104 (2025) 105077

Contents lists available at ScienceDirect

Poultry Science

journal homepage: www.elsevier.com/locate/psj

Exploring effects of dietary coffee pericarp addition on growth, meat quality, gut flora in white-feather broilers

Linxian Shan [a,1], Jun He [a,1], Ruijuan Yang [a], Jinya Dong [a], Zezhu Du [a], Shengjie Duan [a], Yanmei Li [a], Xiuli Lu [a], Yan Shen [a], Jianyang Fu [a], Shengmei Gao [a], Xiaocui Du [b], Chongye Fang [a,*]

[a] College of Food Science and Technology, Yunnan Agricultural University, Kunming, 650100, China
[b] College of Agronomy and Biotechnology, Yunnan Agricultural University, Kunming, 650100, China

ARTICLE INFO

Key words:
Coffee pericarp
White-feather broiler
Meat quality
Volatile compounds
Gut microbiota

ABSTRACT

The incorporation of coffee pericarp into poultry diets enhances chicken meat quality by modulating intestinal flora. This study investigates the effects of coffee pericarp on chicken meat quality through an analysis of growth performance, physical parameters, chemical composition, volatile compounds, and gut microbiome. The results demonstrate that adding coffee pericarp to the diet reduces drip loss and pH while improving meat color and increasing the levels of crude protein, amino acids, unsaturated fatty acids, and volatile compounds. Furthermore, coffee pericarp influences the metabolism of these compounds by increasing the relative abundance of beneficial bacteria, such as Firmicutes and Bacteroidetes, thereby enhancing meat quality. In conclusion, incorporating 2.5 % fermented coffee pericarp effectively regulates beneficial bacteria and significantly boosts the volatile compound content in white feather broilers, which is crucial for improving meat flavor and the economic viability of poultry production.

Introduction

Livestock and poultry meat is an essential component of the human diet (Erian and Phillips, 2017). As living standards improve, consumer demand for higher meat quality has intensified, becoming a significant concern for both producers and consumers (Kleyn and Ciacciariello, 2021). Globally, the demand for chicken meat remains substantial (Estevez-Moreno and Miranda-de, 2022). In recent years, China's livestock and poultry industry has rapidly developed, with significant increases in the demand for feed and animal food. Current research on feed additives and conventional feed substitutes primarily focuses on plant sources, their extracts, probiotics, and their metabolites. These studies predominantly address the regulation of immune and antioxidant functions, contributing to reduced feed costs and improved meat quality (Tang et al, 2021; Jin et al., 2021). Therefore, the strategic development and utilization of new feed materials and alternatives to conventional feeds show considerable promise for enhancing meat quality and lowering feed costs in the short term.

Coffee, a beverage made from roasted and ground coffee beans, ranks among the world's three major beverage crops. Currently, coffee sales are rising annually, accompanied by an expansion in planting areas; global coffee production stands at approximately 1,005,000 metric tons. With increased coffee bean production, the volume of coffee by-products generated during processing is also growing (Silva et al., 2021). The coffee production chain generates millions of tons of by-products each year, which constitute approximately 50 % of fresh coffee fruit (Iriondo-DeHond et al., 2019). These by-products are rich in carbohydrates, proteins, pectin, polyphenols, and other bioactive components, representing low-cost, renewable resources (Bondam et al., 2022). Current methods for treating coffee by-products are largely inadequate and fail to add value to the production chain. Common practices, such as discarding these by-products as waste or incinerating them, not only waste resources but also degrade soil quality and pose carcinogenic risks, leading to environmental pollution (Machado et al., 2023). Recently, there has been an increasing focus on the biofunctionality and technological applications of coffee by-products, spurred by

* Corresponding author.
E-mail addresses: 2032431254@qq.com (L. Shan), hjun202203@163.com (J. He), ffccyy2@163.com (C. Fang).
[1] These authors contributed equally to this work. Linxian Shan and Jun He, as the co-first authors, have provided detailed information regarding their specific contributions in the Authorship contribution statement.

https://doi.org/10.1016/j.psj.2025.105077
Received 25 January 2025; Accepted 18 March 2025
Available online 20 March 2025

advancements in extraction techniques and the potential for their reuse in the food and pharmaceutical industries (Sorita, Leimann and Ferreira, 2023). The most studied by-products include coffee pericarp, coffee pulp, and coffee silver skin, all of which contain valuable compounds. Certain methods can convert the environmentally damaging portions of these by-products into feedstock for extracting bioactive compounds and polymers (Reichembach and Petkowic, 2020; Silveira et al., 2019). Therefore, recovering bioactive compounds from coffee by-products not only enhances the value chain but also reduces waste management costs associated with industrial residues.

Coffee pericarp, comprising the outer skin and pulp of the coffee berry, is a significant source of solid waste generated during wet processing, accounting for 40-50 % of the fresh fruit's weight (Iriondo-DeHond et al., 2019). It is rich in proteins, carbohydrates, fiber, and various bioactive compounds, including flavonoids, polyphenols, polysaccharides, anthocyanins, caffeine, and chlorogenic acid, with total dietary fiber constituting approximately 70 %, making it an excellent source of dietary fiber (Dong et al., 2020; Oliveira et al., 2021). This paper aims to investigate the effects of coffee pericarp on the meat quality and volatile components of white feather broilers and its correlation with intestinal flora. The objective is to explore the nutritional potential of coffee pericarp, improve utilization efficiency, and elucidate its regulatory mechanisms on broiler meat quality, ultimately aligning with the dietary health and nutritional needs of chickens, providing a theoretical basis for these findings.

Materials and methods

Preparation of Coffee Pericarp

Freshly harvested coffee fruits (Arabica strain, provided by Puerh Canglong Coffee Factory) were cleaned and mechanically processed to separate the coffee pericarp. To prepare the fermented coffee pericarp, after cleaning the empty tank, sterilization was performed (≥100°C, 15 min), a ratio of 25:1 (m/v) was established between coffee pericarp and a Lactobacillus plantarum (CGMCC NO.18215) bacterial solution, which was fermented at room temperature for three days. After undergoing natural and air drying, the coffee pericarp was pulverized, passed through a 60-mesh sieve, and the resulting powder was collected as an additive (F: Phenolic 1.85±0.09 g/100g). In a control experimental group, Lactobacillus plantarum was not added, but the remaining procedures were consistent with the experimental treatment (UF: Phenolic 1.97±0.03 g/100g).

Experimental design and feeding management

A total of 100 one-day-old male white-feathered broilers (Shandong Dacheng Collective Hunan Shuncheng Industrial Co., Ltd.) with similar body weights, were randomly divided into 5 groups with 2 replicates per group and 10 broilers per replicate. The experiment was randomly assigned to five treatment groups: a blank control group (CK: base diet), UF-L (base diet + 2.5 % coffee pericarp), UF-H (base diet + 5 % coffee pericarp), F-L (base diet + 2.5 % fermented coffee pericarp), and F-H (base diet + 5 % fermented coffee pericarp). One week prior to the experiment, the chicken coop was thoroughly sterilized and cleaned. The experimental facility was a semi-closed rearing room, with temperature, humidity, and ventilation manually controlled. The initial temperature was set at 32°C, decreasing by 2-3°C weekly until reaching 20°C. Continuous illumination was maintained for 24 h daily throughout the experiment. Chickens were fed using plastic feeders and provided drinking water from plastic buckets once daily. Daily feed and water intake were recorded, and body weights were measured weekly over a 42-day experimental period. At 7 days of age, chickens were vaccinated against Newcastle disease via eye drops, followed by vaccination against infectious bursal disease via drinking water at 12 days of age. Strict adherence to routine management protocols ensured optimal hygiene in the chicken house.

Composition and nutrient levels of the basal diet

The specific composition and nutritional levels of the basal diet are presented in Table 1.

Measurement of growth performance and sample collection

Daily feed and water intake were monitored, and broiler weights were recorded every seven days. At 42 days of age, all groups underwent a 12-hour fasting period before weighing. Feed was then measured, and the average daily gain (ADG; Eq. (1)), average daily feed intake (ADFI; Eq. (2)), and feed-to-gain ratio (F/G; Eq. (3)) were calculated. Six broilers from each group were randomly selected for slaughter. These broilers were euthanized through exsanguination using a conventional neck cut. The breast muscle was collected, placed in a sterile bag, and stored at 4°C for further analysis. The cecal contents were also collected, placed in sterile test tubes, rapidly frozen with liquid nitrogen, and stored at -80°C for subsequent testing.

$$\text{ADFI}\ (g/d) = \text{Total feed intake}/\text{Number of experimental days}; \quad (1)$$

$$\text{ADG}\ (g/d) = (\text{Final weight} - \text{Initial weight})/\text{Number of experimental days}; \quad (2)$$

$$\text{F/G}\ (g/g) = \text{ADFI}/\text{ADG}; \quad (3)$$

Meat quality evaluation

The left pectoral muscle was analyzed for pH, color difference, shear force, crude protein, and fat content, while the right pectoral muscle was examined for amino acids, fatty acids, and volatile compounds.

Measurement of Meat pH. pH measurements were taken at 45 minutes and 24 hours post-slaughter using a portable pH meter (MATTHAUS, Germany), with each sample measured in triplicate.

Table 1
Composition and nutritional profile of basal diet

Ingredients (%)	Day 1 to 21	Day 22 to 42
Corn	58.50	60.10
Limestone	1.60	1.35
Soybean oil	3.04	4.50
Soybean meal	28.00	30.00
Fermented soybean meal	5.00	-
Sodium chloride	0.22	0.26
Threonine	0.10	0.09
Lysine	-	0.10
Methionine	0.14	0.15
Calcium monohydrogen phosphate	1.40	1.45
Premix[a]	2.00	2.00
Total	100.00	100.00
Nutrients		
Metabolizable energy[b]/MJ kg^{-1}	12.33	12.76
Crude protein	21.04	18.97
Crude fat	5.60	7.06
Calcium	1.00	0.90
Methionine	0.43	0.40
Lysine	1.08	1.01
Total phosphate	0.65	0.60

[a] Premix provides vitamin A 9 500 IU, vitamin D3 500 IU, vitamin E 20 IU, vitamin K 1.2 mg, vitamin B1 2.2 mg, vitamin B2 5.0 mg, vitamin B6 2.0 mg, niacin 30 mg, pantothenic acid 12.0 mg, folic acid 0.8 mg, biotin 0.18 mg, iodine 0.35 mg, selenium 0.30 mg, manganese 100 mg, iron 80 mg, copper 8 mg, and zinc 75 mg. The premix contained no antibiotics or chemically synthesized antibacterial agents. The values presented are measured quantities in percentages.

[b] Values for metabolizable energy were computed, while the levels of other nutrients were determined through measurement.

Determination of Flesh Color. Meat color was assessed at 45 minutes and 24 hours post-slaughter using a colorimeter (CR-410, Minolta, Japan). Each sample underwent three measurements, recording brightness (L*), redness (a*), and yellowness (b*) values.

