104 (2025) 105043

Contents lists available at ScienceDirect

Poultry Science

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

Exploring the effects of feeding methods on the growth and meat flavor of Wenchang chicken

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ARTICLE INFO

Keywords:
Wenchang chicken
Rearing system
Growing performance
Meat quality

ABSTRACT

Wenchang chicken, renowned for its high-quality meat, is the economic meat breed in Hainan Province, China. This study compared cage-rearing (CR) and free-range (FR) groups in terms of growth performance, slaughter performance, meat quality, IMP (inosine monophosphate) content, AAs, FAs, serum lipid metabolites, and transcriptomic and metabolomic analyses. The CR group showed increased body weight, live weight, and abdominal fat but lower leg muscle percentage and breast muscle redness, suggesting flavor differences. CR chickens had higher IMP, threonine (Thr), and pentadecanoic, oleic, and linoleic acids, while glutamate (Glu) and alpha-linolenic acid were lower compared to FR. Glycine was elevated, but histidine, myristic, and tricosanoic acids were lower in CR leg muscle. Serum analysis revealed higher total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), lipoprotein lipase (LPL), hormone-sensitive lipase (HSL), fatty acid synthase (FAS), thyroid-stimulating hormone (TSH), leptin (LEP), and adiponectin (ADP) in the CR group. Transcriptomic and metabolomic studies identified 252 differentially expressed genes and 34 metabolites linked to metabolic pathways. In summary, CR system can improve production performance, FR system is considered more flavorful. The results can act as a theoretical basis for selecting a suitable rearing method for this unique breed.

Introduction

According to the Food and Agriculture Organization of the United Nations(FAO) 2023 Statistical Yearbook, global chicken meat production reached 121.59 million tons in 2021, making it the leading meat product worldwide. Wenchang chicken is a key poultry breed in Hainan Province. According to the data on Wenchang chicken from the Chicken Industry Association and Hainan Provincial Department of Agriculture and Rural Affairs, 123 million Wenchang chickens were sold in 2023. Although Wenchang chicken represents just 0.51 % of the national local chicken varieties, it accounts for 3.63 % of the national output of yellow-feathered broilers. Its output accounted for 77.12 % of the province's total poultry value in 2023. Furthermore, Wenchang chicken production comprises 94.6 % of the total broiler output in Hainan Province (Fan, 2024).

At present, cage-rearing (CR) and free-range (FR) systems are the two main rearing systems for chicken (Damaziak et al., 2021). CR is a practice developed to address the demands of modern large-scale, industrialized, and standardized production, with a focus on enhancing efficiency. In contrast, the FR system represents a traditional method that emphasizes animals' ability to move freely and grow in a natural environment.

Previous studies have demonstrated that CR and FR systems considerably impact chicken production performance, slaughter outcomes, and meat flavor. CR is particularly advantageous for broilers, offering a more optimized feed ratio, standardized feeding management, and effective disease prevention measures (Craig & Swanson, 1994). As a result, CR typically yields a higher feed conversion rate compared to FR environments (Cartoni Mancinelli et al., 2020). In contrast, FR chickens exhibit better meat and egg quality and align more closely with animal welfare standards, which enhances their immune performance (Damaziak et al., 2021). However, FR chicken has a lower overall production performance and less effective feeding management compared to chicken raised in cages. Thus, while CR enhances efficiency and production metrics, FR offers benefits in quality and welfare (Lin et al., 2023).

Numerous studies have investigated the impact of rearing systems on chicken meat quality. However, research focusing on Wenchang chicken

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https://doi.org/10.1016/j.psj.2025.105043
Received 8 January 2025; Accepted 15 March 2025
Available online 15 March 2025

regarding muscle composition and growth performance across different rearing systems is limited. Wenchang chicken undergoes a brooding period from 1 to 35 days, typically in a CR environment. During the rapid growth phase from 36 to 113 days, the chickens were reared in an FR environment. The period from 120 to 160 days is the fattening phase, representing a critical stage for meat flavor development. This final fattening stage was selected as the focus of this study to explore how CR and FR influence the meat quality and flavor compounds of Wenchang chicken. First, the meat production and slaughter performance were measured. Second, the serum indexes and the content of muscle flavor substances were determined. Finally, the candidate genes and metabolites affecting the quality of Wenchang chicken were identified and the potential underlying mechanisms were explored. The aim of this study is to explore the effects and regulation mechanisms of feeding methods on the growth and meat flavor of Wenchang chicken. The results lay a foundation for the selection of a suitable rearing system for Wenchang chicken.

