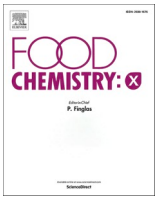
Food Chemistry: X 20 (2023) 100938

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



Moringa oleifera leaf improves meat quality by modulating intestinal microbes in white feather broilers

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ARTICLE INFO

Keywords:
Moringa oleifera
White feather broiler
Meat quality
Intestinal microbe

ABSTRACT

Moringa oleifera addition to animal diets can improve the growth performance, intestinal health, and immunity of animals, without adverse effects. We investigated the effects of *Moringa oleifera* on the growth performance, meat quality, and intestinal health of broilers. *Moringa oleifera* and fermented *Moringa oleifera* could improve the flesh color and breast muscle tenderness of broilers ($p < 0.05$). The contents of essential amino acids, unsaturated fatty acids, ΣMUFA, P/S and n-3 ratio in breast muscle of broilers were dose-increased, and the effect of fermented *Moringa oleifera* was better. *Moringa oleifera* and fermented *Moringa oleifera* regulated chicken flavor metabolism by increasing the relative abundance and Short-chain fatty acid (SCFA) contents of Bacteroides, Spirillum, and lactic acid bacteria. Overall, supplementation with 1 % fermented *Moringa oleifera* can significantly increase essential amino acid and unsaturated fatty acid contents in broilers and participate in the synthesis and transformation of amino acids and fatty acids regulated by beneficial bacteria.

1. Introduction

In recent years, the Chinese livestock and poultry industry has rapidly developed, and the dietary structure and animal food demand have risen remarkably. Every year, food, and forage grass, which are consumed by livestock and poultry products, are constantly improving. However, China will face the pressure of a shortage of raw materials due to a large population and low land availability. Moreover, this has become one of the factors restricting the development of China's feed and breeding industry (Gurbuz & Irem, 2022). It is reported that the direct or indirect consumption of grain resources used for livestock and poultry breeding in China is about 30 % each year, which is highly dependent on imported raw materials and will face raw material shortages in case of trade problems (Heath, Ravikumar, Hansen & Kupets, 2022). Due to restrictions in grain-based formula mode and weak technology, there are not many natural feed resources that are reasonably developed and utilized, and it is urgent to develop new feed materials.

Moringa oleifolia is a tropical deciduous tree belonging to Moringa family. It is rich in protein, crude fiber, vitamins, minerals, unsaturated fatty acids, etc (Zunica et al., 2021). Its roots, stems, leaves, flowers, fruits, seeds, etc. have practical value and rich nutrition, and are widely used in agriculture, industry, medicine, and another field (Abdel-Daim, El-Tawil, Bungau & Atanasov, 2019). Moringa is native to the Himalayas in northern India and has been introduced and promoted in at least 30 countries. China introduced Indian traditional moringa seeds from

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https://doi.org/10.1016/j.fochx.2023.100938
Received 13 June 2023; Received in revised form 2 October 2023; Accepted 8 October 2023
Available online 11 October 2023

Myanmar in 2002, and tested them in arid and semi-arid areas, achieving good results, and has been extended to other provinces, such as Guangxi, Sichuan, Hainan, Yunnan, and other places have planting bases. Moringa oleifera is rich in amino acids, including aspartic acid, glutamic acid, threonine, lysine, valine, glycine, isoleucine, phenylalanine, methionine, alanine, etc. The feeding value of moringa leaf is higher than that of alfalfa hay, and the feeding value of moringa stalk is equivalent to that of Leymus chinensis (Sun et al., 2017). Moringa oleifera contains flavonoids, polysaccharides, phenolic acids, and other active substances, with antioxidant, anti-inflammatory, antiviral, antibacterial and other effects. Moringa moringa, as an unconventional feed, has less anti-nutritional factors (tannins and saponins) and will not cause toxic reactions in livestock and poultry (Bakwo, Nyobe, Ngui, Minka & Mune, 2022). Moringa oleifolia is widely used in livestock and poultry feed, which can improve the growth performance, reproductive performance, antioxidant function and immune function of livestock and poultry, regulate intestinal health, improve the quality of livestock products, and increase the economic benefits of breeding (Kashyap et al., 2022).

Studies have reported the effects of moraxilla on growth performance and slaughter performance in broiler production and demonstrated that it can affect the growth and development of broiler (Khan et al., 2017). Adding 1 % and 2 % fermented moringa significantly increased the weight of liver, pancreas, spleen, bursae, and thymus of broilers (Chen, Chang, Zheng, Cai & Liu, 2020). Adding 1 % moringa leaf powder to the diet improved performance parameters and immune response while reducing total live intestinal bacteria and coliform populations in broilers (Abu, Ibrahim, Eid & Hassan, 2020). These studies suggest that moringa and processed moringa products can be developed to maintain or improve poultry productivity and safety. The main purpose of this study was to explore the effects of moringa oleifera and fermented moringa oleifera on meat quality of broilers based on the determination of amino acids and fatty acids in breast muscle and the determination of growth indexes, and to find the best supplemental amount of fermented moringa oleifera. In addition, the correlation between moringa oleifera and fermented moringa oleifera on meat quality of broilers was evaluated through comprehensive 16sRNA sequencing metabolomics analysis, which provides clues for further understanding the potential mechanism of dietary moringa oleifera products on meat quality of broilers.

2. Materials and methods

2.1. Preparation of experimental materials

Moringa group is the sample of *moringa leaf* extraction (1:10), supernatant, vacuum concentration, and freeze-drying.Fermented *moringa*:After cleaning the empty tank, sterilization was performed (≥100 °C, 15 min), *moringa oleifera leaves* (*Moringa leaf* from Yunnan Tianyou Technology Development Co., Ltd)were mixed with pure water (1:10), sterilized (≥100 °C, 15 min), cooled (≤5 °C), inoculated (0.002 %), fermented (38-42℃), filtered (200 mesh gauze filtration, plate and frame filtration), and the supernatural liquid was vacuum concentrated. Freeze drying means liquid fermentation of moringa oleifolia.

2.2. Experimental design and feeding management

A total of 200 one-day-old male white feathered broilers(Shandong Dacheng Collective Hunan Shuncheng Industrial Co., Ltd.) with similar body weigh were randomly divided into five treatment groups: blank control group (CK: Soybean meal), MOF-L (Soybean meal + 0.5 % moringa leaves), MOF-H (Soybean meal + 1 % moringa leaves), MOR-L (Soybean meal + 0.5 % fermented moringa leaves) and MOR-H (Soybean meal + 1 % fermented moringa leaves). Broilers were placed in a stainless-steel cage, with natural ventilation and free feeding. The relative indoor humidity was kept at 50 %, and the temperature was kept at 34 °C (1–7 days), which was gradually reduced to 23 °C at a rate of 3 °C per week until the end of the experiment. During the experiment, feed intake, body weight, average daily feed intake (ADFI), average daily gain (ADG), and feed-to-gain ratio (FCR) were recorded weekly for each cage on a weekly basis. The experiment lasted 42 days. The formulated basal diets met the nutritional requirements of poultry (NRC, 1994, Washington DC, USA), and were applied in the initial period (0–21 d) and fattening period (22–42 d). The procedures for care and use of animals were approved by the Animal Care Committee of the College of Animal Science and Technology, Yunnan Agricultural University (Ethics Review Number: 202209012) . The composition and nutrient levels of base diets are shown in Table 1.

2.3. Sample collection

Samples were collected on day 42. After fasting for 12 h, the broiler was weighed, hung on the hook, hung on the conveyor belt, and after automatic anesthesia (voltage: 30–50 V) shock, the neck behind the lower forehead was cut with a knife, and all the trachea, blood vessels and esophagus of the neck were cut off. The bleeding time was (3–5 min), and the sampling operation was performed (Averos, Balderas, Cameno & Estevez, 2020). The pH value, color difference, shear force and other physical indexes of left pectoral muscle were measured. Fatty acid and amino acid composition were measured in the pectoralis major muscle of the right pectoral muscle. The cecal contents were collected and placed in a cryogenic vial, quick-frozen with liquid nitrogen, and stored in a −80 °C refrigerator for subsequent detection.

2.4. Measurement of growth performance

At 42 days of age, all groups were fasted for 12 h, and each replicate group was weighed and settled. Average body weight (BW), average daily gain (ADG), average eye intake (ADFI), and feed/gain ratio (F/G)

Table 1
Composition and nutrient level of the basic diet (air-dry).

Raw material composition /%	1–21 days of age	22–42 days of age
Corn	58.50	60.10
Soybean meal	1.60	1.35
Soybean oil	3.04	4.50
Soyabean meal	28.00	30.00
Fermented soybean meal	5.00	–
Salt	0.22	0.26
Threonine	0.10	0.09
Lysine	–	0.10
Methionine	0.14	0.15
Calcium monohydrogen phosphate	1.40	1.45
Raw material composition /%	1–21 days of age	22–42 days of age
Premix feed[1]	2.00	2.00
Total	100.00	100.00
Nutrient level /%[2]		
Metabolizable energy /MJ·kg [1]	15.93	16.21
Crude protein	21.04	18.97
Crude fat	5.60	7.06
Total phosphorus	0.65	0.60
Available phosphorus	0.47	0.40
Calcium	1.00	0.90
Methionine	0.43	0.40
Methionine + cystine	0.76	0.72
Lysine	1.08	1.01
Threonine	0.83	0.77

Note: [1]premix provides: vitamin A $_9$ 500 IU, vitamin D_3 500 IU, vitamin E 20 IU, vitamin K 1.2 mg, vitamin B_1 2.2 mg, vitamin B_2 5.0 mg, vitamin B_6 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. Premix does not contain any antibiotics and chemical synthetic antibacterial drugs, are measured value.

were calculated as follows:

Average daily feed intake (ADFI)(g) = total feed amount/test days.

Average daily gain (ADG)(g)= (final weight-initial weight)/test days.

Feed/gain ratio F/G = total material consumption/total weight gain.

2.5. Determination of meat quality

A sample of the left pectoral muscle was taken for the determination of flesh color, pH value, drip loss, cooking loss, shear force, and fat and protein indexes (Li et al., 2016; Siekmann et al., 2018).

2.5.1. pH value measurement

The pH values of the pectoral muscles were measured (45 min and 24 h after slaughter) using a carcass pH-star (MATTHAUS, Germany). Each meat sample was measured three times.