Measurement of Meat Tenderness and Cooking Loss in Meat Products. A scalpel was used to excise a muscle sample from the breast, measuring 5 cm × 1 cm × 2 cm. The pre-cooking weight of each sample was recorded (W_1, g). The muscle sample was then placed in a sealed bag and immersed in a water bath at 80°C for 15 minutes. After cooling to 25°C-30°C, excess surface moisture was removed with absorbent paper, and the post-cooking weight (W_2, g) was recorded. Cooking loss was calculated using Equation (4). Subsequently, shear force measurements were taken on the cooked samples using a digital muscle tenderizer (C-LM3), with each sample measured three times.

$$Cookingloss(\%) = (W_1 - W_2)/W_1 \times 100; \quad (4)$$

Measurement of Drip Loss. A separate muscle sample, measuring 3 cm × 2 cm × 1 cm, was also excised from the breast and weighed (W_3, g). This sample was suspended in a refrigerator at 4°C using a paper clip and wrapped in cling film to prevent direct contact. After 24 hours, it was weighed again (W_4, g), and the drip loss was calculated using Equation (5).

$$Driploss(\%) = (W_3 - W_4)/W_2 \times 100; \quad (5)$$

Determination of Crude Protein and Crude Fat Contents. Crude fat: Determined using the Soxhlet extraction method as per GB5009.6-2016, "Determination of Fat in Food under National Food Safety Standards." Crude protein: Measured by sulfuric acid-catalyzed digestion and the Kjeldahl method according to GB5009.5-2016, "Determination of Protein in Food under National Standards for Food Safety."

Determination of Ash Content and Moisture Content. The ash content of the chicken was analyzed following the Chinese National Standard for Food Safety GB 5009.4-2010, "Determination of Ash Content in Foods." Moisture content was determined using the direct drying method outlined in GB 5009.3-2010, "Determination of Moisture Content in Foods."

Determination of Amino Acids. Amino acids were quantified using an LC-MS/MS system (Shimadzu Nexera X2 A8-30AD; QTRAP5500, AB SCIEX) equipped with a Waters UPA8 BEH Amide column (1.7 μm, 2.1 × 100 mm). Chromatographic conditions included mobile phase A, a 0.1 % formic acid aqueous solution with 10 mM ammonium formate, and mobile phase B, an acetonitrile solution with 0.1 % formic acid. The sample was injected into the autosampler at 4°C, with the column maintained at 40°C and a flow rate of 300 μL/min; the injection volume was 2 μL. The relevant liquid-phase gradients were as follows: from 0 to 1 min, 90 % mobile phase B; from 1 to 9 min, a linear transition from 90 % to 40 % mobile phase B; held at 40 % for 9 to 10.5 min; then returning to 90 % mobile phase B from 10.6 to 12 min. Mass spectrometric analysis was performed on a QTRAP 5500 mass spectrometer (AB SCIEX) in positive ion mode. The ESI source parameters were: source temperature, 550°C; ion source gas 1 (GAS1), 40; ion source gas 2 (GAS2), 50; curtain gas (CUR), 50; and ion spray voltage floating (ISVF), 5500 V (Andries et al., 2021).

Determination of Fatty Acids. Fatty acids were analyzed using a gas chromatography-mass spectrometry (GC-MS) system (Thermo Trace 1300 and Thermo ISQ 7000; Thermo Fisher Scientific, USA) equipped with a Thermo TG-FAME capillary column (50 m × 0.25 mm ID × 0.20 μm). The chromatographic conditions included shunt injection with an injection volume of 1 μL and a shunt ratio of 8:1. The injection port temperature was set at 250°C, the ion source temperature at 300°C, and the transmission line temperature at 280°C. The temperature program commenced at 80°C, held for 1 minute, ramped to 160°C at a rate of 20°C/min and held for 1.5 minutes, then increased to 196°C at 3°C/min and held for 8.5 minutes, followed by a final ramp to 250°C at 20°C/min and a hold of 3 minutes. Helium was used as the carrier gas, with a flow rate of 0.63 mL/min. Mass spectrometry was conducted using the Thermo ISQ 7000 mass spectrometer, employing electron bombardment ionization (EI) in selected ion monitoring (SIM) mode, with an electron energy of 70 eV (Yao et al., 2022).

Determination of Volatile Compounds. Chicken breast was processed at 80°C for 30 minutes, after which volatile compounds were analyzed using a solid-phase microextraction gas chromatography-mass spectrometry (SPME-GC-MS) system. SPME conditions included a Triplus RSH autosampler, a DVB/Carbon WR/PDMS 80 μm extraction head (50/30 μm), and an extraction temperature of 90°C. The extraction protocol involved shaking for 5 minutes, followed by a 20-minute extraction and a 10-minute incubation at a shaking speed of 250 rpm. The chromatography and mass spectrometry conditions utilized a Thermo Fisher Trace 1610-TSQ 9610 GC-MS with a DB-wax column (30 m × 0.25 mm × 0.25 μm), employing shunt injection with a 1 μL injection volume and a shunt ratio of 5:1. The inlet temperature was set to 240°C, with the ionization source temperature at 280°C and the mass spectrometry temperature at 240°C for the transmission line. The temperature program initiated at 50°C, increased to 100°C at 5°C/min, reached 150°C at 3°C/min, and finally ascended to 240°C at 10°C/min, holding for 2 minutes. Helium served as the carrier gas in full scan mode, with an ionization voltage of 70 eV and a mass range of 40-400 m/z. The mass spectrometry data were analyzed using the Deconvolution Plugin software for peak extraction, detection, deconvolution, and alignment. Substance characterization was performed utilizing the Wiley (including NIST 2020) spectral libraries for mass spectral and retention time index matching (Sun et al., 2021).

Gut microbiome analysis of 16S gene

Total DNA was extracted from samples using the FastDNA® Spin Kit for Soil, and PCR amplification targeted the V3-V4 region of the 16S rRNA gene. The upstream primer was 338F (5′-ACTCCTACGGGGAGGCAGCAG-3′), and the downstream primer was 806R (5′-GGACTACHVGGGTWTCTAAT-3′). PCR was performed with NEB Q5 high-fidelity DNA polymerase, using a reaction system consisting of 5 μL of 5 × Reaction Buffer, 5 μL of 5 × High GC Buffer, 2 μL of dNTP (10 mM), 2 μL of template DNA, 1 μL of forward primer (10 μM), 1 μL of reverse primer (10 μM), and 8.75 μL of water. Amplification products were analyzed via 2 % agarose gel electrophoresis; the target fragment was excised and purified using the Axygen Gel Recovery Kit. Purification was further performed with the Quant-iT PicoGreen dsDNA Assay Kit, and quantification was conducted on a BioTek Microplate Reader (FLx800). Subsequent library construction and sequencing were executed with the TruSeq Nano DNA LT Library Prep Kit. Data processing and refinement were carried out using QIIME2 version 2019.4, following the tutorial guidelines (https://docs.qiime2.org/2019.4/tutorials/), enabling comprehensive microbiome analysis (Jiang et al., 2023).

Statistical analysis

Microsoft Office Excel 2021 was used to record, analyze, and visualize the data. Statistical analyses were conducted on SPSS 23.0 software, and the analyses included one-way analysis of variance (ANOVA) and Tukey's post-hoc multiple comparison tests. Results were expressed as means ± standard error (X ± SEM), with $P > 0.05$ indicating non-significant differences and $P < 0.05$ indicating significant differences. The 16S rDNA sequencing data were analyzed based on the Illumina TruSeq Nano DNA LT Library Prep Kit sequencing platform, some of the results were visualized using GraphPad Prism 9 software.

Results

Growth performance

The addition of varying doses of coffee pericarp influenced broiler growth performance (Table 2). Initial body weight and average daily

Table 2
Effects of different treatments of coffee pericarp on the growth performance of white-feathered broilers

Items	Group					SEM	P-value
	CK	UF-L	UF-H	F-L	F-H		
Initial BW, g	36.92	36.42	36.57	39.60	36.42	0.48	0.169
Final BW, g	1016.92[b]	1082.08[a]	949.85[c]	1083.82[a]	932.83[c]	13.86	<0.001
ADFI, g/day	87.21	91.30	91.57	82.30	75.42	8.20	0.974
ADG, g	23.33[b]	24.90[a]	21.75[c]	24.86[a]	21.34[c]	0.33	<0.001
F/G ratio	3.94[b]	3.67[c]	4.22[a]	3.32[d]	3.54[c]	0.060	<0.001

[a-d] Mean values within a row with different superscripts are different values at $P < 0.05$.
[1]BW = body weight; ADFI = average daily feed intake; ADG = average daily gain; F/G = feed/gain ratio.
[2]CK =base diet (no coffee pericarp); UF-L = base diet+ 2.5 % coffee pericarp; UF-H = base diet+ 5 % coffee pericarp; F-L = base diet + 2.5 % fermented coffee pericarp; F-H = base diet + 5 % fermented coffee pericarp.
[3] SEM = standard error of the mean (n = 6).

feed intake did not differ significantly between groups ($P > 0.05$). However, final body weight in the high-dose coffee pericarp groups (UF-H and F-H) was lower than in the low-dose and CK groups ($P < 0.001$). In terms of average daily gain (ADG), the UF-L and F-L groups exhibited significantly higher values compared to the CK group ($P < 0.001$), while the UF-H and F-H groups were significantly lower ($P < 0.001$). Additionally, the feed-to-weight ratio (F/G) was highest in the UF-H group, with the UF-L, F-L, and F-H groups all showing significantly lower ratios than the CK group ($P < 0.001$). These results indicate that the inclusion of coffee pericarp in broiler diets affects growth performance.

Meat quality

The addition of pericarp to the diets of white-feathered broilers produced varying effects on chicken breast meat parameters (Table 3). Compared with the CK group, the drip loss of chicken breast meat in the coffee peel treatment group was reduced, with the F-L group showing a significant reduction ($P<0.05$). This indicates that coffee peel enhances the water-holding capacity of chicken breast muscle. The pH, an important indicator of meat freshness, tended to decrease in the coffee peel treatment group compared to the CK group, with a significant reduction in the F-H group ($P<0.05$) after 24 hours. This suggests that coffee peel supplementation lowers the pH of chicken meat. Additionally, the F-H group exhibited significantly higher values for L*45min, a*45min, b*45min, a*24h, and b*24h compared to the CK group ($P<0.001$), indicating that fermented coffee peels significantly enhance the brightness, redness, and yellowness of chicken breast meat. Furthermore, coffee pericarp elevated the crude protein content in chicken breast meat compared to the CK group ($P<0.001$). No significant differences ($P>0.05$) were observed regarding the impact of coffee pericarp on cooking loss, shear force, pH45min, L*24h, moisture, ash, and crude fat content of chicken breast meat.

Profile of amino acids

To further investigate the amino acid composition of chicken breast meat, targeted amino acid metabolomics analysis was conducted. A total of 35 amino acids and their derivatives, including 9 essential and 11 non-essential amino acids, were identified (Table 4). In the fermentation high-dose group, levels of anserine, creatinine, cystine, and serine were significantly elevated compared to the CK group($P<0.05$). Overall, while the addition of coffee pericarp increased total amino acid (TAA) levels in the diets of white-feathered broiler chickens, the changes were not statistically significant ($P>0.05$).

