameter	NC	T25	T50	T100	SEM	P-value
NH_3	11.75^{a}	10.38^{ab}	9.38^{b}	9.31^{b}	1.33	0.031
H_2S	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.						

4. Discussion

The present study demonstrated that supplementation of LFRO in drinking water effectively mitigated several negative impacts of chronic tropical heat stress in yellow-feather chickens. Under sustained severe-to-very severe heat stress (THI predominantly 28.9–31.1), LFRO significantly improved final body weight, body weight gain, and feed conversion ratio over the entire 70-day period, with the strongest responses observed at 100 mg/L. The reduction in feed intake coupled with a markedly lower FCR (1.77 in T100 versus 1.98 in NC) indicates that LFRO enhanced nutrient utilization efficiency rather than simply stimulating appetite. This improvement was further reflected in the significantly higher European Production Efficiency Index (EPEI) and Broiler Performance Efficiency Factor (BPEF) in the T100 group. These findings confirm that LFRO can partially counteract the typical depression of growth efficiency caused by chronic heat stress in slow-growing local genotypes reared under open-sided tropical conditions.

The performance benefits are biologically plausible given the combined phytochemical and postbiotic properties of LFRO. Red onion is naturally rich in quercetin and organosulfur compounds that exert

antioxidant and anti-inflammatory effects, while lactic fermentation increases the bioavailability of these compounds and supplies organic acids and other metabolites that support gut integrity and metabolic resilience (Kothari et al. 2019; Huynh et al. 2014; Hai et al. 2024). Such mechanisms are particularly valuable under heat stress, where oxidative damage and impaired nutrient absorption are major limiting factors (Wan et al. 2021; Shah et al. 2022; Wang et al. 2024). The observed improvement in FCR without adverse effects on mortality further supports the safety and practical utility of LFRO as a drinking-water additive.

Although LFRO did not alter overall alpha- or beta-diversity of the cecal microbiota, it selectively modulated the relative abundance of several bacterial genera. This targeted rather than broad-scale microbial shift is consistent with the performance data, as modest changes in key taxa involved in fermentation and short-chain fatty acid production can improve nutrient utilization and gut homeostasis without requiring large-scale restructuring of the microbial community (Liu et al. 2023; Wang et al. 2024).

A further practical advantage was the significant reduction in ammonia (NH_3) emission from excreta at 50 and 100 mg/L LFRO. Lower NH_3 release likely resulted from improved nitrogen retention and reduced proteolytic fermentation in the hindgut. This effect is highly relevant for tropical poultry production, where high ammonia levels impair bird welfare, litter quality, and barn air conditions (Such et al. 2021; Fang et al. 2024). The absence of changes in other gases (H_2S, CO_2, methyl mercaptan, and acetic acid) suggests that LFRO primarily influenced nitrogen-related metabolic pathways under the present conditions.

Collectively, these results indicate that LFRO at 50–100 mg/L offers a promising natural strategy to improve feed efficiency, modulate beneficial gut bacteria, and reduce environmental emissions in heat-stressed yellow-feather chickens. Further dose-response and multi-omics studies are warranted to elucidate the precise molecular links between microbial modulation and host performance.

5. Conclusion

Supplementation of Lactic-fermented red onion bulb extract in drinking water at 50–100 mg/L significantly improved FCR, EPEI, and BPEF while reducing ammonia emission from excreta in yellow-feather chickens reared under chronic tropical heat stress. The extract also induced selective modulation of cecal microbiota without disrupting overall microbial diversity. These findings demonstrate that LFRO is a safe and effective natural drinking-water additive that can partially alleviate the negative effects of heat stress in tropical poultry production. The optimal inclusion level appears to be 100 mg/L for maximum performance benefits.

Declarations

Author contributions (CRediT)

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.

Conflict of Interest

The authors declare that they have no conflict of interest.

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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.