2.5.2. Determination of flesh color

The brightness value (L*), redness value (a*), and yellowness value (b*) of the pectoral muscle (at 45 min and 24 h after slaughter) were measured using a chromometer (CR-410, Minolta, Japan). Each meat sample was measured three times.

2.5.3. Measurement of drip loss

Meat samples of the same size were taken from the muscles of each breast muscle and weighed (W_1). One end of a wire was hooked to the meat sample, and the other end was hooked to the bottom of the plastic cup, so that the sample was suspended in the cup. After 24 h of suspension (4 °C), the surface of the meat was wiped clean with paper and weighed (W_2), and the drip loss of the breast muscle was calculated according to the following formula:

$$Drip\ loss(\%) = (W_1 - W_2)/W_2 \times 100\%$$

2.5.4. Determination of cooking loss

Meat samples of the same size were taken from the muscles of each breast muscle and weighed (W_3), wrapped in tin foil, boiled in a water bath for 15 min, removed, and cooled to room temperature. Remove moisture from the surface of the meat with absorbent paper and weigh (W_4), and the cooking loss of the broiler breast muscle was calculated according to the following formula:

$$Cooking\ loss(\%) = (W_3 - W)_4/W_3 \times 100\%$$

2.5.5. Shear force measurement

After the cooking loss was determined, the pectoralis muscle was cut into three meat samples of the same size and thickness, which were placed perpendicular to the muscle fiber axis on a C-LM3 digital muscle tenderness instrument for cutting, and the values were recorded.

2.5.6. Determination of chemical composition

(1) Crude protein was determined by referring to the Kjelhardt nitrogen determination method in GB5009.5-2016 “Determination of Protein in Food under National Standards for Food Safety”.

(2) Crude fat was determined by referring to GB5009.6-2016 “Determination of Fat in Food under National Food Safety Standards” using the Soxhlet extraction method (Montowska, Kowalczewski, Rybicka & Fornal, 2019).

2.6. Determination of amino acids and fatty acids

Fatty acids were determined using a Thermo Trace 1300 (Thermo Fisher Scientific, USA) gas phase system and a Thermo TG-FAME capillary column (50 m × 0.25 mm ID*0.20 μm) under the following chromatographic conditions: the injection volume was 1 μL, the shunt ratio was 8:1, the inlet temperature was 250 °C, the ion source temperature was 300 °C, and the transmission line temperature was 280 °C. The initial temperature of programmed heating was 80 °C, which was held for 1 min. The temperature was then increased to 160 °C at 20 °C/min and maintained for 1.5 min. The temperature was then increased to 196 °C at 3 °C/min and maintained for 8.5 min. Finally, the temperature was increased to 250 °C at 20 °C/min and maintained for 3 min. The carrier gas was helium, and the carrier gas flow rate was 0.63 mL/min.

Mass spectroscopy was performed with a Thermo TSQ 9000 mass spectrometer (Thermo Fisher Scientific, USA) and an electron bombardment ionization (EI) source in SIM Scanning mode under an electron energy of 70 eV (Kim et al., 2013). The method was used to determine the content of fatty acids in breast muscle and short chain fatty acids in cecum of white feather broilers.

2.7. DNA isolation and 16S rRNA gene sequencing

The microbial composition in the cecum of white feather broilers was determined in this experiment. The FastDNA®SpinKitforSoil kit was used to extract total DNA, and the DNA purity and concentration were detected with a NanoDrop2000. DNA integrity was determined by 0.8 % agarose gel electrophoresis with a voltage of 5 V/cm and a time of 20 min. The sample genomic DNA was used as the template for PCR amplification. The V3–V4 region of 16S rRNA was used for amplification, using the upstream primer 338F (5′- ACTCCTACGGGAGGCAGCAG-3′) and the downstream primer 806R (5 ’-GGACTACHVGGGTWTC-TAAT-3′). NEB Q5 DNA high-fidelity polymerase was used for PCR, and the system consisted of Q5 high-fidelity DNA polymerase and ThermoTG-FAME capillary column. 5*Reaction Buff, 5ul; 5* High GC Buffer, 5ul; dNTP (10 mM), 2ul; Template DNA,2ul; Forward primer (10uM),1ul; Reverse primer (10uM); 1ul; Water,8.75ul. The PCR reaction was carried out by fully denaturing the sample at 98 °C for 30 s on the PCR instrument, and then the amplification cycle was entered (Videnska et al., 2019). In each cycle, the template was denatured at 98 °C for 15 s, and then the temperature was lowered to °C for 30 s to fully anneal the primer and template. The sample was held at 72 °C for 30 s for extension and the completion of a cycle. This cycle was repeated 25 to 27 times, allowing large amounts of amplified DNA to accumulate. The product was kept at 72 °C for 5 min, to complete the extension, and the final product was stored at 4 °C. Gel electrophoresis (2 % agarose) was performed using the results of the amplification, and the target fragment was cut and recovered with an Axygen gel recovery kit. Purification was carried out with a Quant-iT PicoGreen dsDNA Assay Kit, and quantification was performed on a Microplate reader (BioTek, FLx800). Library construction was performed using a TruSeq Nano DNA LT Library Prep Kit. Double-ended sequencing was performed on a MiSeq machine with the MiSeq Reagent Kit V3 (600 cycles) at 2 × 250 bp. Microbiome bioinformatics data was analyzed using QIIME2 version 2019.4, and according to the tutorial (https://docs.qiime2.org/2019.4/tutorials/) to modify and improve the process analysis of microbial groups (Wang, Nesengani, Gong, Yang & Lu, 2018).

2.8. Data processing and analysis

Illumina TruSeq Nano DNA LT Library Prep Kit sequencing platform was used to sequence 16S rDNA data, and univariate analysis of variance was performed in combination with GraphPad Prism 9 and SPSS23.0 analysis software. $P > 0.05$ meant no statistical significance, and $P < 0.05$ meant significant difference.

3. Results

3.1. Growth performance

There were differences in the growth performance of broilers supplemented with moringa at different doses. As shown in Table 2, there

Table 2
Effects of Moringa oleifera at different doses on growth performance of white-feathered broilers.

Items[1]	Group[2]					SEM[3]	P-value
	CK	MOF-L	MOF -H	MOR-L	MOR-H		
Initial BW, g	82.79	85.21	78.63	83.95	84.39	1.45	0.67
Final BW, g	1386.41	1193.39	1095.08	1332.15	1314.51	43.12	0.198
ADFI, g	853.45	705.75	626.45	772.91	733.57	37.45	–
ADG, g	36.21	30.78	28.28	34.67	34.17	1.18	0.201
F:G ratio	23.78	23.32	24.77	22.67	21.65	1.00	0.911

[a–b]Means within a row with different superscripts are different 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 (SBM); MOF-L = SBM + 0.5 % moringa leaves, MOF-H = SBM + 1 % moringa leaves; MOR-L = SBM + 0.5 % fermented moringa leaves and MOR-H = SBM + 1 % fermented moringa leaves.
[3] SEM = standard error of the mean (n = 5).

was no significant difference between initial and final body weight (P > 0.05). In terms of ADFI, the MOF-H group had the lowest value, but there was no significant difference among all groups (P > 0.05). In terms of ADG, the MOF-H group had the lowest average daily gain, but there was no significant difference among all groups (P > 0.05). In terms of F/G, the MOR-H group had the lowest value, and the MOF-H group had the highest value, but there was no significant difference among all groups (P > 0.05). The results showed that a high dose of *Moringa oleifera* could affect the growth performance of white-feathered broilers.

3.2. Meat quality

3.2.1. Quality of breast muscle

As can be seen from Table 3, compared with the control group, the cooking loss of breast muscle of broilers treated with *Moringa oleifera* decreased, indicating that *Moringa oleifera* had a better hydraulic effect on breast muscle of broilers (P > 0.05). In terms of flesh color and pH 24 h control group had the highest values (P < 0.05), and a* 45 min, a*24 h and b*24 h had an increasing trend (P > 0.05). Compared with the control group, *Moringa oleifera* increased protein content in the breast muscle of white feathered broilers, but there was no significant difference (P > 0.05). The *Moringa oleifera* diet had different effects on hydraulic power, pH, flesh color, and protein, but did not affect the drip loss and fat content of broilers.

3.2.2. Amino acid profile of breast muscle

Table 4 shows that 22 kinds of amino acids were measured in this experiment, including 8 kinds of essential amino acids and 10 kinds of umami amino acids. Compared with the control group, moringa can increase the content of essential amino acids (alanine, isoleucine, leucine, methionine, tryptophan, etc.) (p < 0.05), and the fermentation effect of moringa is better. There were significant differences between leucine and isoleucine among branched-chain amino acids (p < 0.05). In terms of functional amino acids, isoleucine, leucine, tryptophan, and methionine were significantly increased (p < 0.05), and the effect was more effective at low doses.

3.2.3. Meat fatty acid composition

A total of 47 fatty acids were detected in the breast muscle of white feathered broilers, and the contents of 17 fatty acids were changed compared with the control group (P < 0.05). C16:0, C18:0, C18:1n-9c, and C18:2n-6 fatty acids were the main fatty acids in broiler treatment groups, and the contents of 4 fatty acids accounted for 79.18 %, 79.32 %, 73.95 %, 67.17 % and 69.47 % of the total fatty acids, respectively. Compared with the control group, the proportions of saturated fatty acids (C15:0, C16:0, C18:0, C20:0, and C22:0) in breast muscle of broilers were decreased (P < 0.05), and the proportions of unsaturated fat (C15:1, C16:1, C18:1n7, C20:1t, C18:2n6, C18:3n3) were increased in a dose-dependent manner. σSFA decreased, while the proportion of ΣMUFA, P/S and n-3 increased significantly (P < 0.05). The results showed that fermented *Moringa oleifera* was more beneficial to improve the proportion of fatty acids in the breast muscle of white feather broilers (P > 0.05) (Table 5).

3.3. Cecal microbial diversity

3.3.1. Species composition analysis of cecum

To investigate the effects of different doses of *Moringa oleifera* on

Table 3
Effects of Moringa oleifera at different doses on meat quality of white feathered broilers.