Profile of fatty acids

The metabolomics analysis of the fatty acid composition in the breast muscle of white-feathered broilers is presented in Table 5. Results indicated that coffee pericarp supplementation led to significant alterations in the muscle's fatty acid profile, with variations dependent on treatment and additive levels. Notably, the n-3 polyunsaturated fatty acid (n-3 PUFA) content was significantly reduced in the experimental group compared to the control. Conversely, saturated fatty acids (SFA),

Table 3
Effects of different treatments of coffee pericarp on the physical parameters and chemical composition of broiler breast meat

Items	Group					SEM	P-value
	CK	UF-L	UF-H	F-L	F-H		
Drip loss (%)	27.47[a]	24.05[ab]	19.70[ab]	15.86[b]	25.13[ab]	1.23	0.012
Cooking loss (%)	19.98	15.27	20.67	21.96	19.17	1.02	0.304
Shear force (kgf)	4.86	4.81	4.80	4.52	4.84	0.07	0.505
pH45min	6.38	6.28	6.41	6.32	6.25	0.03	0.367
pH24h	6.29[a]	6.02[ab]	6.07[ab]	6.08[ab]	5.94[b]	0.04	0.046
L*45min	42.56[b]	43.34[b]	44.68[a]	44.60[a]	44.77[a]	0.18	<0.001
a*45min	2.37[bc]	1.82[c]	1.81[c]	2.98[ab]	3.45[a]	0.14	<0.001
b*45min	6.90[b]	6.26[c]	7.41[ab]	6.31[c]	7.70[a]	0.12	<0.001
L*24h	44.50	44.57	44.53	44.57	44.58	0.03	0.936
a*24h	1.73[b]	1.36[b]	1.81[b]	2.75[a]	3.17[a]	0.14	<0.001
b*24h	5.05[c]	5.61[b]	5.32[bc]	5.81[b]	7.04[a]	0.14	<0.001
Moisture (%)	72.88	73.06	72.60	73.25	72.84	0.11	0.429
Ash content (%)	2.78	3.08	2.24	2.40	2.62	0.13	0.305
Crude protein (g/100 g)	15.53[a]	15.91[a]	17.04[a]	26.15[b]	28.40[b]	1.16	<0.001
Crude fat (g/100 g)	3.69	4.55	3.63	4.52	3.04	0.32	0.542

[a-c] Mean values within a row with different superscripts are different values at $P < 0.05$.
[1] L*: luminance; a*: redness; b*: yellowness.
[2] CK = base diet (no coffee pericarp); UF-L = base diet + 2.5 % coffee pericarp; UF-H = base diet + 5 % coffee pericarp; F-L = base diet+ 2.5 % fermented coffee pericarp; F-H = base diet + 5 % fermented coffee pericarp.
[3] SEM = standard error of the mean (n = 6).

Table 4
Effects of different treatments of coffee pericarp on the amino acid content of broiler breast meat

Items	Group(μg/g)					SEM	P-value
	CK	UF-L	UF-H	F-L	F-H		
1-methylhistidine	0.73	0.77	0.67	0.77	0.63	0.03	0.431
2-aminobutyric acid	29.87	23.82	25.41	27.75	23.45	0.90	0.093
3-methylhistidine	0.65	0.66	0.63	0.67	0.71	0.01	0.179
4-aminobutyric acid	0.03	0.05	0.00	0.02	0.00	0.01	0.697
Alanine	7.55	7.98	7.17	7.03	7.94	0.21	0.534
aminoadipic acid	0.85	0.69	0.88	1.19	0.94	0.08	0.389
Anserine	242.68[b]	245.38[b]	249.07[b]	250.81[b]	273.34[a]	3.43	0.008
Arginine	2.07	3.71	2.85	3.14	3.70	0.26	0.245
Asparagine	0.65	1.09	1.02	0.77	0.82	0.07	0.328
Aspartate	0.36	0.32	0.32	0.37	0.30	0.02	0.800
carnosine	55.82[a]	49.71[b]	52.05[ab]	51.19[b]	48.06[b]	0.77	0.001
citrulline	0.26	0.25	0.25	0.24	0.26	0.01	0.538
creatinine	0.70[b]	0.75[b]	0.67[b]	0.77[b]	1.32[a]	0.08	0.005
cystine	0.09[b]	0.10[ab]	0.10[ab]	0.10[ab]	0.11[a]	0.00	0.022
glutamate	11.04	11.14	8.76	9.99	10.13	0.35	0.193
glutamine	8.95	9.74	8.25	8.73	9.47	0.23	0.275
glutathione	35.80	29.29	27.58	30.41	33.65	1.14	0.120
glycine	10.69	15.63	14.60	12.57	14.16	0.71	0.196
histidine	1.04	0.93	0.92	0.92	0.79	0.03	0.132
hydroxyproline	3.66[a]	2.97[ab]	1.94[b]	2.63[ab]	4.16[a]	0.25	0.010
isoleucine	0.06	0.10	0.08	0.10	0.06	0.01	0.357
leucine	3.30	5.19	3.87	4.80	3.74	0.31	0.279
L-homoserine	3.86	5.71	4.25	7.04	6.02	0.47	0.175
lysine	1.83	4.50	2.68	2.36	2.15	0.33	0.062
methionine	0.02	0.02	0.03	0.03	0.03	0.01	0.969
ornithine	0.14	0.19	0.18	0.13	0.18	0.01	0.513
phenylalanine	2.36	2.71	2.45	2.51	2.58	0.08	0.731
proline	1.57	1.72	1.51	1.56	1.75	0.07	0.777
sarcosine	0.27	0.31	0.36	0.37	0.23	0.02	0.233
serine	3.43[b]	4.99[a]	5.15[a]	5.13[a]	4.20[ab]	0.22	0.018
Taurine	0.23	0.20	0.21	0.14	0.13	0.02	0.556
threonine	2.01	3.15	2.42	3.81	3.34	0.25	0.117
tryptophan	2.24	2.60	2.30	2.33	2.64	0.08	0.439
tyrosine	3.16	3.51	3.02	3.26	3.08	0.13	0.842
valine	1.81	3.19	2.30	2.80	2.28	0.23	0.397
EAA	14.69	22.40	17.05	19.67	17.60	1.07	0.205
NEAA	49.57	59.93	52.72	52.66	55.65	1.56	0.303
TAA	439.82	443.09	433.93	446.47	466.31	4.23	0.127

[a-c] Mean values within a row with different superscripts are different values at $P < 0.05$.
[1]EAA: essential amino acid; NEAA: non-essential amino acid; TAA: total amino acid.
EAA=sum of (Ile, Leu, Lys, Met, Phe, Thr, Trp, Val, His); NEAA=sum of (Ala, Arg, Asn, Asp, Cys, Glu, Gln, Gly, Pro, Ser, Tyr);
TAA: total amino acid.
[2] CK = base diet (no coffee pericarp); UF-L = base diet + 2.5 % coffee pericarp; UF-H = base diet + 5 % coffee pericarp; F-L = base diet + 2.5 % fermented coffee pericarp; F-H = base diet + 5 % fermented coffee pericarp.
[3] SEM = standard error of the mean (n = 6).

monounsaturated fatty acids (MUFA), n-6 PUFA, and total PUFA content were increased in the UF-H, F-L, and F-H groups, although these changes were not statistically significant ($P > 0.05$).

Analysis of volatile compounds

Gas chromatography-mass spectrometry (GC-MS) analysis of volatile compounds in the cooked breast muscle of white-feathered broilers revealed significant differences in volatile component structures between the control and treatment groups. As shown in Fig. 1 (A and B), the primary classifications of volatile compounds included aldehydes, ketones, alcohols, esters, hydrocarbons, and acids, with aldehydes and ketones being the most prevalent (Fig. 1A). The 10 volatile components with the highest relative contents comprised three aldehydes and two ketones, demonstrating differences in relative abundance across treatments (Fig. 1B). Further analysis (Table 6) identified a total of 55 volatile compounds, including 11 aldehydes, 12 ketones, 11 alcohols, 6 esters, 8 hydrocarbons, 1 acid, and 6 other compounds. The addition of coffee pericarp to the diet significantly altered the volatile composition of chicken meat. Compared with the control group, the levels of aldehydes, ketones, and alcohols increased significantly in all experimental groups, except for the UF-L group ($P<0.05$). Additionally, the content of esters significantly increased in the UF-H and F-L groups ($P<0.05$). These findings suggest that coffee pericarp markedly influences the composition and structure of volatile components in chicken breast.

Intestinal microbiome analysis

Species richness analysis. Alpha diversity analysis based on 16S rRNA gene sequencing revealed no significant differences in microbial diversity among the three groups. The species coverage index (Fig. 2A), with a mean value nearing 1, indicated that the sequencing depth adequately captured most microbial communities. The estimated species richness index, Chao1 (Fig. 2B), also showed no significant differences, with values ranging from 1,000 to 1,500. Shannon's index (Fig. 2C), reflecting species richness and evenness, varied between 7.0 and 7.8 without significant group differences. The Pielou_e species evenness index (Fig. 2D) ranged from 0.6 to 0.8, similarly showing no significant variation among groups. Collectively, these indices suggest a lack of significant differences in microbial diversity, although subtle variations in community structure and species abundance may exist.

Microbial Community Structure Analysis. The 16S rRNA gene sequencing revealed significant differences in microbial community structure between the control group and each dose group. As illustrated

Table 5
Effects of different treatments of coffee pericarp on the fatty acid content of broiler breast meat

Items	Group (μg/g)					SEM	P-value
	CK	UF-L	UF-H	F-L	F-H		
C14:0	3.44	3.59	4.63	5.33	4.64	0.36	0.454
C16:0	342.66	336.77	415.06	443.82	388.8	20.96	0.473
C18:0	175.45	170.94	192.11	191.59	168.1	4.28	0.220
C20:0	1.13	1.15	1.34	1.39	1.18	0.05	0.354
C22:0	0.65^{ab}	0.60^{ab}	0.68^{a}	0.64^{ab}	0.56^{b}	0.01	0.021
∑SFA	523.33	513.06	613.81	642.77	563.28	25.23	0.454
C16:1T	0.44	0.41	0.46	0.47	0.48	0.02	0.675
C16:1	33.12	31.12	41.13	53.50	47.09	4.47	0.530
C18:1N9C	288.83	280.84	336.17	398.47	336.03	20.90	0.443
∑MUFA	322.39	312.38	377.76	452.44	383.60	25.33	0.462
C18:3N3	4.29	4.17	5.48	6.39	5.64	0.45	0.521
C20:5N3	5.23^{a}	4.56^{ab}	5.22^{a}	4.71^{ab}	3.98^{b}	0.14	0.004
C22:5N3	18.51^{a}	15.00^{b}	16.01^{ab}	15.78^{ab}	14.99^{b}	0.41	0.011
C22:6N3	18.17^{a}	14.55^{c}	16.47^{ab}	14.31^{c}	14.62^{bc}	0.43	<0.001
n-3PUFA	46.19^{a}	38.27^{b}	43.19^{ab}	41.19^{ab}	39.23^{b}	0.87	0.003
C18:2N6	258.99	263.62	313.11	331.76	311.82	14.79	0.471
C18:3N6	1.52	1.61	1.80	2.19	1.70	0.10	0.297
C20:3N6	22.26^{b}	23.30^{ab}	25.98^{a}	23.70^{ab}	22.51^{b}	0.45	0.034
C20:4N6	122.12^{a}	112.69^{ab}	116.00^{ab}	113.55^{ab}	101.49^{b}	2.20	0.018
C22:5N6	9.76	9.14	9.23	9.08	9.34	0.34	0.858
n-6PUFA	414.65	410.36	466.12	480.29	446.86	14.78	0.537
∑PUFA	460.84	448.63	509.31	521.48	486.09	15.09	0.557
PUFA/SFA (%)	88.06	87.77	83.51	81.56	87.31	1.24	0.385
Total	1306.56	1274.06	1500.87	1616.69	1432.97	64.89	0.489

[a-c] Mean values within a row with different superscripts are different values at $P < 0.05$.
[1] SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; n-3PUFA: n-3 polyunsaturated fatty acid; n-6PUFA: n-6 polyunsaturated fatty acid; PUFA: polyunsaturated fatty acid.
[2] CK = base diet (no coffee pericarp); UF-L = base diet + 2.5 % coffee pericarp; UF-H = base diet + 5 % coffee pericarp; F-L = base diet + 2.5 % fermented coffee pericarp; F-H = base diet + 5 % fermented coffee pericarp.
[3] SEM = standard error of the mean (n = 6).