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Figures

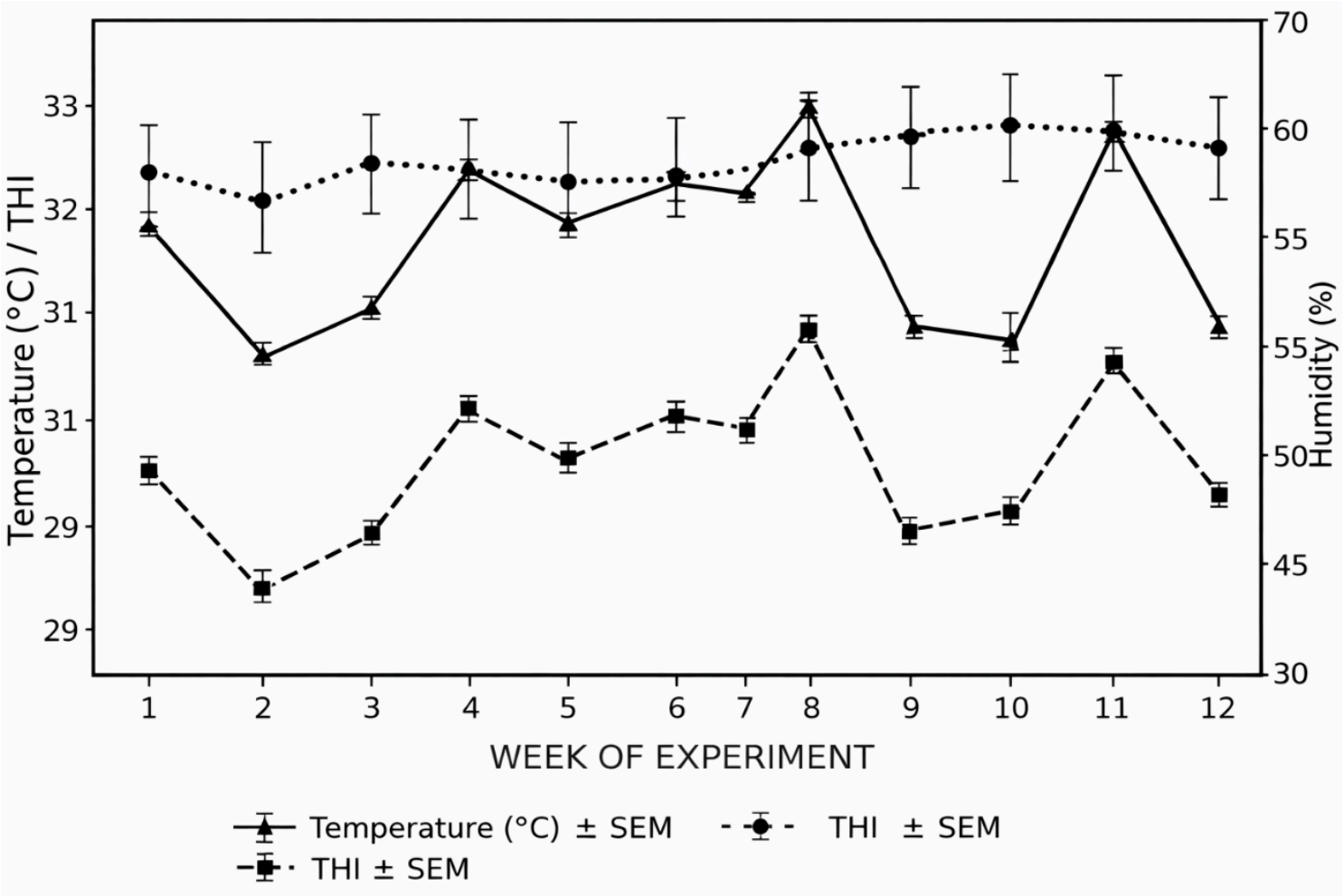


Figure 1

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