Items[1]	Group[2]					SEM[3]	P-value
	CK	MOF-L	MOF -H	MOR-L	MOR-H		
Drip loss, %	29.09	28.56	30.41	30.78	30.09	2.69	0.995
Cooking loss, %	24.05	23.44	21.33	23.69	22.93	1.46	0.795
Shear force, N	2.95	3.49	3.66	3.12	2.93	0.32	0.482
$pH_{45\ min}$	6.21^{b}	6.59^{a}	6.49^{ab}	6.40^{ab}	6.44^{ab}	0.11	0.110
$pH_{24\ h}$	6.70^{a}	6.41^{b}	6.45^{b}	6.44^{b}	6.37^{b}	0.02	<0.01
$L^*_{45\ min}$	38.19	39.20	35.78	37.05	38.87	1.40	0.535
$a^*_{45\ min}$	4.55	6.14	5.29	5.45	4.57	0.72	0.098
$b^*_{45\ min}$	10.02^{ab}	11.55^{a}	10.50^{ab}	8.71^{b}	8.42^{b}	0.79	0.526
L^*_{24h}	34.97	36.06	35.32	37.35	38.77	1.34	0.325
a^*_{24h}	7.64	11.16	11.39	10.85	10.24	1.38	0.419
b^*_{24h}	10.54	13.00	12.33	12.50	12.30	0.77	0.247
Protein g/100 g	21.50	23.12	22.53	22.88	22.18	0.56	0.324
Fat g/100 g	1.98	1.88	2.03	2.04	1.80	0.19	0.910

[a–b]Means within a row with different superscripts are different at $P < 0.05$.
[1] L*: luminance; a*: redness; b*: yellowness.
[2] CK = base diet (SBM); MOF-L = SBM + 0.5 % moringa leaves, MOF-H = SBM + 1 % moringa leaves; MOR-L = SBM + 0.5 % fermented moringa leaves and MOR-H = SBM + 1 % fermented moringa leaves.
[3] SEM = standard error of the mean (n = 5).

Table 4
Effects of Moringa oleifera at different doses on amino acids in breast muscle of white-feathered broilers (%).

Items[1]	Group[2]					SEM[3]	P-value
	CK	MOF-L	MOF-H	MOR-L	MOF-H		
Glycine	7.21^{a}	5.84^{c}	6.49^{b}	6.30$^{b, c}$	6.00$^{b, c}$	0.17	<0.001
Alanine	8.52	8.56	8.83	9.05	9.09	0.16	0.077
Serine	7.84^{a}	7.33$^{a, b}$	8.67$^{b, c}$	8.26^{c}	7.80^{a}	0.08	<0.001
Proline	2.15^{a}	2.05$^{a, b}$	1.87$^{b, c}$	1.79^{c}	2.21	0.05	0.002
Valine	3.50$^{b, c}$	3.87^{a}	3.48$^{b, c}$	3.67^{b}	3.43^{c}	0.06	<0.001
Threonine	5.12^{a}	4.21^{b}	4.84^{a}	5.01^{a}	4.55$^{a, b}$	0.17	0.013
Isoleucine	2.88^{d}	3.32^{a}	2.96$^{c, d}$	3.22$^{a, b}$	3.10$^{b, c}$	0.06	<0.001
Leucine	5.32^{c}	5.97$^{a, b}$	5.77$^{a, b}$	6.15^{a}	5.63$^{b, c}$	0.14	0.007
Asparagine	3.20^{b}	3.45^{a}	3.13^{b}	3.41^{a}	3.51^{a}	0.06	0.001
Asparagin	3.54	3.29	3.32	3.22	3.18	0.07	0.050
Glutamine	6.84^{a}	6.16^{c}	6.88^{a}	6.57$^{a, b}$	6.23$^{b, c}$	0.11	0.001
Lysine	6.25^{a}	5.50^{c}	6.35^{a}	5.71^{c}	5.97^{b}	0.08	<0.001
Glutamic	7.86^{a}	6.84^{b}	7.77^{a}	7.82^{a}	7.69^{a}	0.11	<0.001
Methionine	2.26	2.42	2.37	2.51	2.27	0.07	0.122
Histidine	15.90$^{b, c}$	19.18^{a}	15.63$^{b, c}$	14.74^{c}	17.31^{b}	0.52	<0.001
Phenylalanine	2.80^{c}	2.99$^{a, b}$	2.88$^{b, c}$	3.09^{a}	2.77^{c}	0.06	0.005
Arginine	2.50$^{b, c}$	2.75$^{a, b}$	2.81^{a}	2.93^{a}	2.37^{c}	0.09	0.003
Tyrosine	4.90^{c}	4.87^{c}	4.74^{c}	5.25^{b}	5.52^{a}	0.06	<0.001
Tryptopha	0.89^{b}	0.99^{a}	0.90^{b}	0.96^{a}	0.98^{a}	0.01	<0.001
EAA	28.79	29.94	29.46	30.55	29.67	0.41	0.093
FAA	39.54$^{a, b}$	38.39$^{b, c}$	39.57$^{a, b}$	40.29^{a}	37.88^{c}	0.37	0.002
TAA	2070.16^{a,b}	1747.90^{c}	2076.28^{a,b}	2326.49^{a}	1839.87^{b,c}	85.72	0.005

EAA = Sum of (Phe, Met, Lys, Thr, Trp, Leu, Ile, Val).
FAA = Sum of (Arg, Gln, Leu, Ile, Val, Glu, Trp, Met, Hcy, Gly).
$^{a-c}$Means within a row with different superscripts are different at $P < 0.05$.
[1] EAA: essential amino acids; FAA: flavor amino acids; TAA: total amino acids.
[2] CK = base diet (SBM); MOF-L = SBM + 0.5 % moringa leaves, MOF-H = SBM + 1 % moringa leaves; MOR-L = SBM + 0.5 % fermented moringa leaves and MOR-H = SBM + 1 % fermented moringa leaves.
[3] SEM = standard error of the mean (n = 5).

intestinal microbial health, the cecal microbial flora of broilers was analyzed by 16S rRNA sequencing. A total of 20,733 OTUs (operational taxonomic units) were generated in all samples. The figure shows a total of 792 OTUs in five groups, The number of unique OTUs in CK, MOF-L, MOF-H, MOR-L, and MOR-H groups were 3011 (14.53 %), 3060 (14.76 %), 2723 (13.14 %), 3149 (15.19 %) and 3980 (19.2 %), respectively. Increased species richness (Fig. 1A). Alpha diversity, which includes the Chao, Shannon, Simpson, and Observed species indices, refers to the diversity within a particular region or ecosystem, compared to the control group, The indexes of Chao, Shannon, Simpson and Observed species of *Moringa oleifera* were down-regulated ($P < 0.05$), while those of fermented *Moringa oleifera* were up-regulated ($P < 0.05$). The dose relationship was shown (Fig. 1D–1G). The species accumulation curve showed that the samples were reasonable, and the sample size was sufficient to reflect the species richness (Fig. 1B). A wide and smooth abundance grade curve indicated a high abundance and uniform distribution of cecal microbial species (Fig. 1C). β-Diversity was used to analyze the similarity of cecal microflora among different groups. The CK group was completely separated from MOF-L, MOF-H, MOR-L, and MOR-H groups, indicating that *Moringa oleifera* changed the bacterial community structure in the diet of white feather broiler. Crossover was observed between the MOR-L and MOR-H groups, indicating that their cecal microbiota structure was similar (Fig. 1H–1I).

3.3.2. Microbial community structure analysis

At the phylum level, Firmicutes and Bacteroidetes were the main phyla in the five groups. The relative abundance values of firmicutes of CK, MOF-L, MOF-H, MOR-L, and MOR-H were 76.39 %, 69.84 %, 61.36 %, 74.04 %, and 76.45 %, respectively. The relative abundance values of Bacteroides were 18.85 %, 18.52 %, 20.70 %, 19.10 %, and 14.96 % (Fig. 2A), respectively. Compared with the CK group, the relative abundance of actinomycetes and B/F in the MOF-H group was increased, and the relative abundance of Verrucomicrobacteria in the MOR-L group was increased ($p < 0.05$) (Fig. 2C–2H). On the genus level, the dominant genera of the five groups are Bacteroides, Oscillospira, Faecalibacterium, [Ruminococcus], and Lactobacillus, and the relative abundance of water products in each treatment group is significantly different (Fig. 2B). Compared with the control group, MOF-L increased the relative abundance of Coprobacillus and Koalobacterium, MOF-H increased the relative abundance of Bacteroides and Spirillum, and Spirillum and Lactobacillus showed dose-dependent increases in the MOR-L and MOR-H groups (Fig. 2I–2N) ($P < 0.05$).

3.3.3. Species difference analysis of different doses of Moringa oleifera on cecum microbial community

To find out which bacteria were responsible for statistical differences in microbial communities between groups, LEfSe analysis was used to identify biomarkers. A total of 17 orders and 27 families were identified in all the samples, and 25 characteristic bacteria genera were identified by LDA scores. Most of the specific taxonomic groups were from moringa, indicating that the treatment of fermented moringa had a strong effect on the intestinal microflora (Fig. 3A). The species of Firmicutes (Firmicutes) and Clostridia (Clostridia) in the CK group were significantly different (LDA > 4). The species with a significant difference in the MOF-L group were Ruminococcaceae and Faecalibacterium (LDA > 4). Coriobacteriia (Actinobacteria) and Bacteroides (Bacteroides) were significantly different in the MOF-H group (LDA > 4). Species with significant differences in the MOR-L group were Lachnospiraceae (Trichococcus) and Butyricimonas (*Clostridium butyricum*) (LDA > 4). The species with a significant difference in the MOR-H group were Lactobacillaceae and Bacilli (LDA > 4) (Fig. 3B). Our results showed that the characteristic flora increased with the increase of moringa dosage, especially the content of beneficial bacteria.

3.4. Functional prediction analysis of cecum microflora

The predictive KEGG pathway from metagenomic sequences was analyzed by PICRUSt. The metagenome of white feathered broilers shows host gene enrichment and regulation involved in metabolism, genetic information processing, environmental information processing,

Table 5
Effects of Moringa oleifera at different doses on fatty acids in breast muscle of white feathered broilers (%).