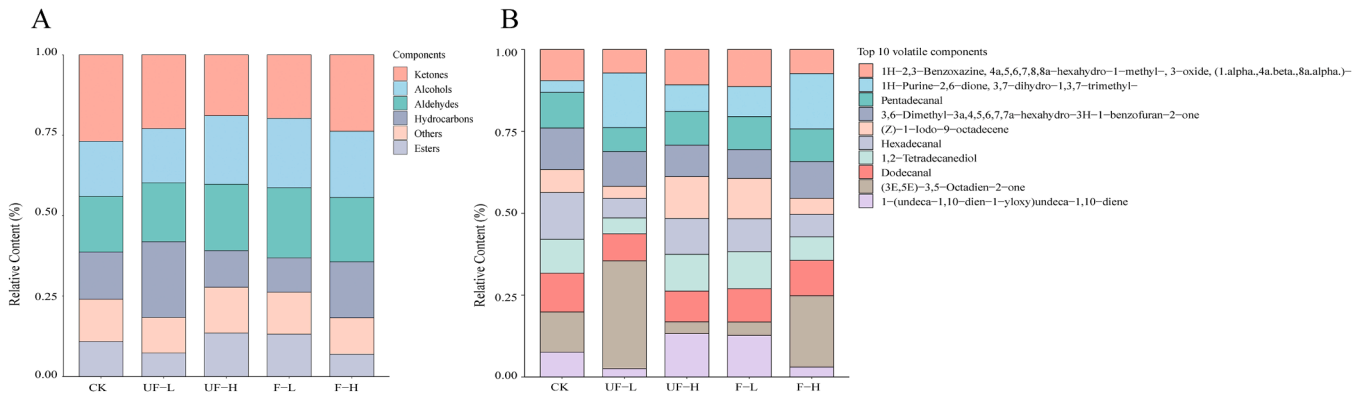

Fig. 1. Effects of different coffee pericarp treatments on the composition and structure of volatile components. (A) Composition of volatile components; (B) Relative content of the top 10 volatile components. CK: base diet (no coffee pericarp); UF-L: base diet + 2.5 % coffee pericarp; UF-H: base diet + 5 % coffee pericarp; F-L: base diet + 2.5 % fermented coffee pericarp; F-H: base diet + 5 % fermented coffee pericarp.

in Fig. 3, the predominant phyla across all samples included Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, Verrucomicrobia, and Tenericutes, with Thick-walled Bacteria, Bacteroidetes, and Ascomycetes being the most dominant (Fig. 3A). The Firmicutes/Bacteroidetes (F/B) ratio, a key indicator of gut microbiota health, was elevated in all treatment groups, increasing in a dose-dependent manner in the UF treatment group (Fig. 3B).At the genus level (Fig. 3C), the relative abundances of Faecalibacterium, Oscillospira, Bacteroides, Ruminococcus, and Lactobacillus varied across treatments, suggesting that coffee pericarp may influence the composition and structure of gut microbial communities.

Analysis of Species Differences in Microbial Community Composition. Beta diversity analysis based on 16S rRNA gene sequencing, illustrated through PCoA and NMDS two-dimensional ordination plots, showed complete separation of the CK group from the UF-L, UF-H, F-L, and F-H groups. This indicates that coffee pericarp significantly alters the intestinal microbial community structure in white-feathered broiler chickens (Fig. 4A, B). The Venn diagram (Fig. 4C) displays the unique and shared operational taxonomic units (OTUs) among experimental groups, with a total of 12,194 OTUs generated across all samples. The diagram shows that the CK, UF-L, UF-H, F-L, and F-H groups had 2,206 (18.09 %), 2,165 (17.75 %), 2,253 (18.48 %), 2,391 (19.61 %), and 2,244 (18.40 %) OTUs, respectively.

LEfSe analysis further identified signature microflora (Fig. 4D, E). At the levels of phylum, order, family, and genus, seven taxa were identified in the CK group, nine in the UF-L group, six in the UF-H group, twelve in the F-L group, and five in the F-H group. Notably, the low-dose coffee pericarp treatments (UF-L, F-L) exhibited a richer diversity of differential taxa, indicating a stronger effect on intestinal flora. The top two LDA scores were for Verrucomicrobiae (LDA score = 4.68) and

Table 6
Effects of different treatments of coffee pericarp on volatile components of broiler breast meat

Items		Group (%)					SEM	P-value
		CK	UF-L	UF-H	F-L	F-H		
Aldehydes	2-Undecenal	5.17^{b}	5.96^{ab}	6.15^{ab}	7.09^{ab}	8.96^{a}	0.43	0.021
	Nonanal	6.22	6.40	6.19	7.64	6.88	0.28	0.478
	(E)-7-dodecenal	4.13^{b}	2.15^{b}	11.46^{a}	11.62^{a}	3.98^{b}	1.12	<0.001
	Hexadecanal	5.20^{b}	1.80^{b}	12.12^{a}	10.87^{a}	3.35^{b}	1.15	<0.001
	Tetradecanal	2.29^{b}	1.01^{b}	14.73^{a}	12.86^{a}	2.44^{b}	1.59	<0.001
	2,4-Decadienal	4.81^{b}	6.95^{ab}	5.47^{b}	6.33^{b}	9.78^{a}	0.52	0.003
	2,4-Nonadienal	4.52^{c}	6.98^{b}	5.13^{bc}	5.78^{bc}	10.92^{a}	0.63	<0.001
	Hexanal	4.98^{b}	7.01^{ab}	6.10^{b}	6.59^{ab}	8.65^{a}	0.38	0.007
	2-Octenal, 2-butyl-	1.68^{c}	2.43^{c}	8.73^{ab}	12.35^{a}	8.15^{b}	1.13	<0.001
	Dodecanal	4.31^{b}	2.47^{b}	10.40^{a}	10.88^{a}	5.28^{b}	0.95	<0.001
	Pentadecanal	3.96^{b}	2.20^{b}	11.39^{a}	10.89^{a}	4.89^{b}	1.04	<0.001
	Subtotal	47.26^{c}	45.35^{c}	97.87^{a}	102.90^{a}	73.28^{b}	6.68	<0.001
Ketones	1,7,7-Trimethylbicyclo[2.2.1]heptan-2-one	3.60^{d}	4.37^{cd}	6.62b^{c}	8.47^{ab}	10.28^{a}	0.71	<0.001
	2-Pentyl-2H-furan-5-one	4.32^{b}	5.16^{b}	6.79^{b}	7.10^{ab}	9.97^{a}	0.58	0.001
	(R)-2-phenyl-2,3-dihydropyridin-4(1H)-one	23.93	5.76	2.12	1.20	0.34	3.06	0.045
	1,2-Propanedione, 1-phenyl-	8.13^{a}	5.24^{b}	5.85^{ab}	6.37^{ab}	7.75^{ab}	0.37	0.029
	5-Ethynyl-4-methyl-2-oxabicyclo[2.2.2]oct-5-ene-3-one	4.52^{b}	1.76^{b}	12.34^{a}	11.46^{a}	3.26^{b}	1.22	<0.001
	(3E,5E)-3,5-Octadien-2-one	4.46^{b}	9.85^{a}	3.99^{b}	4.39^{b}	10.64^{a}	0.85	<0.001
	6,7-Dodecanedione	2.90^{c}	6.76^{b}	4.46^{bc}	5.43^{bc}	13.78^{a}	1.04	<0.001
	1-(3,4-Dihydro-2H-chromen-4-yl)ethanone	5.43^{bc}	3.67^{c}	8.65^{ab}	9.40^{a}	6.19^{abc}	0.63	0.001
	1H-Purine-2,6-dione, 3,7-dihydro-1,3,7-trimethyl-	1.30^{b}	4.98^{ab}	8.98^{a}	9.83^{a}	8.23^{a}	0.97	0.005
	2-Dodecanone	4.75^{b}	3.03^{b}	10.40^{a}	9.82^{a}	5.33^{b}	0.83	<0.001
	3,6-Dimethyl-3a,4,5,6,7,7a-hexahydro-3H-1-benzofuran-2-one	4.61^{b}	3.17^{b}	10.66^{a}	9.42^{a}	5.47^{b}	0.82	<0.001
	Geranylacetone	5.31^{c}	2.98^{c}	8.77^{ab}	10.41^{a}	5.87^{bc}	0.75	<0.001
	Subtotal	73.26^{ab}	56.71^{b}	89.61^{ab}	93.30^{a}	87.11^{ab}	4.54	0.029
Alcohols	(1R*,6R*,9S*)-9-Methylbicyclo[4.3.0]nonan-1-ol	4.03^{b}	4.42^{b}	4.14^{b}	7.83^{ab}	12.92^{a}	1.05	0.004
	1-Octanol	5.38^{c}	5.83^{bc}	6.31^{abc}	7.82^{ab}	7.99^{a}	0.33	0.007
	(8Z)-14-Methyl-8-hexadecen-1-ol	2.70^{b}	1.23^{b}	13.54^{a}	13.43^{a}	2.43^{b}	1.53	<0.001
	1,2-Tetradecanediol	3.77^{b}	1.45^{b}	12.49^{a}	12.13^{a}	3.50^{b}	1.31	<0.001
	1-Pentanol	5.25^{b}	7.23^{ab}	5.98^{b}	5.98^{b}	8.89^{a}	0.38	0.001
	1-Undecen-3-ol	5.64^{b}	7.05^{ab}	5.68^{b}	6.50^{ab}	8.47^{a}	0.33	0.012
	1-Heptadecanol	3.56^{c}	1.69^{c}	11.15^{ab}	11.85^{a}	5.08^{bc}	1.20	0.001
	1-Hexanol	3.46	4.53	12.38	5.08	7.89	1.59	0.437
	1-Octadecanol	3.68^{bc}	2.29^{c}	10.13^{ab}	11.19^{a}	6.05^{abc}	1.07	0.004
	11-Dodecenol	5.08^{b}	3.32^{b}	9.24^{a}	9.76^{a}	5.92^{ab}	0.74	0.001
	n-Tridecan-1-ol	4.18^{b}	2.52^{b}	9.90^{a}	10.46^{a}	6.28^{ab}	0.91	0.001
	Subtotal	46.73^{bc}	41.56^{c}	100.93^{a}	102.03^{a}	75.42^{ab}	7.37	<0.001
Esters	S-Methyl N,N-diethylthiocarbamate	5.67^{bc}	2.99^{c}	5.66^{bc}	8.65^{ab}	10.36^{a}	0.74	<0.001
	1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	9.27	5.94	6.35	7.99	3.79	0.70	0.103
	Eicosyl acetate	4.32^{b}	2.12^{b}	11.58^{a}	11.34^{a}	3.97^{b}	1.11	<0.001
	Hexadecanoic acid, 1-methylethyl ester	4.63^{bc}	2.52^{c}	13.10^{a}	10.65^{ab}	2.44^{c}	1.31	0.001
	Hexadecanoic acid, butyl ester	2.19^{b}	3.35^{ab}	13.25^{a}	12.63^{a}	1.92^{b}	1.60	0.006
	Methyl 2,8-Dimethylundecanoate	3.68^{b}	1.08^{b}	14.18^{a}	11.25^{a}	3.14^{b}	1.44	<0.001
	Subtotal	29.75^{b}	18.02^{b}	64.13^{a}	62.51^{a}	25.60^{b}	5.49	<0.001
Hydrocarbons	Methane, sulfonylbis-	3.21^{b}	4.14^{b}	7.97^{ab}	5.78^{b}	12.24^{a}	0.97	0.002
	1-(Cyclohexoxy)ethoxycyclohexane	3.17^{b}	0.85^{b}	14.93^{a}	12.53^{a}	1.85^{b}	1.65	<0.001
	(1-Ethyloctyl)cyclohexane	5.75^{b}	7.37^{ab}	5.52^{b}	6.05^{b}	8.65^{a}	0.36	0.004
	Dodecane	7.21^{ab}	13.15^{a}	1.76^{b}	1.90^{b}	9.32^{ab}	1.33	0.002
	Dodecane, 4-methyl-	7.34^{ab}	13.52^{a}	1.69^{b}	1.73^{b}	9.05^{a}	1.34	0.001
	Dodecane, 5-methyl-	7.30^{ab}	11.99^{a}	2.35^{b}	2.40^{b}	9.29^{a}	1.15	0.002
	(R)-3-neopentylcyclohex-1-ene	3.52^{c}	6.21^{b}	5.63^{bc}	7.06^{b}	10.92^{a}	0.67	<0.001
	(Z)-1-Iodo-9-octadecene	2.57^{b}	1.09^{b}	14.11^{a}	13.15^{a}	2.41^{b}	1.56	<0.001
	Subtotal	40.05	58.33	53.96	50.59	63.72	2.95	0.091
Others	Bis-(3,5,5-trimethylhexyl) ether	5.26^{b}	6.61^{ab}	5.82^{b}	6.72^{ab}	8.91^{a}	0.39	0.009
	1-(undeca-1,10-dien-1-yloxy)undeca-1,10-diene	2.77^{b}	0.77^{b}	14.65^{a}	13.65^{a}	1.49^{b}	1.75	<0.001
	Hexanoic acid	4.65^{b}	4.75^{b}	5.41^{b}	6.09^{b}	12.43^{a}	0.86	0.001
	Nicotinamide	6.94	4.55	4.29	8.16	9.39	0.80	0.174
	(2-phenylphenyl)-(p-tolyl)amine	7.36^{ab}	5.78^{ab}	10.53^{a}	6.57^{ab}	3.10^{b}	0.80	0.022
	1H-2,3-Benzoxazine, 4a,5,6,7,8,8a-hexahydro-1-methyl-, 3-oxide, (1.alpha.,4a. beta.,8a.alpha.)-	3.47^{b}	2.15^{b}	11.97^{a}	12.15^{a}	3.60^{b}	1.25	<0.001
	N-Ethylmorphine	5.34^{b}	2.75^{b}	14.69^{a}	8.16^{ab}	2.40^{b}	1.36	0.002
	Subtotal	35.78^{b}	27.36^{b}	67.37^{a}	61.51^{a}	41.32^{b}	4.26	<0.001
	Total	272.83^{c}	247.34^{c}	473.88^{a}	472.83^{a}	366.45^{b}	26.63	<0.001