Materials and methods

Animal and rearing systems

The Wenchang chickens employed in this study were raised in the Yongfa Breeding Farm of the Hainan Academy of Agricultural Sciences, China. 200 113-day-old Wenchang hens with no significant weight differences were randomly divided into CR and FR groups. Each group consisted of five replicates, with 20 hens per replicate. The temperature, humidity, and lighting conditions during the rearing process were kept consistent between the two groups. The pre-test and formal test were performed on day 7 and 40, respectively.

Body weight and body size measurements

On day 40 of the experiment, the Wenchang chickens were fasted for 12 h before measurements. Drinking water was provided normally during the fast. After fasting, the chickens were weighed and their body sizes were measured according to the national standard "Poultry Production Performance Noun Terminology and Measurement Calculation Method" (NY/T 823-2020).

Slaughter performance analysis

After fasting for 12 h, four chickens with no significant weight differences were selected from each replicate. The live weight, slaughter weight, half-eviscerated weight, eviscerated weight, breast muscle weight, leg muscle weight, and abdominal fat weight were measured separately.

Meat quality analysis

Raw meats of the left-side breast and left-side leg muscles were uniformly collected to measure the flesh color, pH value, drip loss, and shear force, following the standards outlined in the national standard “Performance Terminology and Measurement for poultry” (NY/T 823-2020).

Detection of serum biochemical indicators

Chickens with no significant difference from the average body weight were selected from each replicate, and 2 mL of blood was collected from the subwing vein. The blood was left to stand for 15 min and then centrifuged at 3,000 rpm for 15 min. The supernatant was collected and stored in an Eppendorf tube at −20 °C for the determination of the serum index. Serum biochemical indexes included total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), lipoprotein lipase (LPL), hormone-sensitive lipase (HSL), fatty acid synthase (FAS), thyroid-stimulating hormone (TSH), leptin (LEP), and adiponectin (ADPN). The kits used for measurements were from the Beijing Huaying Institute of Biotechnology, with the operation steps strictly in accordance with the manufacturer’s instructions. The serum biochemical indexes were measured using a biochemical analyzer (Hitachi 7180, Hitachi, Japan).

Inosine monophosphate measurements

To quantify inosine monophosphate (IMP), raw meat muscle samples were ground with a meat grinder, precisely weighed to 5 g, and placed in a centrifuge tube with 20 mL of 5 % perchloric acid. The mixture was vortexed three times, with each vortex followed by a 10-min rest in a 4 °C freezer. After the final vortex, samples were centrifuged at 8,000 rpm for 15 min and filtered into a conical flask. The precipitate was then resuspended in perchloric acid, vortexed, centrifuged again, and the combined supernatant was adjusted to pH 6.5 using sodium hydroxide. The volume was brought to 100 mL and filtered through a 0.22 μm filter for analysis. IMP detection was performed under the following conditions: YMC-Pack ODS-AM column (4.6 × 250 mm, 5 μm), a detection wavelength of 254 nm a flow rate of 1 mL/min, a column temperature of 25 °C, an injection volume of 5 μL, and mobile phases A (50 mM ammonium formate) and B (methanol) at a ratio of 97:3. Isocratic elution was run for 8 min.

Amino acid measurements

For the measurements of amino acids (AAs), approximately 2 g of raw meat muscle sample was hydrolyzed in 15 mL of 6 mol/L hydrochloric acid with 3 to 4 drops of phenol. After freezing briefly and cycling between vacuum and nitrogen three times, the tubes were sealed and hydrolyzed at 110 °C for 22 h. The hydrolysate was then filtered, diluted to 50 mL, and dried. The resulting residue was dissolved in sodium citrate buffer (pH 2.2), passed through a 0.22 μm membrane filter, and stored for subsequent analysis. Amino acid detection was conducted using liquid chromatography under the following conditions: a Poroshell SB-AQ column (Agilent, USA), mobile phases comprising 0.1 % formic acid in water and methanol, an injection volume of 2 μL, and a column temperature of 30 °C.