Items[1]	Group[2]					SEM[3]	P-value
	CK	MOF-L	MOF -H	MOR-L	MOF -H		
C14:0	0.47^{b}	0.56$^{a, b}$	0.45^{b}	0.57^{a}	0.46^{b}	0.02	0.043
C15:0	0.33^{a}	0.28^{b}	0.33^{a}	0.29$^{a, b}$	0.31$^{a, b}$	0.01	0.044
C16:0	30.25^{a}	27.78^{b}	29.44^{a}	29.30^{a}	29.76^{a}	0.45	0.025
C18:0	19.59^{a}	15.90^{b}	19.83^{a}	15.26^{b}	17.61$^{a, b}$	0.82	0.029
C20:0	0.16^{a}	0.14^{b}	0.16^{a}	0.15$^{a, b}$	0.15$^{a, b}$	0.00	0.056
C22:0	0.03^{a}	0.02^{b}	0.01^{c}	0.00^{c}	0.00^{c}	0.00	<0.01
C24:0	0.01^{a}	0.01$^{a, b}$	0.01^{b}	0.00^{c}	0.00^{c}	0.00	<0.01
C15:1	0.54$^{a, b}$	0.47^{b}	0.66^{a}	0.59$^{a, b}$	0.68^{a}	0.04	0.025
C16:1	2.51$^{b, c}$	3.65$^{a, b}$	2.26^{c}	3.93^{a}	2.92$^{a, b, c}$	0.28	0.046
C18:1n9c	18.69^{a}	17.27 ^{a}	14.25^{b}	10.66^{c}	11.76$^{b, c}$	0.01	0.014
C18:1n7	2.56^{c}	10.96$^{a, b}$	6.37$^{b, c}$	15.66^{a}	12.31^{a}	1.44	<0.01
C20:1T	0.16^{b}	0.12^{c}	0.19$^{a, b}$	0.18$^{a, b}$	0.21^{a}	0.01	<0.01
C22:1n9	0.21^{a}	0.14^{c}	0.20 $^{a, b}$	0.17$^{b, c}$	0.20$^{a, b}$	0.01	0.001
C24:1	0.31$^{a, b}$	0.22^{c}	0.33 ^{a}	0.26$^{b, c}$	0.28$^{a, b, c}$	0.02	0.013
C18:2n6	10.64$^{a, b}$	12.25^{a}	10.42^{b}	11.96$^{a, b}$	10.34^{b}	0.37	0.046
C18:3n6	0.11$^{a, b, c}$	0.13^{a}	0.10^{c}	0.13 $^{a, b}$	0.10$^{b, c}$	0.01	0.021
C18:3n3	0.28^{b}	0.42^{a}	0.27^{b}	0.45^{a}	0.30^{b}	0.03	0.005
C20:2	0.42^{a}	0.34^{b}	0.43^{a}	0.33^{b}	0.34^{b}	0.01	<0.01
C20:3n6	1.00^{a}	0.69^{c}	0.86^{b}	0.69^{c}	0.87^{b}	0.03	<0.01
C20:3n3	0.05^{a}	0.04^{b}	0.06^{a}	0.04^{b}	0.05^{b}	0.00	<0.01
C20:4n6	4.40^{b}	3.50^{c}	5.44^{a}	3.46^{c}	4.45^{b}	0.18	<0.01
C20:5n3	0.27^{a}	0.20^{b}	0.28^{a}	0.19^{b}	0.28^{a}	0.01	<0.01
C22:5n3	0.68^{b}	0.55^{c}	0.93^{a}	0.55^{c}	0.74^{b}	0.03	<0.01
C22:6n3	0.44^{b,c}	0.34^{d}	0.60^{a}	0.39^{c,d}	0.49^{b}	0.02	<0.01
Total Fas	3068.31^{c}	4233.06^{a,b}	2848.89^{c}	4394.11^{a}	3406.83^{b,c}	220.56	0.003
ΣSFA	51.61^{a}	45.31^{a}	50.98^{a}	46.23^{b}	48.97^{a,b}	1.17	0.02
ΣMUFA	28.74^{b}	35.15^{a}	27.98^{b}	34.48^{a}	31.73^{a,b}	1.08	0.001
ΣPUFA	19.65^{b}	19.54^{b}	21.05^{a}	19.29^{b}	19.30^{b}	0.28	0.007
P/S	0.38	0.43	0.41	0.43	0.39	0.02	0.318
n-3	1.72^{b,c}	1.56^{d}	2.13^{a}	1.62^{c,d}	1.85^{b}	0.04	<0.01
n-6	16.48	16.83	17.22	16.52	16.10	0.29	0.274
n-6/n-3	9.58$^{b, c}$	10.81^{a}	8.11^{d}	10.31$^{a, b}$	8.70$^{c, d}$	0.31	<0.01

$^{a-c}$Means within a row with different superscripts are different at $P < 0.05$.
[1] SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.
[2] CK = base diet (SBM); MOF-L = SBM + 0.5 % moringa leaves, MOF-H = SBM + 1 % moringa leaves; MOR-L = SBM + 0.5 % fermented moringa leaves and MOR-H = SBM + 1 % fermented moringa leaves.
[3] SEM = standard error of the mean (n = 5).

cellular processes, human diseases, and biological system pathways. Metabolism mainly involved amino acid metabolism, carbohydrate metabolism, energy metabolism, glycan biosynthesis and nucleotide metabolism, lipid metabolism, cofactor, and vitamin metabolism, terpenoid compounds and polyketoic acid metabolism and other pathways (Fig. 4A). In addition, compared with the control group, toF-L involved three pathways including Retinol_metabolism, Calcium_signaling_pathway and Atrazine_degradation. Moof -H involves the up-regulation of Calcium_signaling_pathway and Atrazine_degradation of 3 pathways and the downregulation of 12 pathways. MOR-L involves the up-regulation of Atrazine_degradation and Flavonoid_biosynthesis pathways and the downregulation of the Bisphenol_degradation pathway. MOR-H is involved in the up-regulation of the pathways of Toxoplasmosis, Atrazine_degradation, and Endocytosis (Fig. 4B–4E). The results showed that Moringa mainly controlled metabolism through atrazine degradation. It was more beneficial for 0.5 % unfermented moringa and 1 % fermented moringa to control the cecal microbe composition of white fowl.

3.5. Short-chain fatty acid (SCFA) analysis

To investigate the influence factors of *Moringa oleifera* at different doses on cecal microbe composition of broilers, acetic acid, propionic acid, butyric acid, valeric acid, isobutyric acid, and isovaleric acid were detected. Compared with the control group, the content of SCFAs in the cecum of white-plumed broilers in the moringa treatment group was significantly increased ($P < 0.05$), and fermented moringa presented a dose-dependent increase ($P < 0.05$) (Fig. 5A–5F). The heat map of SCFAs and intestinal microbes in Spelman correlation analysis showed that the changes in the cecal microbial composition of broilers were related to SCFAs (Fig. 5G). Acetic acid, propionic acid, and isobutyric acid were strongly correlated in the SCFA differential metabolite correlation heat map. The molecular network map of KEGG metabolites showed that acetic acid, propionic acid, and butyric acid were the key metabolites.

3.6. Correlation analysis of intestinal microorganisms and amino acid composition

The changes in amino acids and fatty acids in the pectoral muscle might be related to the changes in the intestinal flora. Spearman correlation was used to analyze the correlation between amino acids and fatty acids in pectoral muscle and intestinal microorganisms (at the genus level). The results showed that 12 genera were positively correlated with breast muscle amino acid and fatty acid composition, and 12 genera were negatively correlated with breast muscle amino acid and fatty acid composition. In the correlation analysis between amino acids and microorganisms in chest muscle, the main positive regulatory bacteria were unidentified_Christensenellaceae, [Ruminococcus] and Dehalobacterium, while the negative regulatory bacteria were Clostridium (Fig. 6A). In the correlation analysis between breast muscle fatty acids and microorganisms, the main regulating bacteria groups were Butyricimonas, unidentified_Bacteroidales, and Parabacteroides. The negative regulatory flora were Oscillospira and Subdoligranulum (Fig. 6B). These results indicated that the changes in amino acid and fatty acid composition in the breast muscle of broilers were related to intestinal microbial composition. SCFAs are one of the important