[a-d] Mean values within a row with different superscripts are different values at $P < 0.05$.
[1] CK = base diet (no coffee pericarp); UF-L = base diet+ 2.5 % coffee pericarp, UF-H = base diet+ 5 % coffee pericarp; F-L = base diet + 2.5 % fermented coffee pericarp; F-H = base diet + 5 % fermented coffee pericarp.
[2] SEM = standard error of the mean (n = 6).

Verrucomicrobia (LDA score = 4.65) in the CK group; Clostridiales (LDA score = 5.17) and Ruminococcaceae (LDA score = 5.16) in the UF-L group; Proteobacteria (LDA score = 3.99) and Actinobacteria (LDA score = 3.90) in UF-H; Bacilli (LDA score = 4.64) and Lactobacillales (LDA score = 4.62) in F-L; and Bacteroidia (LDA score = 5.01) and Bacteroidetes (LDA score = 5.00) in F-H.

Functional Prediction Analysis of Cecum Microflora. The 16S rRNA gene sequences from the cecum contents of white feather broilers were

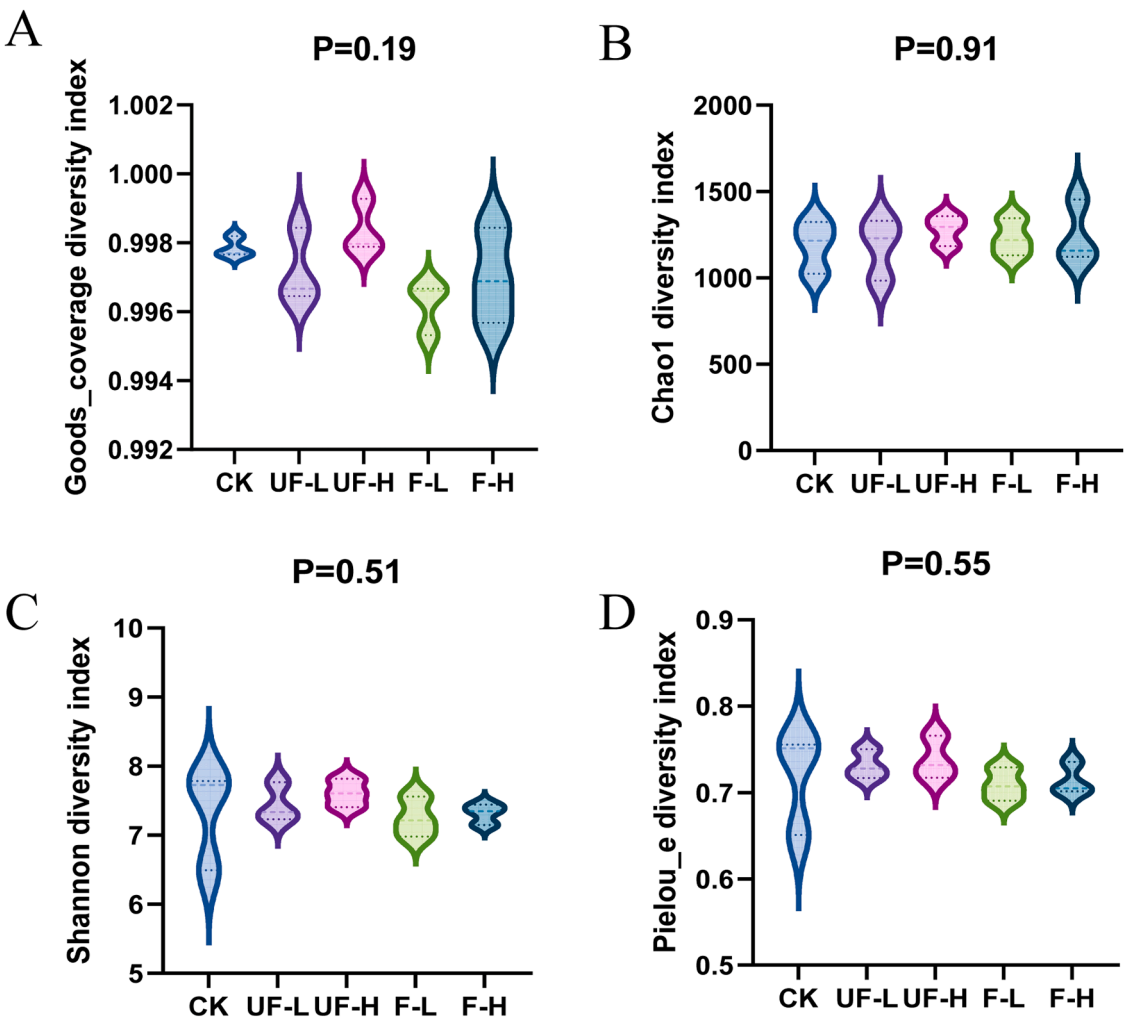

Fig. 2. Alpha diversity indices of the cecum flora. (A) Goods-coverage, (B) Chao1, (C) Shannon, and (D) Pielou-e diversity indices. CK, base diet (no coffee pericarp); UF-L, base diet + 2.5 % coffee pericarp; UF-H: base diet + 5 % coffee pericarp; F-L: base diet + 2.5 % fermented coffee pericarp; F-H: base diet + 5 % fermented coffee pericarp.

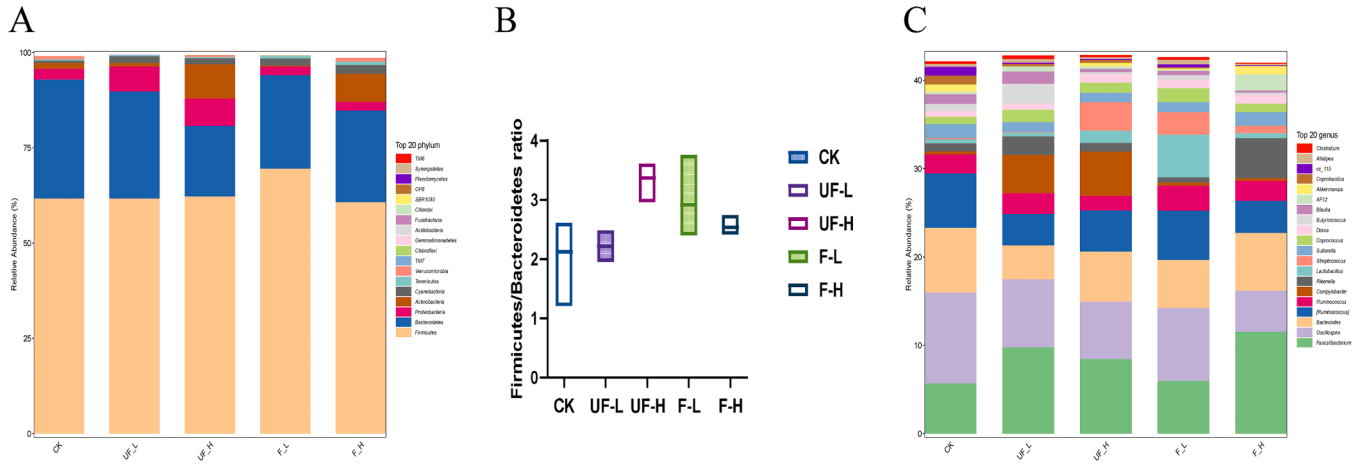

Fig. 3. Effects of different treatments of coffee pericarp on intestinal microbial community composition at the phylum and genus levels in white feather broilers. (A) Phylum-level microbial composition; (B) Firmicutes/Bacteroidetes (F/B) ratio; (C) Genus-level microbial composition. CK: base diet (no coffee pericarp); UF-L: base diet + 2.5 % coffee pericarp; UF-H: base diet + 5 % coffee pericarp; F-L: base diet + 2.5 % fermented coffee pericarp; F-H: base diet + 5 % fermented coffee pericarp.

analyzed for predicted pathways using the KEGG database via PICRUSt. Given the extensive number of functional units (ECs), PCoA was employed to illustrate the functional differences among the samples in reduced dimensional space. As shown in Fig. 5A, the first two coordinates account for 65.8 % of the overall variation, with Coordinate 1 contributing 41.5 % and Coordinate 2 contributing 24.3 %. The analysis