Measurement of fatty acids (FAs) composition

For the extraction of fatty acids (FAs), 5 g of raw meat muscle was mixed with a chloroform-methanol solution (2:1), shaken until the sample turned white, and centrifuged at 3,000 rpm. The lower layer was filtered and dried at 80 °C for 1.5 h, yielding yellow oil droplets. The FAs were then saponified and methylated according to GB 5009.168-2016, with the final content calculated by area normalization.

Preparation of white-cut and salt-baked chicken

To prepare the white-cut chicken, clean chickens were placed in boiling water and gently cooked for about 15 min until fully cooked. The chickens were then quickly transferred to an ice bath to cool down, causing the skin to contract and the meat to become firmer.

For the salt-baked chicken preparation, clean chickens were wrapped in salt chicken paper and buried in preheated coarse salt for 30 min to bake the chickens.

Electronic nose analysis

Breast and leg white-cut and salt-baked chicken muscles were accurately weighed to 9.00 g ± 0.01 g and placed into a 40 mL electronic nose autosampler vial. Clean air served as the carrier gas, with a data acquisition time of 60 s and a flow rate of 1 L/min. The sensor was cleaned for 10 s at a flow rate of 3 L/min. The instrument used was the

electronic nose sensory detector ISENSO SuperNose (ISENSO Technologies, USA). The peak response signal value of the electronic nose sensor was selected as the "eigenvalue" for constructing the original data matrix. Principal component analysis (PCA) was then performed on the data matrix.

Transcriptomics analysis

Total RNA was extracted and the cDNA library was prepared for RNA-Seq analysis via the HiSeq 2000 (Illumina, USA) system. The raw sequencing data were filtered to remove the redundant data. The remaining clean reads were used for the blast search and annotation against the NCBI non-redundant database. DESeq2 was used to analyze the DEGs under the following screening conditions: $|\log2FoldChange| > 1$ and P-value < 0.05. Gene ontology (GO) annotation and functional categorization were performed using Blast2GO to examine the roles of the differentially expressed genes (DEGs).

Untargeted metabolomics analysis

Wenchang chicken raw meat samples were thawed on ice, and metabolites were extracted from 100 mg of each sample using 1 mL of precooled 50 % methanol. The mixture was vortexed for 1 min, incubated at room temperature for 10 min, and stored overnight at −20 °C. After centrifugation at 4,000 g for 20 min, the supernatants were transferred to 96-well plates and stored at −80 °C for LC-MS analysis. Pooled QC samples were prepared by combining 10 μL of each extraction.

LC-MS analysis was conducted using a Vanquish Flex UHPLC system with an ACQUITY UPLC T3 column at 35 °C, a flow rate of 0.4 mL/min, and a mobile phase of water (0.1 % formic acid) and acetonitrile (0.1 % formic acid) under a gradient elution profile. Metabolites were detected with a Q-Exactive mass spectrometer in positive and negative ion modes, collecting precursor and fragment spectra at resolutions of 70,000 and 17,500, respectively. Raw data were converted to mzXML format using MSConvert (Proteowizard), while peak extraction and annotation were performed with XCMS and CAMERA. The identification and quantification of metabolites were conducted using metaX, with reference to the HMDB and KEGG databases.

Combined transcriptomic and metabolomic analysis

DEGs and differential metabolites (DMs) were enriched in KEGG pathways. Venn analysis identified and compared the enriched pathways across different omics datasets. Detailed information on the DEGs and DMs linked to the overlapping pathways was extracted using their IDs. The results were visualized through heat maps and network diagrams using OmicStudio (https://www.omicstudio.cn).

Statistical analysis

Statistica (2023) was used to analyze the data. The mean and standard deviation were calculated for each feature examined within the groups. Checks for sample homogeneity and normal distribution were conducted. One-way analysis of variance was performed to evaluate the data. Statistically significant differences between the groups were identified using T-tests, with $P < 0.05$ and $P < 0.01$ considered as significant and extremely significant, respectively.