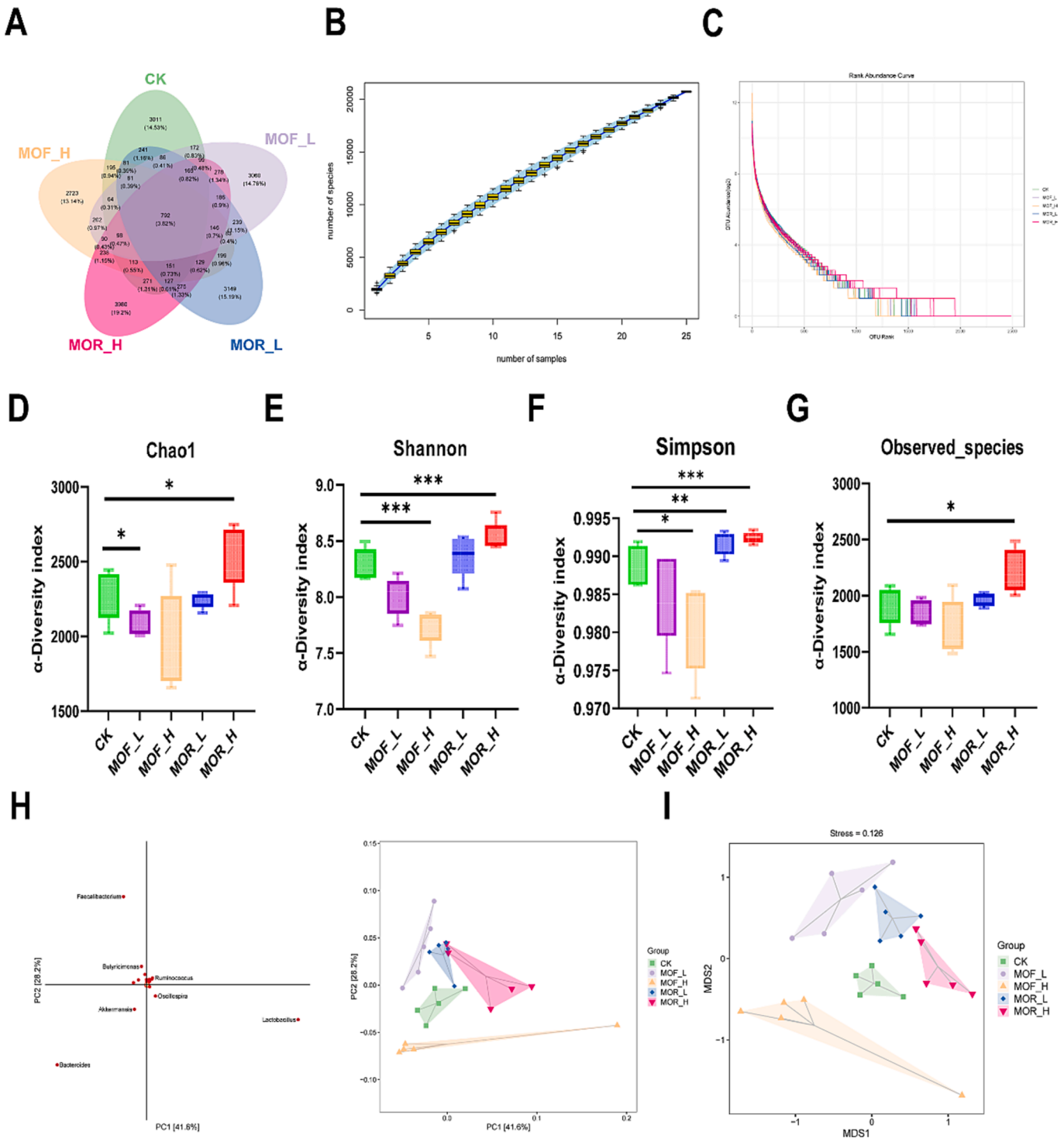

Fig. 1. Effects of *Moringa oleifera* on intestinal microbial community diversity of broilers. (A) Venn diagram; (B) Specaccum species accumulation curve; (C) Abundance grade curve; (D–G) α-diversity: Chao, Shannon, Simpson and Observed species indices; (H–I) β-diversity: PcA and NMDs maps.

products of intestinal microbiota, and amino acids are an important source of SCFAs. Therefore, the correlation between 18 amino acids and 7 SCFAs in cecal contents of five groups of white feather broilers was analyzed, and the results showed that valeric acid and caproic acid in 7 SCFAs were positively correlated with amino acids. Acetic acid, butyric acid, and isobutyric acid are negatively correlated (Fig. 6C). The correlation between fatty acids and SCFAs in broiler breast muscle indicates that acetic acid, butyric acid, and SCFAs are positively correlated with fatty acids, while valeric acid is negatively correlated with fatty acids (Fig. 6D).

4. Discussion

Supplementation of 2.5 % and 5 % moringa leaf to replace part of fish meal and soybean meal in broilers' diets was found to significantly increase daily gain and promote the feed utilization rate of broilers (Zhang et al., 2023). However, some studies suggest that the addition of excessive *Moringa oleifera* to the chicken diet will affect the growth

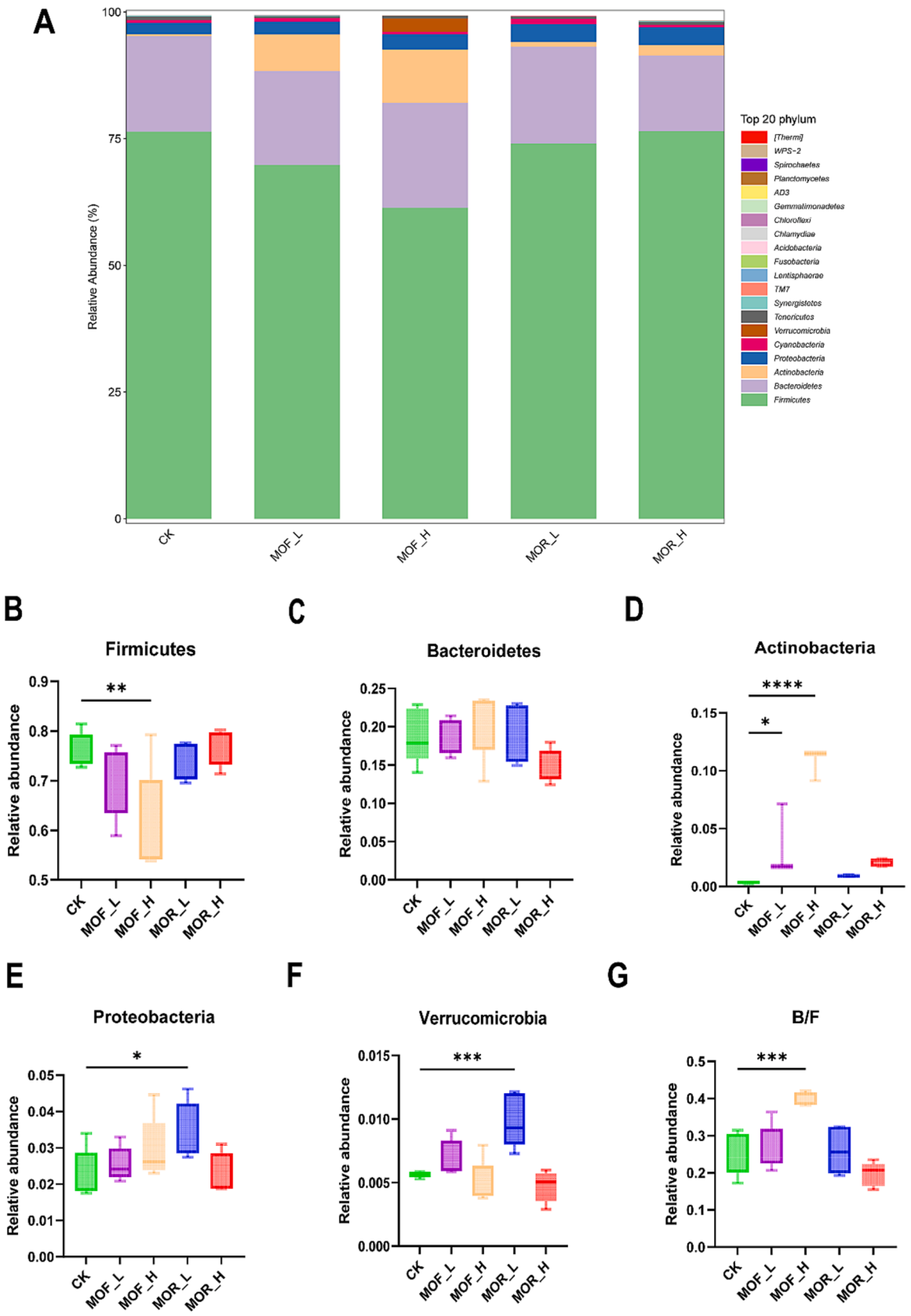

Fig. 2. Effects of *Moringa oleifera* on intestinal microbial community composition of broilers. (A) phylum-level microbial composition; Relative abundance of Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Verrucobacteria, and B/F in (B–G) groups at different doses; (H) Generic microbial composition. Relative abundances of Bacteroides, Spirillae, Spirillae, Coprobacillus, Lactobacillus, and Koala Bacillus in the *Moringa oleifera* group at different doses in the genus class (I–N). Data were analyzed by ANOVA one-way analysis of variance (*P < 0.05, **P < 0.01, ***P < 0.001).

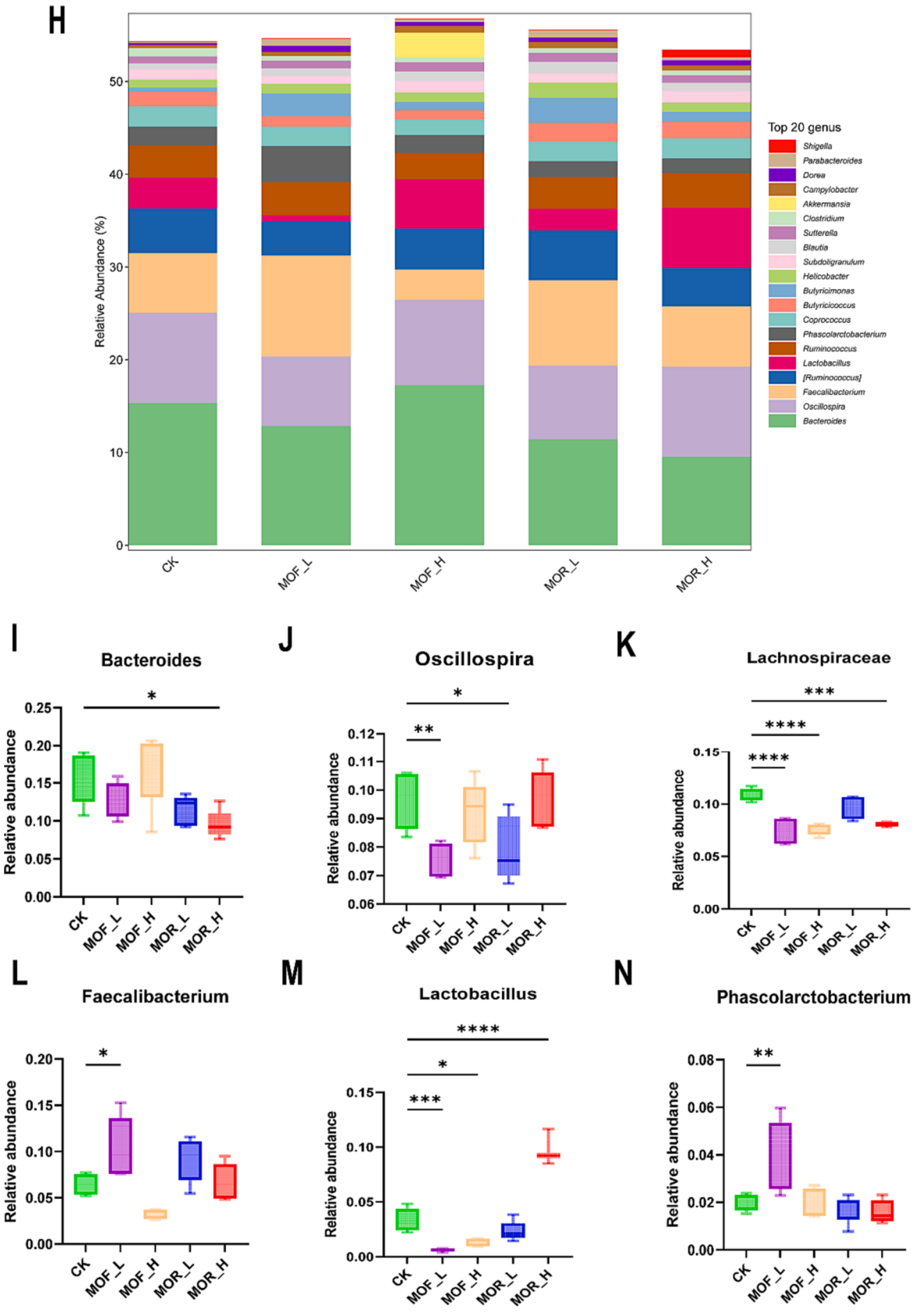

Fig. 2. (*continued*).