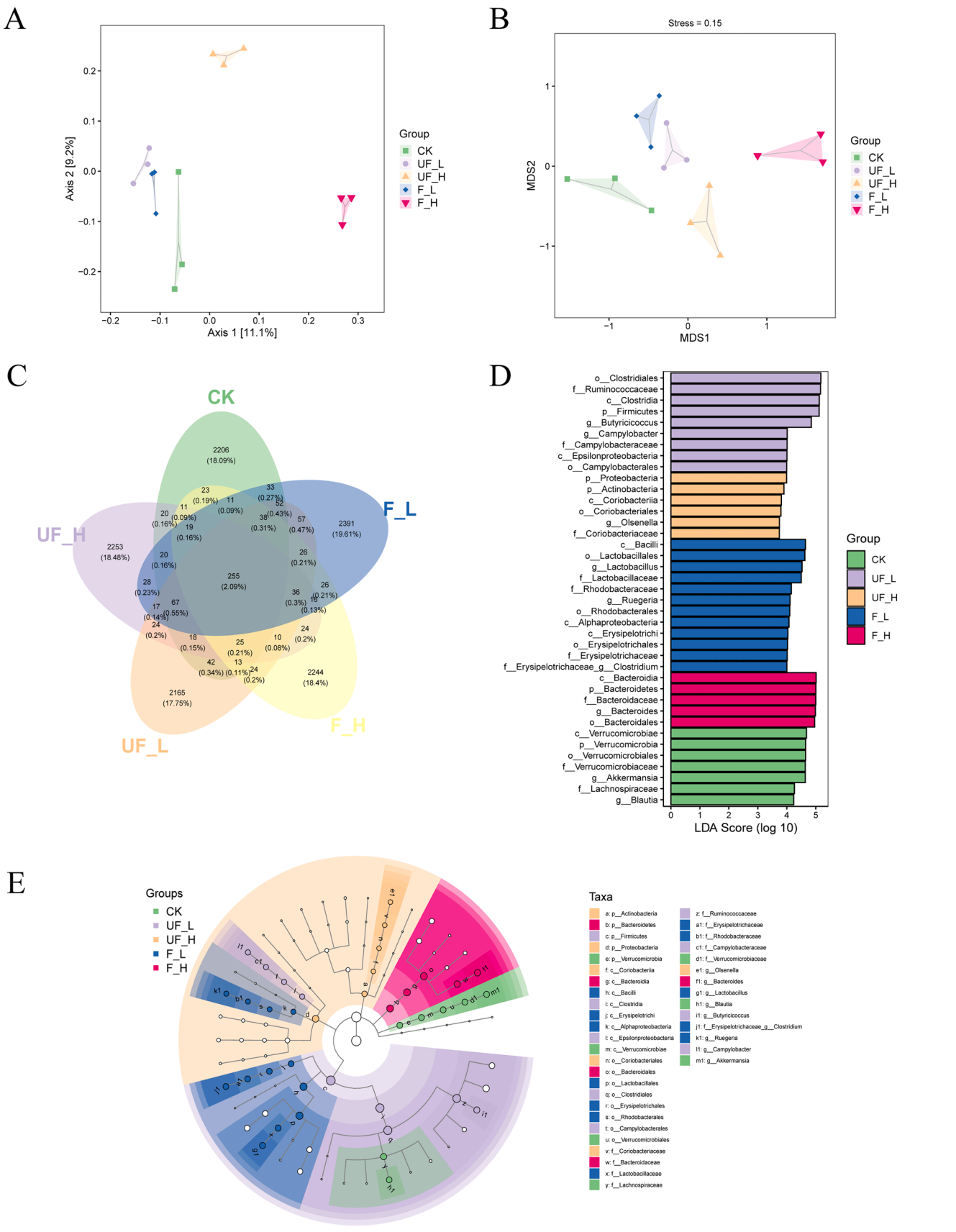

Fig. 4. Effects of different treatments of coffee pericarp on species differences of intestinal microbial community composition in white feather broilers. (A-B) β-diversity: PcA and NMDs maps; (C) Venn diagram; (D) LDA scores; (E) Taxonomic lineage map obtained from LEfSe analysis.

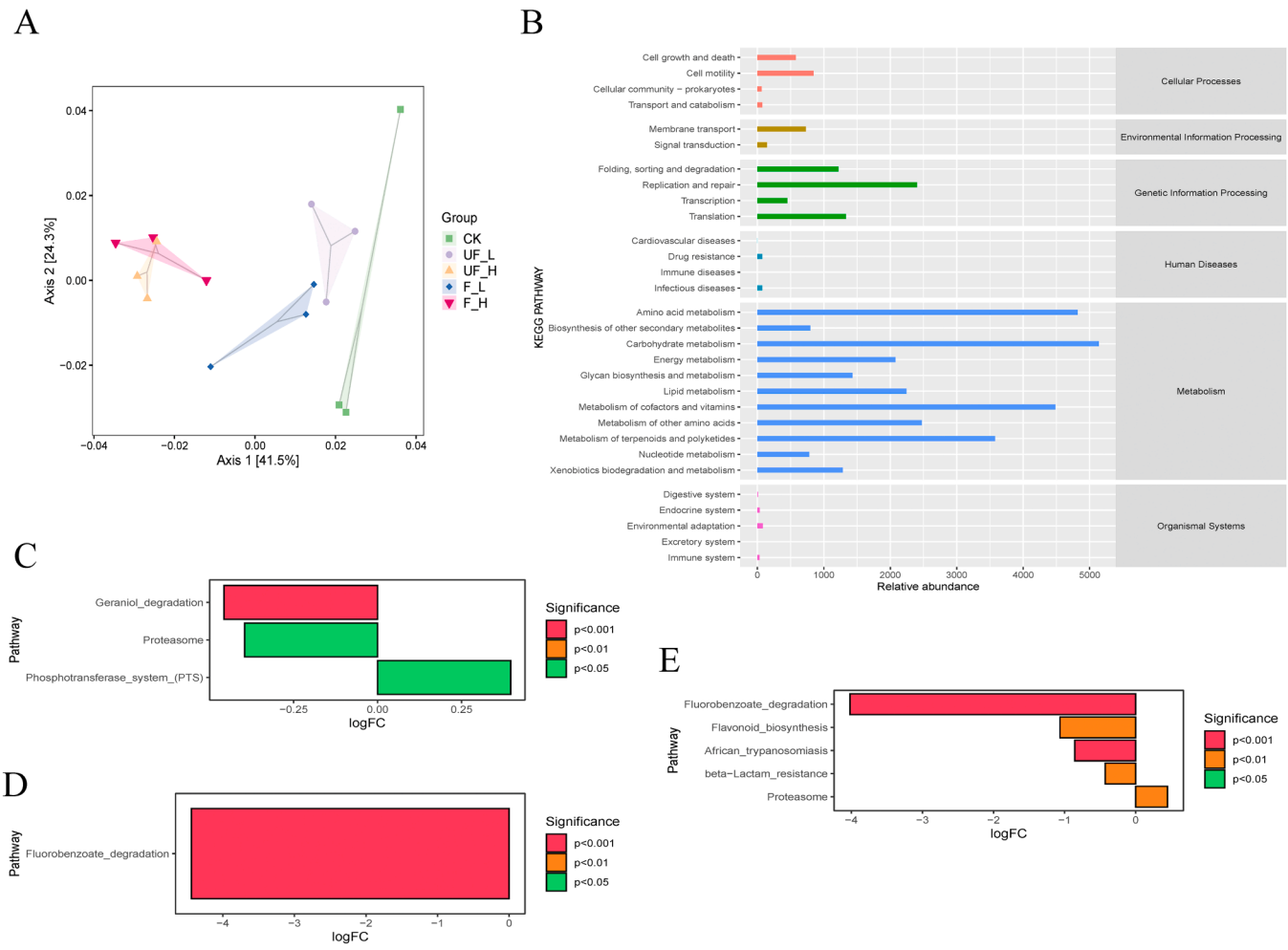

Fig. 5. Predicted KEGG functional pathway abundance analysis. (A) PCoA map; (B) KEGG secondary functional metabolic pathway statistics; (C) KEGG metabolic pathway difference analysis (CK group and UF-H group, CK group and F-L group, CK group and F-H group).

revealed overlapping spectral line plots of the 16S intestinal bacterial composition between the UF-H and F-H dose groups, indicating similar functional compositions within these dimensions. The metabolism of white-feathered broilers primarily encompasses amino acid metabolism, carbohydrate metabolism, energy metabolism, glycan biosynthesis, nucleotide metabolism, lipid metabolism, cofactor and vitamin metabolism, as well as terpenoid and polyketide metabolism pathways (Fig. 5B). Compared to the control group, the UF-H group exhibited down-regulation of the Geraniol degradation and Proteasome pathways, along with up-regulation of the Phosphotransferase system (PTS) pathway. The F-L group showed down-regulation of the Fluorobenzoate degradation pathway, while the F-H group displayed down-regulation of the Fluorobenzoate degradation, Flavonoid biosynthesis, African trypanosomiasis, and beta-Lactam resistance pathways, and up-regulation of the Proteasome pathway (Fig. 5C-E).

Correlation analysis

Changes in the physical and chemical characteristics, amino acids, fatty acids, and volatile components in breast muscle may correlate with alterations in intestinal flora. Mantel and chordogram correlation methods were employed to investigate the relationship between intestinal microbiota levels and the amino acid and fatty acid compositions in the breast muscle of white-feathered broilers, aiming to elucidate the mechanism by which coffee pericarp enhances meat quality. The results indicated significant correlations in the analysis of physicochemical indices with specific amino acids and fatty acids: L* at 45 minutes positively correlated with Anserine (r=0.642, p=0.010), creatinine (r=0.596, p=0.019), and cystine (r=0.543, p=0.036), while negatively correlating with C20:5N3 (r=-0.551, p=0.033) and C20:4N6 (r=-0.600, p=0.018). Similarly, b* at 45 minutes showed positive correlations with creatinine (r=0.681, p=0.005), cystine (r=0.574, p=0.025), and hydroxyproline (r=0.780, p=0.001), and negative correlations with C22:0 (r=-0.620, p=0.014) and C20:3N6 (r=-0.556, p=0.032) (Fig. 6A, B). The correlation analysis between volatile components and genus-level flora revealed that the top 20 bacterial species significantly correlated with volatile components, including Faecalibacterium, Oscillospira, Bacteroides, Ruminococcus, Lactobacillus, and Blautia (Fig. 6C). Furthermore, in the categorical correlation analysis of the top twenty bacterial groups at both the phylum and genus levels with amino acids, fatty acids, and volatile components, the genus-level groups exhibited significant negative correlations with aldehydes, esters, and hydrocarbons among the volatile components (Fig. 6D).