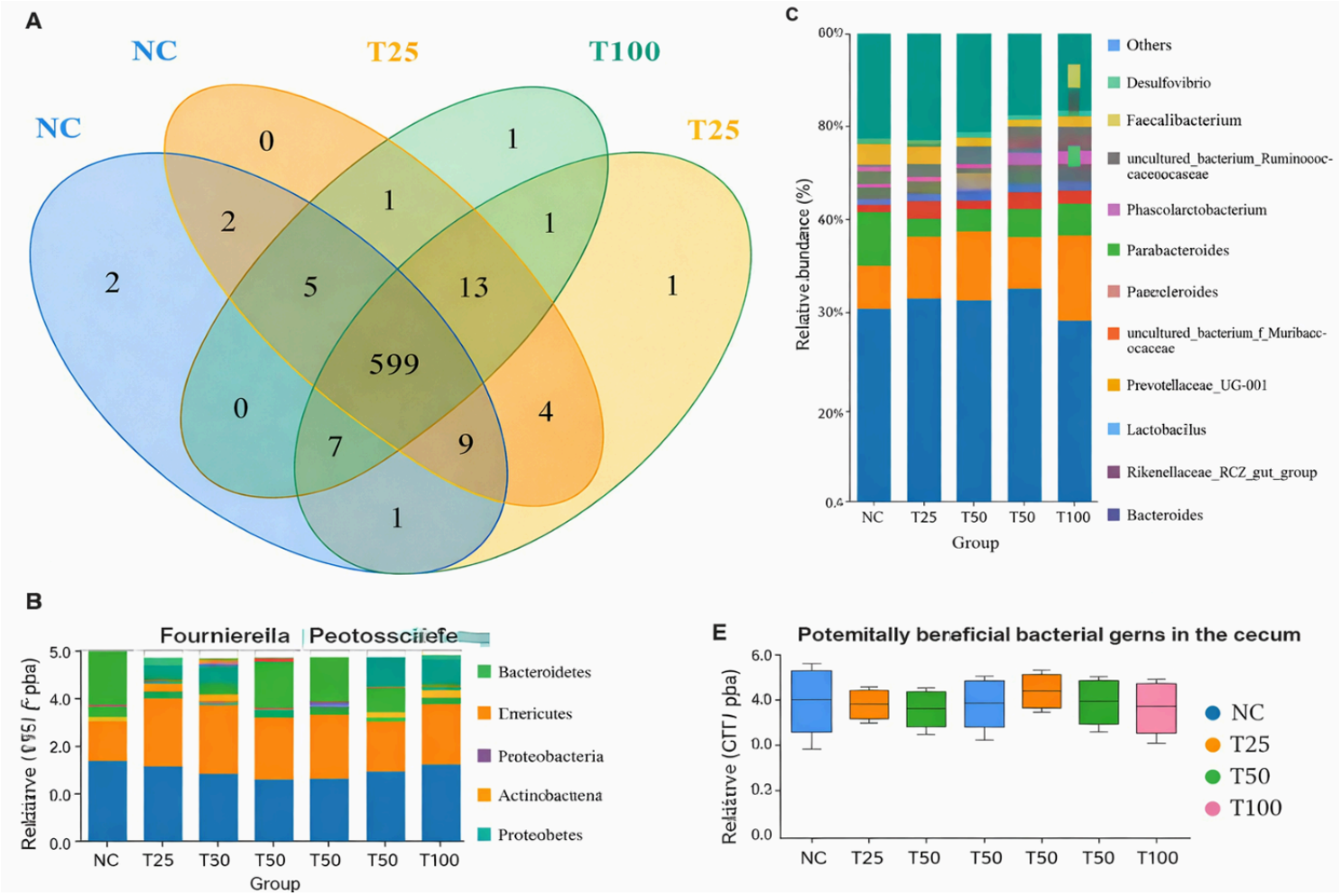


Figure 2

Operational taxonomic unit overlap (A) and taxonomic composition of the cecal microbiota at the phylum (B) and genus (C) levels.