performance of broilers. Moringa leaf supplementation in the diets of broilers was greater than 5 %, which directly reduced feed intake and final weight of broilers (Su & Chen, 2020). It is found that moringa leaf supplementation significantly reduces the daily gain, body length, and body height of broilers during the brooding period. Moringa leaf as a raw material replacing part of the corn-soybean meal diet has little effect

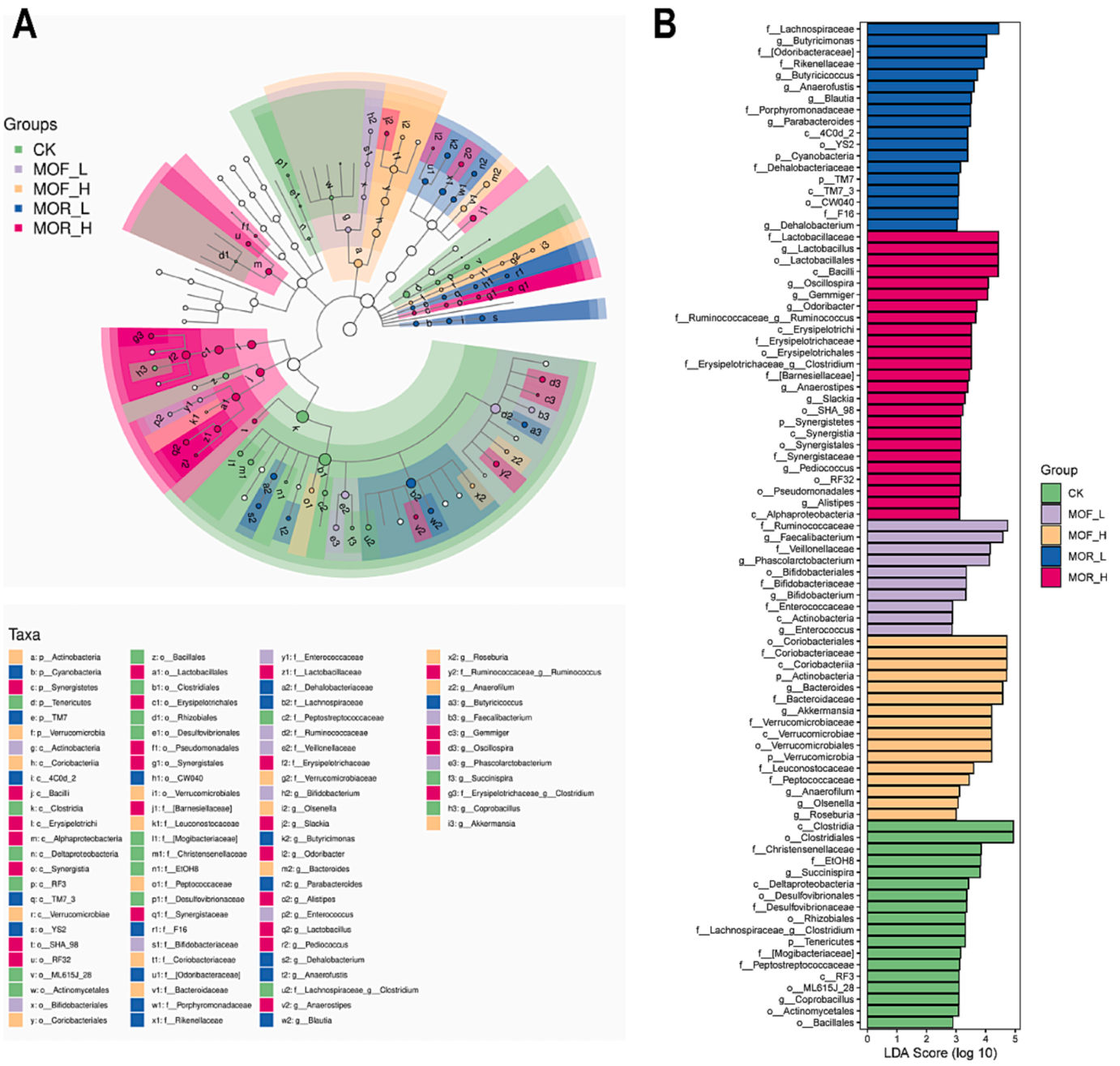

Fig. 3. Combination effect size measurement (LEfSe) and linear discrimination (LDA) analysis of cecal microbiota of broilers (n = 5). (A) The cladchart shows the microbial species that differ significantly between the two groups (species classifications are shown from the inside out: p, c, o, f, and g). (B) Species with significant differences whose LDA score is greater than estimated (default score is 2.0), the length of the histogram represents the LDA score.

on the nutrient conversion rate of broilers, but with moringa leaf supplemental level, feed intake and daily gain of broilers showed a linear decrease trend. The results of this study are consistent with those reported above. In terms of feed/meat ratio, this study found that fermented *Moringa oleifera* showed a dose effect and F/G tended to decrease, but the effect was not significant. Fermented *Moringa oleifera* did not affect the final weight, ADFI, and ADG of broilers, which may be because the contents of some macromolecular substances and anti-nutrient factors decreased after fermented *Moringa oleifera* leaves, which promoted the utilization rate of feed for broilers and thus increased daily gain. Under the experimental conditions, compared with the control group, the supplemental level of fermented *Moringa oleifera* was up to 1 %, but it had no negative effect on the growth and development of broilers, and there was no significant change in each group. Compared with fresh *Moringa oleifera* leaf powder, it did not affect the feeding of chickens. This is contrary to the result that the feed intake of unfermented *Moringa oleifera* decreased with increases in the level of supplementation (Gebregiorgis, Negesse & Nurfeta, 2012). These results indicate that a high level of supplementation of fermented *Moringa oleifera* can improve the utilization rate of feed and improve the daily gain of broilers. This phenomenon may be caused by the fact that the high supplemental level of fermented *Moringa oleifera* group is rich in flavonoids, polyphenols, polysaccharides, and other nutrients, which promote protein synthesis and precipitation of broilers (Aoki, Nakatsuka-Mori, Ueno, Nabeshima & Oyama, 2023). Therefore, in the actual feed production, do not add excessive unfermented moringa, the rational use of fermented moringa will be better.

Meat quality mainly includes appearance, juiciness, flavor, and

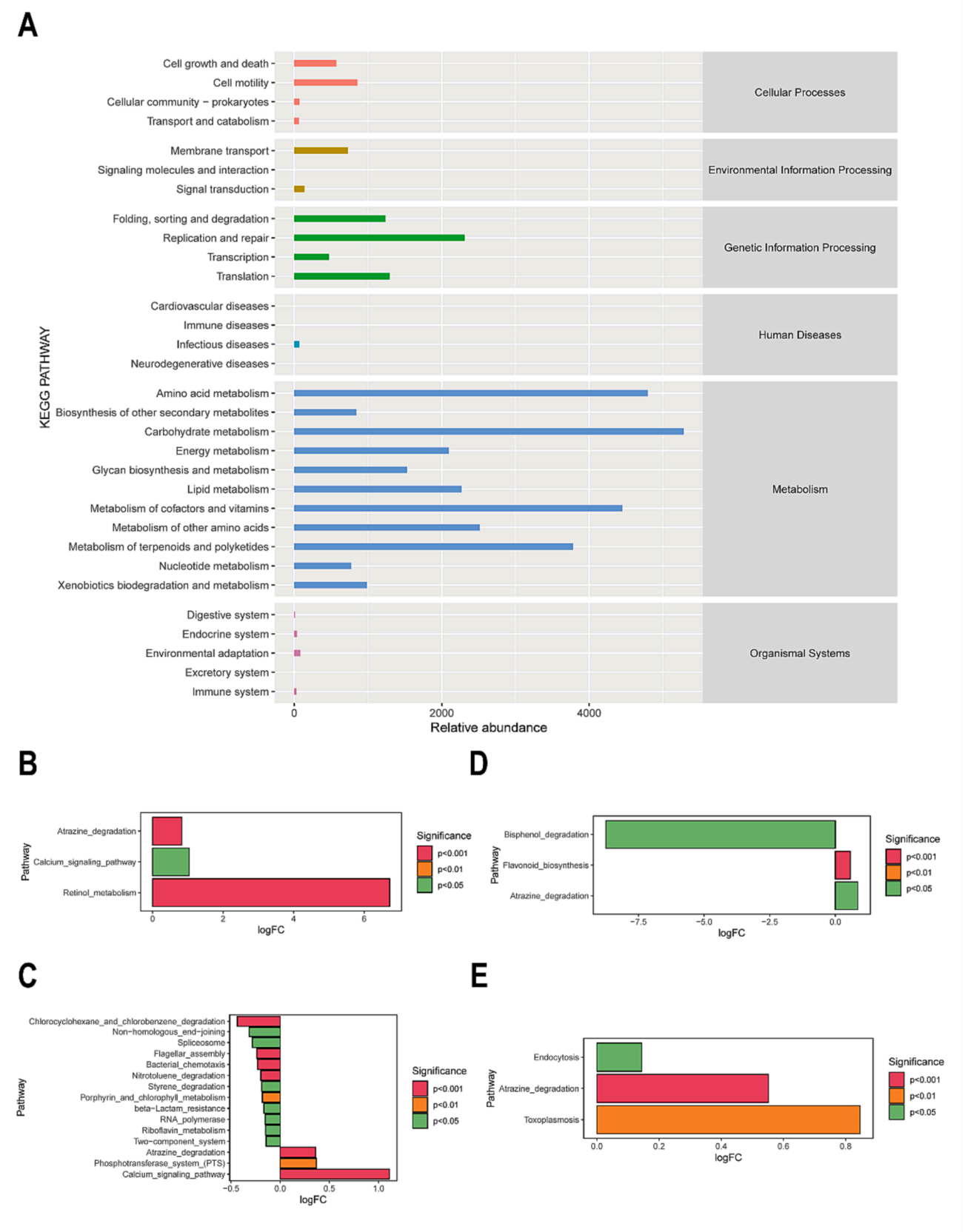

Fig. 4. Predicted KEGG functional pathway abundance analysis. (A) KEGG secondary functional metabolic pathway statistics (B) KEGG metabolic pathway difference analysis (CK group and MOF-L group, CK group and MOF-H group, CK group and MOR-L group, CK group and MOR-H group).