Discussion

The use of feed additives is increasingly attracting attention as consumer concerns regarding food safety and animal welfare rise (Chandra et al., 2021). Corn, wheat, and soybean meal (SBM) are staple nutrients in animal feeds. Incorporating agricultural by-products and specific plant or insect proteins into conventional feeds not only reduces production costs and enhances economic efficiency but also mitigates

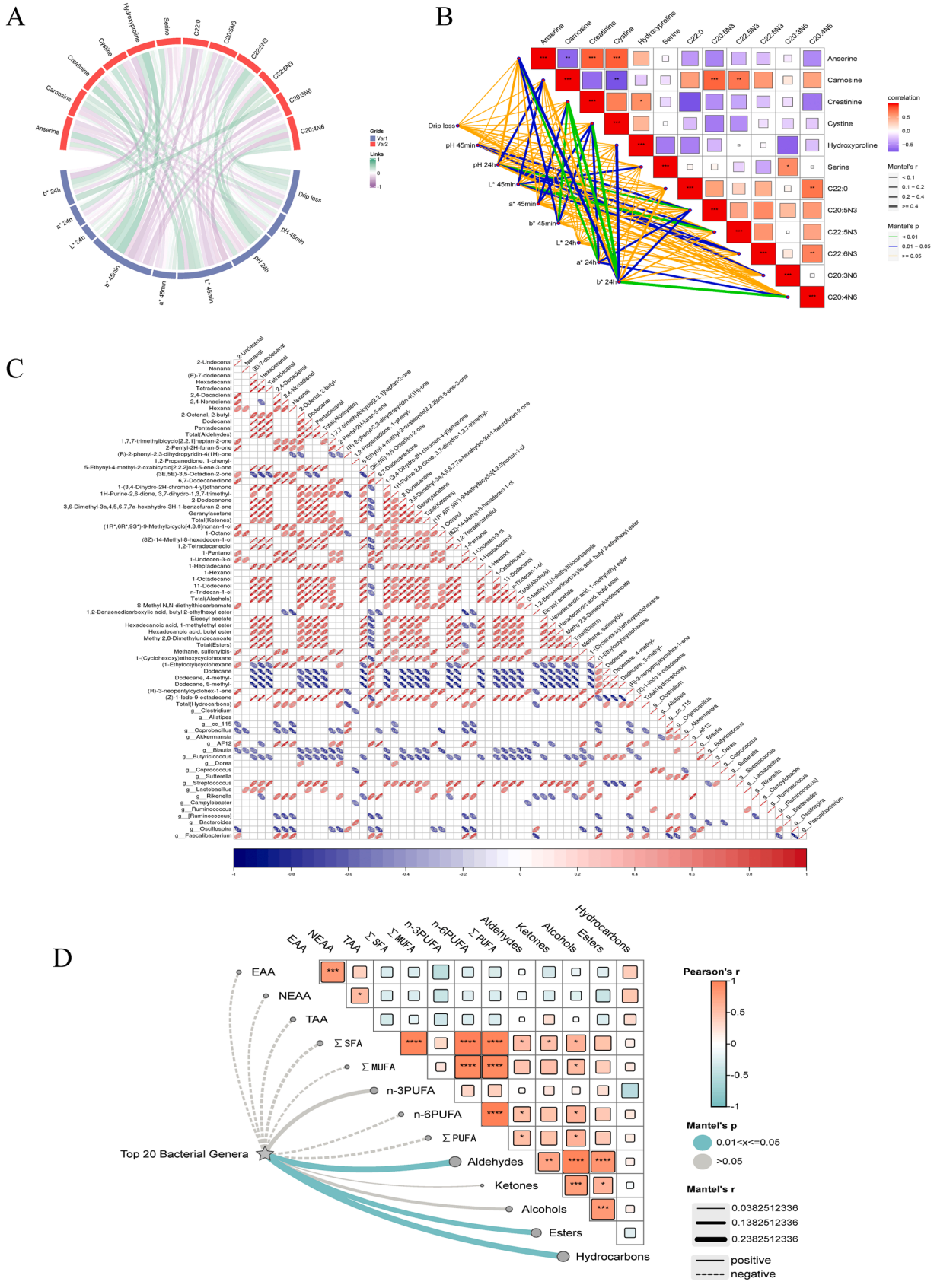

Fig. 6. Correlation analysis between meat quality and intestinal flora.(A) Correlation between the physical properties of chicken and amino acids and fatty acids; (B) Mantel test analysis diagram of physical properties, amino acids, and fatty acids of chicken; (C) Heat map of the correlation analysis between volatile components of chicken and top 20 bacteria at the genus level; (D) Classification correlation analysis of the top 20 floras at the phylum level and genus level with amino acids, fatty acids, and volatile components.

over-reliance on traditional feed resources, thereby improving the sustainability of animal husbandry (Martínez-Zamora et al., 2021; Zhao et al., 2024). This study investigated the potential value of coffee fruit peel powder as a feed additive in broiler rearing. Different doses of coffee pericarp powder were added to the basal diet of white-feathered broilers, revealing that the addition of coffee pericarp powder improved growth performance (final body weight, average daily gain, and feed-to-gain ratio). However, with increasing levels of addition, daily weight gain decreased, and the feed-to-weight ratio increased, potentially linked to the trace amounts of caffeine present in the coffee pericarp.

Meat quality serves as a critical index of the economic traits of livestock and poultry, with key parameters for evaluation including drip loss, shear force, pH, and meat color (Chang et al., 2023). Drip loss measures the meat's ability to retain moisture, influencing its edible qualities such as flavor, aroma, and juiciness, while also directly affecting product yield, which holds significant economic value (He et al., 2023; Tang et al., 2021). This study found that chicken breast meat from white-feather broilers fed coffee pericarp exhibited improved water retention, indicating that coffee pericarp supplementation positively affects the water-holding capacity of chicken meat, particularly in the low-dose fermented group. pH is a crucial indicator of meat freshness; in this study, pH changes at 45 minutes post-slaughter were minimal, yet the F-H group showed a more significant pH variation at 24 hours compared to the control group. Meat color also plays a vital role in determining quality and freshness, significantly influencing consumer preference and purchasing decisions (Jin et al., 2021). The present study demonstrated that the color parameters (L*, a*, and b*) of chicken breast meat varied significantly across all experimental groups, with the addition of fermented coffee pericarp powder markedly enhancing the redness (a*) value compared to the control group. These findings suggest that coffee pericarp powder can improve muscle color, aligning it more closely with consumer expectations.

The nutritional value of meat is primarily assessed through its chemical composition. Moisture content is essential for meat preservation and processing, directly affecting taste and the concentration and bioactivity of nutrients. Ash content reflects mineral levels in meat, which are critical for physiological functions and metabolic activities; essential minerals such as calcium, iron, and zinc are vital for human health. Additionally, protein and fat content are important nutritional indicators of meat (Guo et al., 2024). In this study, coffee pericarp powder did not significantly affect the moisture, ash, or crude fat content of the muscle, indicating that its addition did not alter these parameters. However, the incorporation of fermented coffee pericarp powder significantly increased the crude protein content in chicken breast meat compared to the control group, likely due to the rich presence of polysaccharides, polyphenols, and chlorogenic acid, which may enhance protein synthesis and deposition (Aoki et al., 2023).

The amino acid and fatty acid profiles of meat are closely linked to its nutritional value and flavor (Yu et al., 2021; Deng et al., 2022). Amino acids and fatty acids are vital components in protein synthesis and various metabolic processes, and a balanced diet is crucial for ensuring adequate intake to maintain health (He and Wu, 2022). In this study, both essential and non-essential amino acid levels in chicken breast meat increased with coffee pericarp addition compared to the control group, although these differences were not statistically significant, possibly due to individual variability. The fatty acid profile revealed an upward trend in saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and n-6 polyunsaturated fatty acids (n-6PUFA), alongside a significant decrease in n-3 polyunsaturated fatty acids (n-3PUFA) in the chicken breast meat supplemented with coffee pericarp powder. n-3 Polyunsaturated fatty acids (n-3PUFA) are produced through the proteolytic conversion of alpha(a)-linolenic acid (ALA) via a series of enzymatic reactions in the endoplasmic reticulum and peroxisomes, a process characterized by its complexity (Bhatt et al., 2020). Numerous genes and enzymes are implicated in fatty acid metabolism and its association with meat quality traits; however, there is a notable lack of research exploring the relationship between dietary quality attributes and n-3PUFA content in meat. Some studies suggest that genetic factors in specific animal strains may influence this relationship (Pewan et al., 2020). Thus, we hypothesize that the observed decrease in n-3PUFA content in this experiment is linked to the genetic characteristics of white-feather broiler breeds.

Meat flavor is a critical indicator of quality. Extensive research has focused on the effects of volatile compounds on meat flavor, which is shaped by a series of intricate reactions during cooking, including the Maillard reaction and lipid oxidative degradation (Zhang et al., 2021). The volatile odors and flavor compounds generated during these processes collectively define the overall flavor profile of chicken meat (He et al., 2023; Ramalingam et al., 2019). Various factors, particularly the composition of the animal's diet, significantly influence changes in flavor compounds within animal tissues. In this study, we detected several volatile compounds, including aldehydes, ketones, alcohols, and esters, with aldehydes and ketones being the most abundant contributors to meat flavor. Aldehydes, generated primarily through the Strecker reaction of amino acids and the oxidative degradation of fatty acids, greatly influence meat flavor, imparting greasy, grassy, and citrus notes (Yang et al., 2017). Ketones, resulting mainly from fat oxidation, have a higher threshold than aldehydes; thus, their contribution to meat flavor is likely less significant. Alcohols in chicken meat predominantly arise from the oxidative decomposition of unsaturated fatty acids, with branched alcohols typically containing shorter carbon chains. As the carbon chain length increases, the flavor profile of these alcohols evolves toward fruity and lipid-like aromas (Yao et al., 2023). In total, we identified 11 aldehydes, including nonanal and hexanal, as well as 12 ketones and 11 alcohols. Furthermore, we detected six esters, eight hydrocarbons, and one acid compound across all chicken samples. Notably, the addition of coffee pericarp significantly enhanced the concentrations of aldehydes, ketones, alcohols, and esters in the muscles of both the unfermented low-dose coffee pericarp group and the fermented coffee pericarp group, with the fermented coffee pericarp low-dose group demonstrating greater efficacy.

Gut flora undergo dynamic changes, comprising a complex microbiota unique to each individual. This microbiota plays a vital role in nutrient utilization, growth, and overall health (Mao et al., 2023). Research indicates that the cecum hosts the highest microbial diversity in the gastrointestinal tract of chickens, primarily dominated by Bacteroidetes, Firmicutes, Proteobacteria, and Actinobacteria (Feye et al., 2020; Tan et al., 2019). Our experimental findings corroborate this observation, although variations in the relative abundance of dominant bacterial phyla were noted, potentially due to the inclusion of coffee pericarp powder in the diet. Short-chain fatty acids produced by Bacteroidetes have been shown to influence weight gain and immune function in both birds and mammals (Shen et al., 2019). Bacteroidetes play a crucial role in enhancing host polysaccharide utilization, boosting immune performance, and maintaining intestinal flora balance. The Firmicutes/Bacteroidetes (F/B) ratio is recognized as a significant indicator of gut microbiota health (Zhang et al., 2023). In this experiment, the addition of coffee pericarp powder increased the relative abundance of Firmicutes while decreasing Bacteroidetes, resulting in a higher F/B ratio compared to the control group. Furthermore, LEfSe analysis demonstrated that low-dose coffee pericarp had a more pronounced effect on intestinal flora. These findings suggest that coffee pericarp powder is beneficial for the intestinal health of white-feather broilers. To investigate the relationship between coffee peel-induced changes in intestinal microorganisms and chicken meat quality, we conducted functional metagenomic sequencing of the intestinal microbiota using 16S rRNA sequencing. We also performed correlation analyses between the intestinal flora and the physical and chemical properties of chicken meat. The analysis revealed that potential metabolic pathways associated with the microbiota primarily pertained to amino acid, carbohydrate, and lipid metabolism, directly or indirectly influencing the

biosynthesis of amino acids, fatty acids, and lipids, thereby affecting the physical traits and chemical composition of the chicken meat.