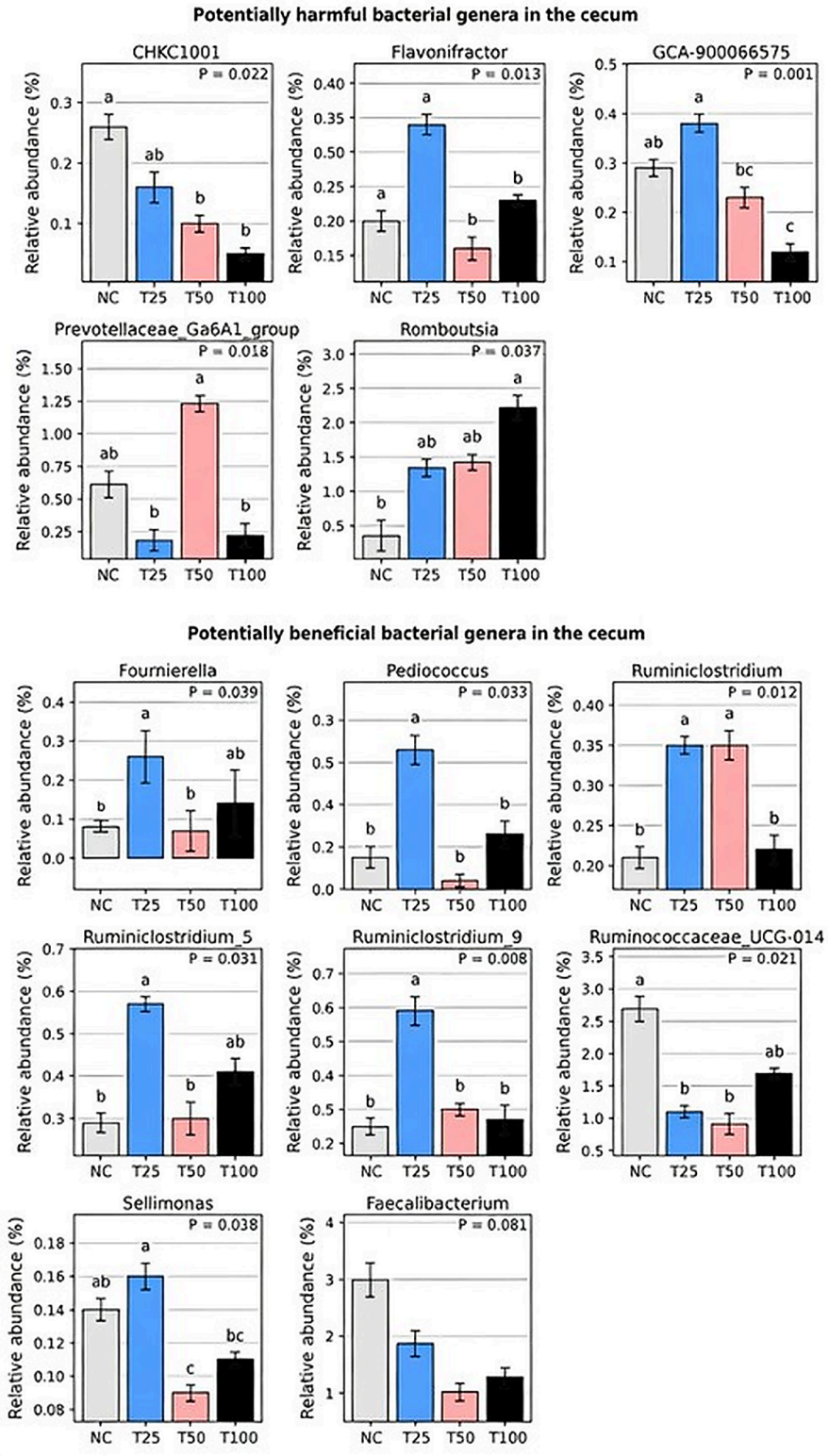


Figure 3

Differentially abundant potentially harmful and potentially beneficial bacterial genera in the cecum of birds receiving fermented red onion extract.