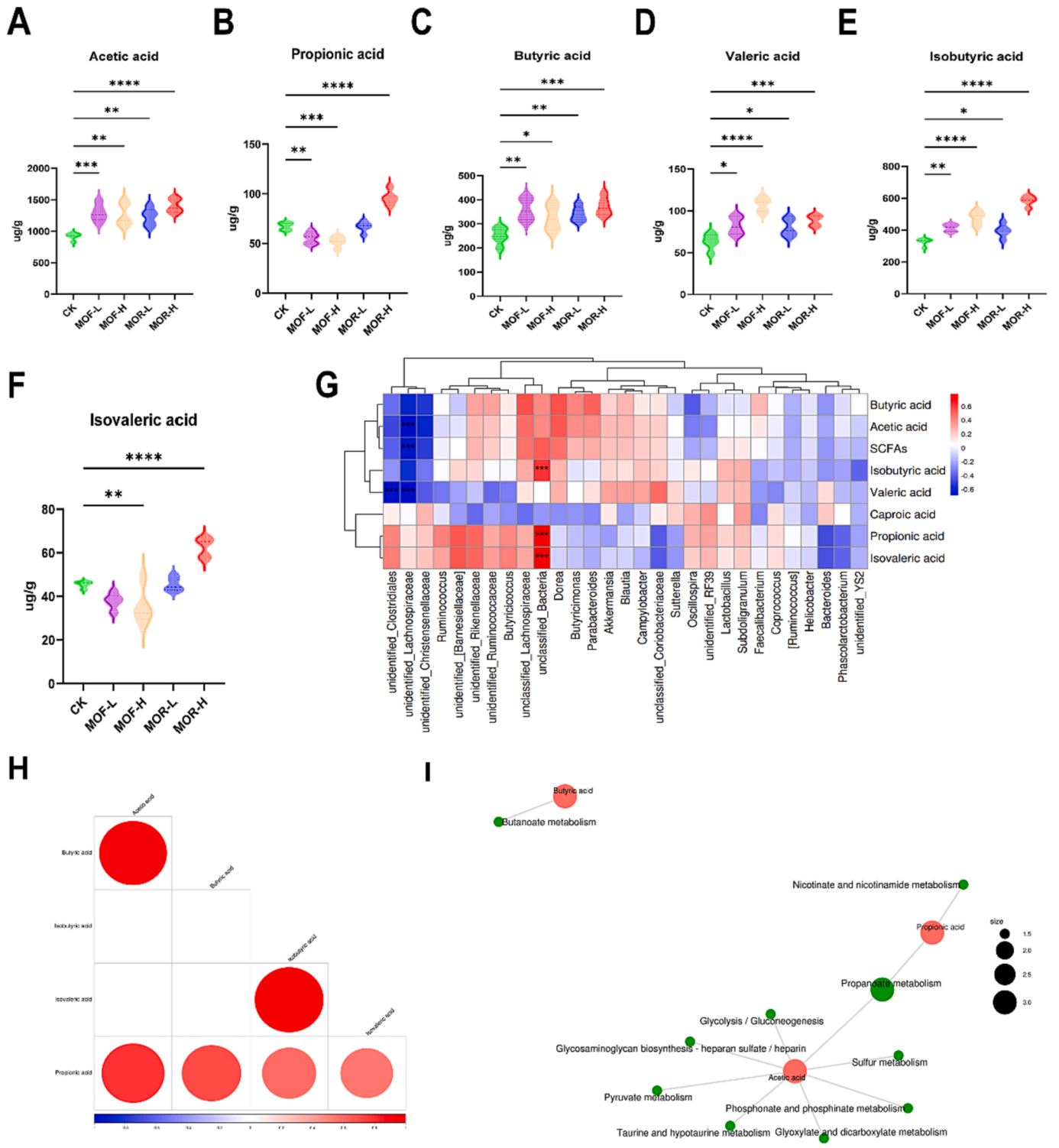

Fig. 5. Effects of *Moringa oleifera* on short-chain fatty acid (SCFAs) content in the cecum of broilers. (A) acetic acid; (B) propionic acid; (C) butyric acid; (D) valerate acid; (E) isobutyric acid; (F) isovaleric acid; (G) Heat map of correlation analysis between SCFAs and intestinal microbes Spearman; (H) SCFAs differential metabolite association heat map; (I) Molecular network diagram of KEGG metabolites.

tenderness. The tenderness of meat quality is closely related to consumer satisfaction, so the tenderness of edible meat affects its commercial value (Pomeranz, 1976). *Moringa oleifera* is rich in nutrients, such as amino acids, mineral elements, proteins, fatty acids, and vitamins, which can promote the growth of animals and improve the quality of animal meat. Meat quality is an important comprehensive index of animal economic traits, and its pH value, flesh color, system hydraulic, shear force, etc., are several core parameters to measure animal meat quality (Wen et al., 2020). pH value is a parameter of the speed and intensity of glycogen glycolysis in meat muscle after slaughter, and hydraulic value is a parameter of the ability of meat to retain water. The level of water will affect the shear strength of the muscle, and the shear force is an indicator of tenderness. The smaller the shear force, the better the meat quality. This study showed that the hydraulic capacity of the

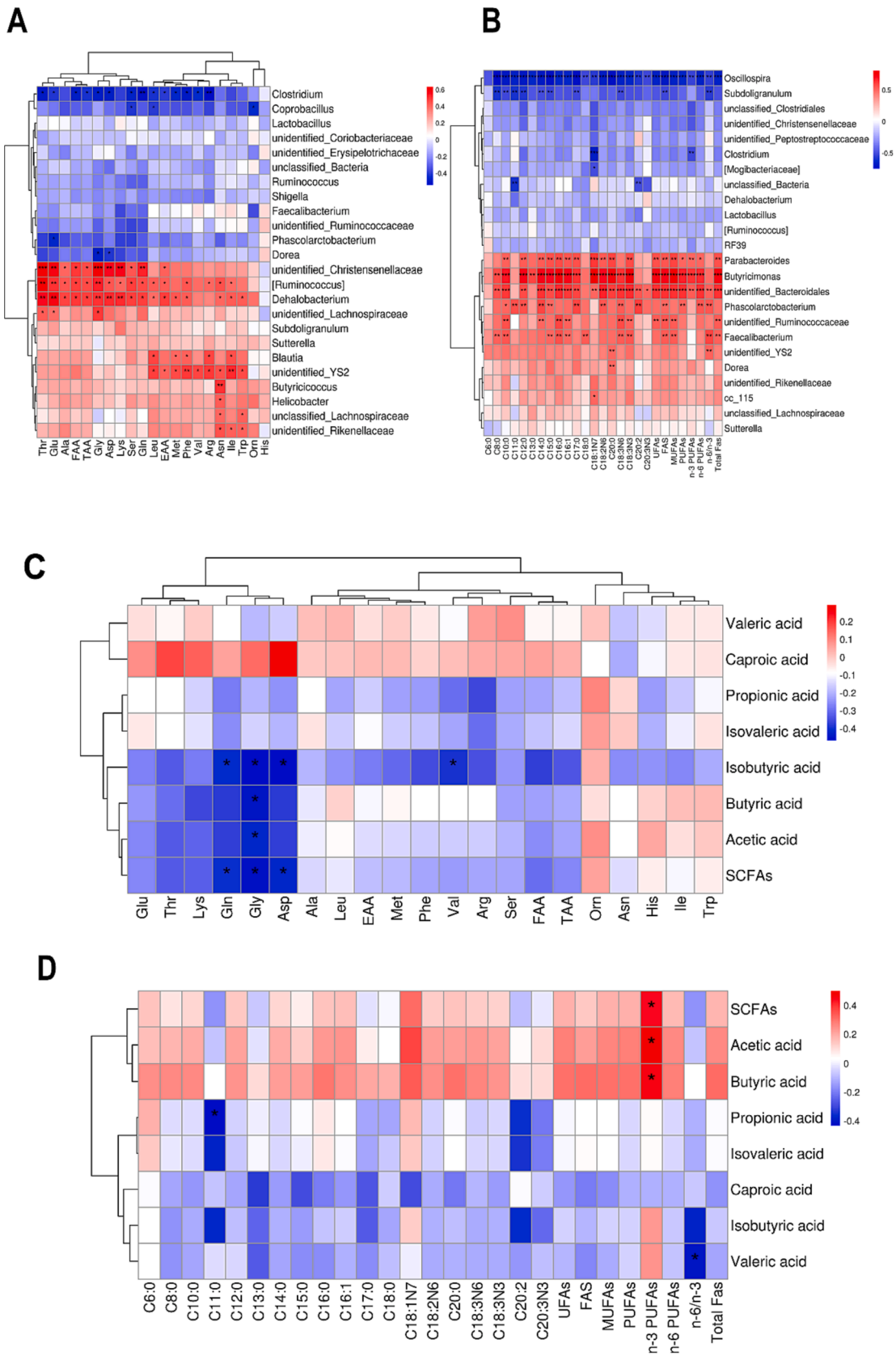

Fig. 6. Spearman correlation analysis of flavor substance. (A) Heat map of correlation analysis between pectoral amino acid composition and intestinal microorganisms Spearman. (B) Heat map of correlation analysis between pectoral fatty acid composition and intestinal microbes Spearman. (C) Heat map of sternal muscle amino acid composition and SCFAs Spearman correlation analysis. (D) Heat map of sternal fatty acid composition and SCFAs Spearman correlation analysis.