Conclusions

This study explored the effects of incorporating coffee pericarp powder into the daily diet of white-feathered broilers. Results indicated that the addition of coffee pericarp significantly improved the color of chicken breast meat, enhancing its sensory quality. Moreover, coffee pericarp positively influenced the amino acid content and increased the levels of unsaturated fatty acids in chicken breast by modulating the intestinal microbial community. It also significantly boosted the content of volatile compounds in chicken breast, improving its nutritional value and flavor. In conclusion, this study provides preliminary evidence that adding coffee pericarp to broiler feed can enhance the flavor and nutritional quality of white-feather broiler meat through a multifaceted evaluation.

Author contributions

Linxian Shan: Data curation, Writing; Jun He: Conceptualization; Ruijuan Yang: Methodology; Jinya Dong: Formal analysis; Zezhu Du, Shengjie Duan, Yanmei Li, Xiuli Lu, Yan Shen, Jianyang Fu and Shengmei Gao: Validation; Xiaocui Du: Project administration, Supervision; Chongye Fang: review & editing, Resources.

Disclosures

All authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was financially supported by Yunnan International Joint Laboratory of Green Health Food (China & Thailand) (grant numbers: 202203AP140011). The animal experimentation protocol adhered to the guidelines and approvals set forth by the Experimental Animal Management and Use Committee of Yunnan Agricultural University (approved # APYNAU 202411003).

References

Andries, A., Rozenski, J., Vermeersch, P., Mekahli, D., Schepdael, A.V., 2021. Recent progress in the LC–MS/MS analysis of oxidative stress biomarkers. Electrophoresis 42, 402–428.
Aoki, H., Nakatsuka-Mori, T., Ueno, Y., Nabeshima, Y., Oyama, H., 2023. Analysis of functional ingredients of tempe-like fermented Moringa oleifera seeds (Moringa tempe) prepared with Rhizopus species. J. Biosci. Bioeng. 135, 306–312.
Bondam, A.F., Silveira, D.D., Santos, J.P., Hoffmann, J.F., 2022. Phenolic compounds from coffee by-products Extraction and application in the food and pharmaceutical industries. Trends Food Sci. Tech. 123, 172–186.
Bhatt, D.L., Budoff, M.J., Mason, R.P., 2020. A Revolution in Omega-3 Fatty Acid Research. J. Am. Coll. Cardiol. 76, 2098–2101.
Chandra, P., Mk, P., Ke, V., Mukhopadhyay, C., Acharya, U.D., Rajan, M.S., Rajesh, V., 2021. Antimicrobial resistance and the post antibiotic era: better late than never effort. Expert. Opin. Drug Saf. 20, 1375–1390.
Chang, Y., Zhang, J., Jin, Y., Deng, J., Shi, M., Miao, Z., 2023. Effects of Dietary Supplementation of Chinese Yam Polysaccharide on Carcass Composition, Meat Quality, and Antioxidant Capacity in Broilers. Animals 13, 503.
Dong, W., Wang, D., Hu, R., Long, Y., Lv, L., 2020. Chemical composition, structural and functional properties of soluble dietary fiber obtained from coffee pericarp using different extraction methods. Food Res. Int. 136, 109497.
Deng, S., Liu, R., Li, C., Xu, X., Zhou, G., 2022. Meat quality and flavor compounds of soft-boiled chickens: effect of Chinese yellow-feathered chicken breed and slaughter age. Poul. Sci. 101, 102168.
Erian, I., Phillips, C.J.C., 2017. Public Understanding and Attitudes towards Meat Chicken Production and Relations to Consumption. Animals 7, 20.
Estevez-Moreno, L.X., Miranda-de, G.C., 2022. Meat consumption and consumer attitudes in Mexico: Can persistence lead to change? Meat. Sci. 193, 108943.
Feye, K.M., Baxter, M.F.A., Tellez-Isaias, G., Kogut, M.H., Rickeet, S.C., 2020. Influential factors on the composition of the conventionally raised broiler gastrointestinal microbiomes. Poul. Sci. 99, 653–659.
Guo, T., Zhang, Q., Wang, X., Xu, X., Wang, Y., Wei, L., Xu, S., 2024. Targeted and untargeted metabolomics reveals meat quality in grazing yak during different phenology periods on the Qinghai-Tibetan Plateau. Food Chem. 447, 138855.
He, P., Lei, Y., Zhang, K., Zhang, R., Bai, Y., Li, Z., Lei, Z., 2023. Dietary oregano essential oil supplementation alters meat quality, oxidative stability, and fatty acid profiles of beef cattle. Meat. Sci. 205, 109317.
He, W., Wu, G., 2022. Oxidation of amino acids, glucose, and fatty acids as metabolic fuels in enterocytes of developing pigs. Amino Acids. 54, 1025–1039.
Iriondo-DeHond, A., García, N.A., Fernandez-Gomez, B., Guisantes-Batan, E., Escobar, F. V., Blanch, G.P., Castillo, M.D., 2019. Validation of coffee by-products as novel food ingredients. Innov. Food Sci. Emerg. Technol. 51, 194–204.
Jin, S., Pang, Q., Yang, H., Diao, X., Shan, A., Feng, X., 2021. Effects of dietary resveratrol supplementation on the chemical composition, oxidative stability and meat quality of ducks (Anas platyrhynchos). Food Chem. 363, 130263.
Jiang, X., Yang, J., Zhou, Z., Yu, L., Yu, L., He, J., Fang, C., 2023. Moringa oleifera leaf improves meat quality by modulating intestinal microbes in white feather broilers. Food Chem. X. 20, 100938.
Kleyn, F.J., Ciacciariello, M., 2021. Future demands of the poultry industry: will we meet our commitments sustainably in developed and developing economies? World's Poult. Sci. J. 77, 267–278.
Martínez-Zamora, L., Peñalver, R., Ros, G., Nieto, G., 2021. Olive Tree Derivatives and Hydroxytyrosol: Their Potential Effects on Human Health and Its Use as. Functiona Ingred. Meat. Foods 10, 2611.
Machado, M., Santo, L.E., Machado, S., Lobo, J.C., Costa, A.S.G., Oliveira, M.B.P.P., Ferreira, H., Alves, R.C., 2023. Bioactive Potential and Chemical Composition of Coffee By-Products: From Pulp to Silverskin. Foods. 12, 2354.
Mao, J., Wang, Y., Duan, T., Yin, N., Dong, C., Ren, X., Qi, J., 2023. Effect of fermented dandelion on productive performance, meat quality, immune function, and intestinal microbiota of broiler chickens. BMC. Vet. Res. 19, 178.
Oliveira, G., Passos, C.P., Ferreira, P., Coimbra, M.A., Gonçalves, I., 2021. Coffee By-Products and Their Suitability for Developing Active Food Packaging Materials. Foods. 10, 683.
Pewan, S.B., Otto, J.R., Huerlimann, R., Budd, A.M., Mwangi, F.W., Edmunds, R.C., Malau-Aduli, A.E.O., 2020. Genetics of Omega-3 Long-Chain Polyunsaturated Fatty Acid Metabolism and Meat Eating Quality in Tattykeel Australian White Lambs. Genes. 11, 587.
Ramalingam, V., Song, Z., Hwang, I., 2019. The potential role of secondary metabolites in modulating the flavor and taste of the meat. Food Res. Int. 122, 174–182.
Silva, M.R., Bragagnolo, F.S., Carneiroet, R.L., Pereira, I.D.O.C., Ribeiro, J.A.A., Rodrigues, C.M., Jelley, R.E., Fedrizzi, B., Funari, C.S., 2021. Metabolite characterization of fifteen by-products of the coffee production chain: From farm to factory. Food Chem. 369, 130753.
Silveira, J.S.D., Durand, N., Lacour, S., Belleville, M.P., Perez, P.A., Loiseau, G., Dornier, M., 2019. Solid-state fermentation as a sustainable method for coffee pulp treatment and production of an extract rich in chlorogenic acids. Food Bioprod. Process. 115, 175–184.
Sorita, G.D., Leimann, F.V., Ferreira, S.R.S., 2023. Phenolic Fraction from Peanut (Arachis hypogaea L.) By-product: Innovative Extraction Techniques and New Encapsulation Trends for Its Valorization. Food Bioproc. Tech. 16, 726–748.
Sun, Y., Zhang, Y., Song, H., 2021. Variation of aroma components during frozen storage of cooked beef balls by SPME and SAFE coupled with GC-O-MS. J. Food Process. Preserv. 45, 15036.
Reichembach, L.H., Petkowic, C.L.O., 2020. Extraction and characterization of a pectin from coffee (Coffea arabica L.) pulp with gelling properties. Carbohydr. Polym. 245, 116473.
Shen, A., Edwards, A.N., Sarker, M.R., Paredes-Sabja, D., 2019. Sporulation and Germination in Clostridial Pathogens. Microbiol. Spectr. 7, 6.
Tang, X., Liu, X., Liu, H., 2021. Effects of Dietary Probiotic (Bacillus subtilis) Supplementation on Carcass Traits, Meat Quality, Amino Acid, and Fatty Acid Profile of Broiler Chickens. Front. Vet. Sci. 8, 767802.
Tan, Z., Luo, L., Wang, X., Wen, Q., Zhou, L., Wu, K., 2019. Characterization of the cecal microbiome composition of Wenchang chickens before and after fattening. PLoS. One 14, 0225692.
Yao, L., Davidson, E.A., Shaikh, M.W., Forsyth, C.B., Prenni, J.E., Broeckling, C.D., 2022. Quantitative analysis of short-chain fatty acids in human plasma and serum by GC-MS. Anal. Bioanal. Chem. 414, 4391–4399.
Yu, Y., Wang, G., Yin, X., Ge, C., Liao, G., 2021. Effects of different cooking methods on free fatty acid profile, water-soluble compounds and flavor compounds in Chinese Piao chicken meat. Food Res. Int. 149, 110696.
Yang, Y., Zhang, X., Wang, Y., Pan, D., Sun, Y., Cao, J., 2017. Study on the volatile compounds generated from lipid oxidation of Chinese bacon (unsmoked) during processing. Eur. J. Lipid Sci. Technol. 119, 1600512.
Yao, H., Jin, Y., Zhang, X., Yang, N., Xu, X., 2023. Influence of pulsed electric field on thawing of frozen pork Physical properties, fat oxidation and protein structure. Food Biosci. 56, 103175.
Zhang, Z., Shan, J., Shi, B., Dong, B., Wu, Q., Zhang, Z., 2023. SeNPs alleviates BDE-209-induced intestinal damage by affecting necroptosis, inflammation, intestinal barrier and intestinal flora in layer chickens. Ecotox. Environ. Saf. 262, 115336.
Zhao, W., Tian, Y., Wang, Y., Du, J., Chen, L., Gu, T., Sun, C., 2024. Dietary effect of Dendrobium officinale leaves on chicken meat quality, fatty acid composition, and volatile compounds profile. Food Chem. X. 22, 101330.
Zhang, J., Kaneko, G., Sun, J., Wang, G., Xie, J., Tian, J., Yu, E., 2021. Key Factors Affecting the Flesh Flavor Quality and the Nutritional Value of Grass Carp in Four Culture Modes. Foods 10, 2075.