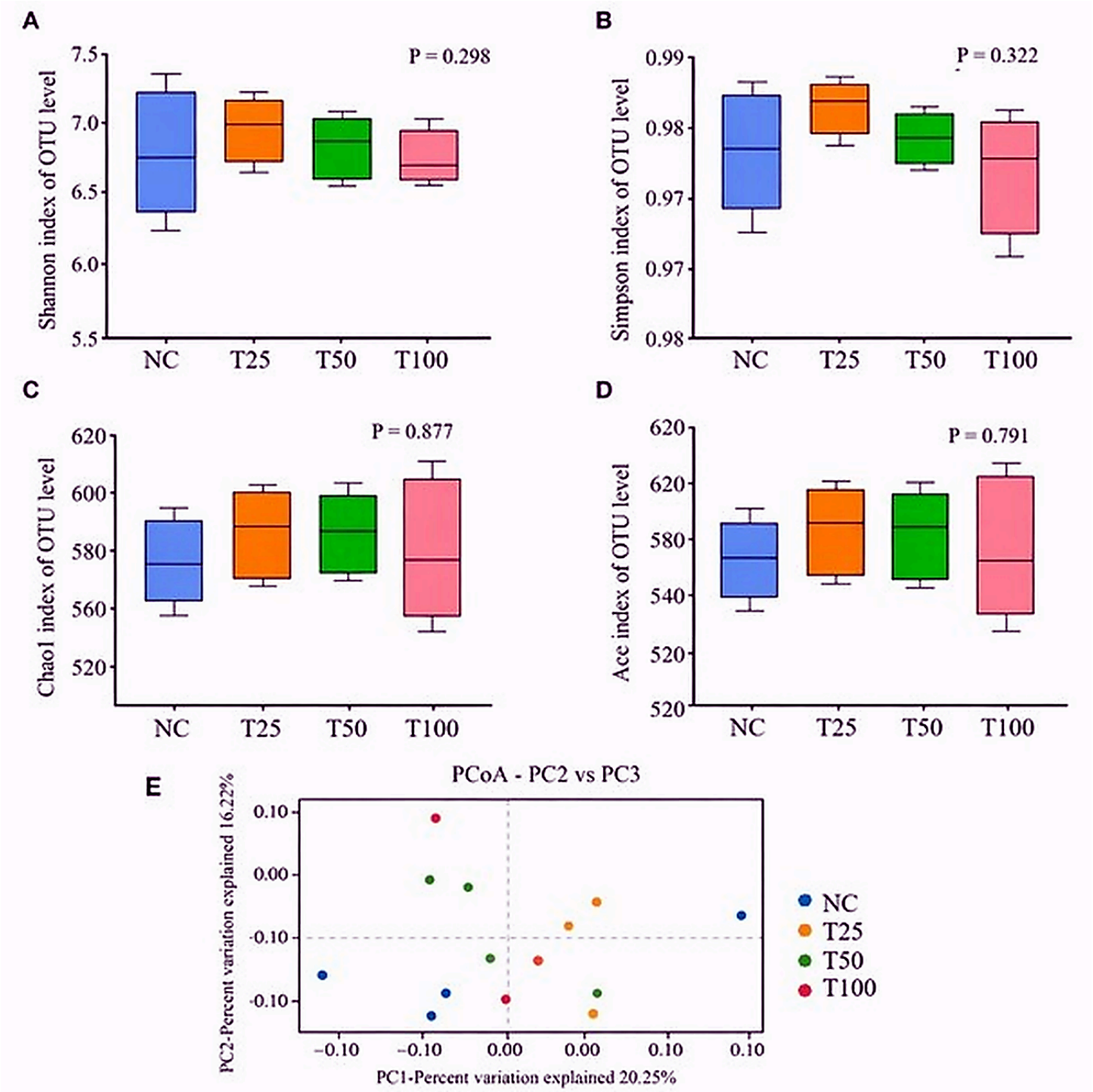


Figure 4

Alpha-diversity indices (A–D) and principal coordinates analysis of cecal microbiota among treatments (E).

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