MOF-H group was the highest, followed by that of the MOR-H and that of the control group was the lowest, indicating that fermented moringa contains rich flavonoids, polyphenols, polysaccharides, and other substances, which can prevent lipid peroxidation by removing oxygen free radicals generated by stress in muscles, enhance the fluidity of cell membrane, reduce permeability, and prevent cell fluid from flowing out easily. The water retention of muscle tissue is maintained at a high level and the rigidity of muscle is prolonged, thus achieving the fresh-keeping effect (Garcia-Perez, Rocchetti, Giuberti, Lucchini & Lucini, 2023). Compared with unfermented moringa oleifera, liquid fermentation of moringa oleifera was more beneficial to improve the tenderness of breast muscle of white feathers broilers. This may be because the decomposed macromolecular nutrients of *Moringa oleifera* after fermentation were more thorough, and its substances improving meat quality increased to a certain extent. Muscle tenderness was positively correlated with the activity of a myofibrillar degrading enzyme, and the higher the activity, the higher the muscle fiber degrading ability and the lower the shear force. The higher the tenderness (Chen et al., 2021). Therefore, fermented *Moringa oleifera* can promote the activity of myofibrillar degrading enzyme in muscle, to improve chicken tenderness. In terms of flesh color, a* 45 min, a*24 h and b*24 h showed an increasing trend. The above results indicated that 1 % fermented *Moringa oleifera* could significantly improve meat quality, tenderness, and meat color of broilers under experimental conditions.

The amino acid content in meat is closely related to the nutritional value of meat protein and meat flavor (Wood et al., 1996). The content of essential amino acids determines the quality of proteins, while flavoring amino acids affect the taste (Yang et al., 2020). Compared with the control group, Moringa oleifera could increase the contents of essential amino acids (alanine, leucine, methionine, and tryptophan) in the breast muscle of broilers, and the effect of fermented *Moringa oleifera* was better, indicating that adding fermented *Moringa oleifera* to broilers' diet can improve meat quality and nutritional value. Flavor amino acids such as serine, alanine, glycine, and arginine can act as precursors to produce subtle flavors and have important effects on meat flavor (Qi, Men, Wu & Xu, 2019). Another major factor affecting flavor is fatty acid composition and content. The composition and content of fatty acids in the muscle are key indicators to evaluate its nutritional value (Wood et al., 2008). Relevant studies have shown that high concentrations of n-3 polyunsaturated fatty acids can improve the concentration ratio of unsaturated fatty acids (PUFA/SFA) and n-3/n-6, which is beneficial to human health. From the perspective of nutrition and health, the composition and content of muscle fatty acids are important indicators to measure meat quality (Saleh et al., 2012). The results of this study showed that with Moringa oleifera supplementation, the proportion of saturated fatty acids (C15:0, C16:0, C18:0, C20:0, and C22:0) in the breast muscle of white feathered broilers decreased ($P < 0.05$). The proportion of unsaturated fat (C15:1, C16:1, C18:1n7, C20:1t, C18:2n6, C18:3n3) was dose dependent. σSFA decreased, while the proportion of ΣMUFA, P/S and n-3 increased significantly ($P < 0.05$). Fatty acids in muscle can form precursors of flavor substances through related reactions; In addition, PUFAs can effectively prevent some chronic diseases, reduce fat, regulate the body's immunity, promote growth and development, and be beneficial to human health (Scaife, Moyo, Galbraith, Michie & Campbell, 1994). The experimental results are consistent with the reported results. Therefore, supplementation of fermented moringa in broiler diets can not only improve the ratio of amino acids and fatty acids in the breast muscle of white-feathered broilers, but also improve the nutrition and flavor of chicken by increasing the content of essential amino acids and fatty acids to a certain extent.

As the core area for animals to digest and absorb feed, the intestinal tract is also an important immune barrier for continuous material exchange. In addition to assisting the host to decompose and digest various nutrients (especially dietary fiber) entering the gut, intestinal bacteria can also synthesize and produce a variety of nutritive functional substances (such as B vitamins and partial functional amino acids, etc.). Probiotics can regulate the structure and diversity of the host gut microbiome (Fan et al., 2021). Studies have shown that moringa root polysaccharides (MRPs) increased the content of short-chain fatty acids and the abundance of beneficial bacteria, such as Bacteroides, while decreasing the abundance of harmful bacteria, such as Proteus (Hermawati, Sari & Partadiredja, 2015). In this experiment, significant increases in Chao, Shannon, Simpson, and Observed species of intestinal microbiota were detected, suggesting that fermenting Moringa oleifera could improve the α-diversity of intestinal microbiota. Regarding the structural changes of microbial communities, Firmicutes and Bacteroidetes were the main phyla in the five groups at the phyla level. Compared with the CK group, the MOF-H group showed an increased relative abundance of actinomycetes and B/F, and MOR-L showed an increased relative abundance of Verrucomicrobacteria. At the genus level, compared with the control group, MOF-L increased the relative abundance of Coprobacillus and Koalella, MOF-H increased the relative abundance of Bacteroides and Spirillum, and Spirillum and Lactobacillus showed dose-dependent increases in the MOR-L and MOR-H groups. This is consistent with previous research.

LEfSe analysis further showed that unfermented *Moringa oleifera* significantly increased the relative abundance of 25 bacterial biomarkers including Ruminococcaceae, Faecalibacterium, Coriobacteriia, and Bacteroides. Bacterial biomarkers significantly increased in fermented moringoleaceae include Lachnospiraceae, Butyricimonas, Lactobacillaceae, and Bacilli. There are various types of probiotics, and the most common species belong to Lactobacillus and Bifidobacterium (Gaggia, Mattarelli & Biavati, 2010). Studies have shown that lactic acid bacteria can retain micronutrients by promoting phytase secretion to promote intestinal metabolism (Mohammadi, Kermanshahi, Nasiri & Majidzadeh, 2020). In addition, lactic acid and short-chain fatty acids (SCFAs) produced by Lactobacillus activate intestinal epithelial cells and immune cells to protect the intestinal barrier and maintain gastrointestinal homeostasis (Iraporda et al., 2015). The synergistic effect of the intestinal microstructure and intestinal microbes can affect intestinal digestion and absorption and regulate body weight and feed intake. Such stimuli include changes in the environment and diet (Shi, Li, Duan & Niu, 2017). To explore the association between *Moringa oleifera*-induced microbial changes and broiler quality, functional metagenomic prediction of intestinal microflora was performed based on 16S rRNA gene sequencing. We found that 31 pathways were altered at the second level of the KEGG pathway, including "Amino acid metabolism", "Carbohydrate metabolism", "Metabolism of cofactors and vitamins", and "Metabolism of terpenoids and polyketides". Spearman correlation analysis further shows that the percentage of bacteria associated with "Amino acid metabolism" and "Lipid metabolism" (Firmicutes, Clostridium, and Lactobacillus) is high. These results suggest that may reconstruct intestinal barrier homeostasis, regulate microbial diversity, and increase the relative abundance of beneficial bacteria in the intestinal tract by regulating the metabolic function of intestinal microflora.

To further confirm the changes in short-chain fatty acid metabolism detected by untargeted metabolomics analysis, short-chain fat metabolism was quantified by targeted metabolomic analysis. Previous studies have shown that probiotics play a protective role in human and animal gut health by regulating short-chain fatty acids produced by bacteria (Kim et al., 2021). In this study, acetic acid, propionic acid, butyric acid, valeric acid, isobutyric acid, and isovaleric acid in the cecum after moringa treatment increased with dose. SCFAs are considered important mediators of communication between the gut microbiome and the immune system (Ratajczak et al., 2021). SCFAs are not only substrates for fatty acid synthesis and gluconeogenesis, but also participate in the function and metabolism of peripheral tissues (Canfora, Jocken & Blaak, 2015). Many studies have reported the possible effects of SCFAs on adipose tissue. The fat deposition pattern (including backfat thickness and intramuscular fat) is considered an important factor affecting meat quality (Li et al., 2023), and butyrate can improve

the meat quality of broilers (Zhang et al., 2011). In addition, SCFAs promote white adipogenesis and inhibit chronic inflammation in mice, which is related to the regulation of free fatty acid receptors (Lu, Fan, Li, Lu, Chang & Qi, 2016). SCFAs is one of the important products of intestinal microbiota, and amino acid is an important source of SCFAs. To further confirm changes in the metabolic function of intestinal microflora, a correlation analysis of amino acids, fatty acids, and SCFAs was conducted. It was found that acetic acid, butyric acid, and SCFAs are positively correlated with fatty acids, while valeric acid is negatively correlated with fatty acids. The results showed that the metabolome of cecal contents in the moringa-treated group was related to the significant increase in n-3 PUFAs.

Limitations of this study. Firstly, to better understand the effect of this new feed ingredient on meat quality regulation of white feathered broilers, it is necessary to evaluate the effect of high-level moringa fermentation supplemental level on meat quality regulation of white feathered broilers in the future. Secondly, although 1 % fermented *Moringa oleifera* can cause major metabolic changes in cecal microorganisms of broilers, the specific components contributing to such changes have not been determined. Finally, although fermented moringa is a sustainable protein alternative to SBM, research is focused on identifying the factors that contribute to the performance-inhibiting effects of high levels of fermented moringa if it is to be a true and complete replacement for SBM and its use as a feed ingredient is adopted by poultry producers worldwide.

5. Conclusion

In short, adding 1 % fermented moringa can improve the utilization rate of chicken to feed to improve the daily gain of broilers. Moringa oleifera and fermented moringa oleifera can improve the tenderness and color of breast meat of white feathers broiler. The supplementation of 0.5 % *Moringa oleifera* and 1 % fermented *Moringa oleifera* could increase the contents of essential amino acids (alanine, isoleucine, leucine, methionine, and tryptophan, etc.) in the human body, and reduce the contents of saturated fatty acids (C15:0, C16:0, C18:0, C20:0, and C22:0) in breast muscle of broilers. Increased unsaturated fat (C15:1, C16:1, C18:1N7, C20:1T, C18:2N6, C18:3N3) content and adjusted P/S closer to 0.4, more in line with the needs of a healthy diet. Fermented *Moringa oleifera* may reconstruct intestinal barrier homeostasis by regulating the metabolic function of intestinal microbiota, promote cecal SCFA synthesis of broilers, regulate microbial diversity, and increase the relative abundance of beneficial bacteria in the intestine. Therefore, this study provides new ideas for improving the quality of broilers and reference data for the development and application of *Moringa oleifera* supplement formulations.

Declaration of Competing Interest

The 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.

Data availability

The data that has been used is confidential.

Acknowledgements

Yunnan International Joint Laboratory of Green Health Food (China & Thailand) (grant numbers: 202203AP140011).

Ethics statement

The animal study was reviewed and approved by the Animal Experiment Ethics Review Committee of Yunnan Agricultural University.

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