Results

Effects of different rearing systems on the growth performance

The growth performance indicators were presented in Table 1. Compared with the FR group, the body weight and pelvis width in the CR group were significantly higher ($P < 0.05$). In addition, the fossil bone length and shank length were extremely significantly longer in the FR group ($P < 0.01$).

Table 1
Effects of different rearing systems on the body weight and body size.

Indicator	CR	FR	P-value
Body weight (g)	1632.47 ± 11.69	1569.89 ± 23.38	0.024
Body slope length (cm)	17.97 ± 0.40	18.65 ± 0.29	0.179
Keel length (cm)	9.93 ± 0.29	10.45 ± 0.19	0.001
Breast width (cm)	6.62 ± 0.10	6.56 ± 0.23	0.819
Breast depth (cm)	9.71 ± 0.28	9.32 ± 0.14	0.438
Pelvis width (cm)	6.71 ± 0.21	6.99 ± 0.21	0.027
Shank length (cm)	6.41 ± 0.10	7.01 ± 0.10	0.005
Shank circumference (cm)	3.52 ± 0.04	3.57 ± 0.09	0.596

Effects of different rearing systems on the slaughter performance

The slaughter performance indicators were reported in Table 2. Compared with the FR group, the CR group showed significant increases in the live weight, abdominal fat weight, and percentage of abdominal fat ($P < 0.05$). In addition, the slaughter weight, half-eviscerated weight, eviscerated weight, breast muscle weight, percentage of half-eviscerated yield, and percentage of eviscerated yield in the CR group exhibited an extremely significant increase ($P < 0.01$). However, the percentage of leg muscle in the CR group was observed to decrease ($P < 0.05$).

Effects of different rearing systems on fat metabolism

The serum lipometabolism indicators were presented in Table 3. Compared with the FR group, the CR group showed significant increases in the levels of TC, TG, LPL, and LEP ($P < 0.05$). In addition, the levels of HSL, FAS, and TSH in the CR group exhibited an extremely significant increase ($P < 0.01$). However, the level of ADPN in the CR group was significantly reduced ($P < 0.05$).

Effect of different rearing systems on the meat quality index

The quality indexes were presented in Table 4. In the breast muscle, compared with the FR group, the redness value a* of the CR group was significantly lower ($P < 0.05$). Moreover, in the leg muscle, the yellowness value b* of the CR group significantly increased ($P < 0.05$), the

Table 2
Effects of different rearing systems on slaughter performance.

Indicator	CR	FR	P-value
Live weight (g)	1632.93 ± 14.08	1561.50 ± 25.76	0.023
Slaughter weight (g)	1484.40 ± 15.47	1405.13 ± 21.98	0.007
Half-eviscerated weight (g)	1365.09 ± 61.18	1247.88 ± 87.11	0.001
Eviscerated weight (g)	1202.09 ± 55.69	1088.69 ± 76.55	0.004
Breast muscle weight (g)	179.53 ± 17.96	156.03 ± 18.03	0.003
Leg muscle weight (g)	215.34 ± 11.96	202.93 ± 22.13	0.246
Abdominal fat weight (g)	106.29 ± 24.77	86.09 ± 24.99	0.032
Dressed percentage (%)	90.91 ± 0.64	90.04 ± 0.59	0.324
Percentage of half-eviscerated yield (%)	83.04 ± 0.57	79.97 ± 0.89	0.008
Percentage of eviscerated yield (%)	73.60 ± 0.54	69.76 ± 0.75	0.001
Percentage of breast muscle (%)	14.99 ± 0.48	14.36 ± 0.41	0.330
Percentage of leg muscle (%)	17.93 ± 0.27	18.64 ± 0.43	0.025
Percentage of lean meat (%)	32.92 ± 0.62	31.94 ± 0.78	0.336
Percentage of abdominal fat (%)	8.50 ± 0.47	7.31 ± 0.50	0.025

Table 3
Effects of different rearing systems on fat metabolism.

Indicator (mmol/L)	CR	FR	P-value
TC	4.54 ± 0.86	2.77 ± 0.20	0.038
TG	5.18 ± 0.89	3.00 ± 0.72	0.025
HDL	1.61 ± 0.20	1.52 ± 0.27	0.798
LDL	1.48 ± 0.42	0.79 ± 0.07	0.112
LPL	19.33 ± 1.17	15.84 ± 0.45	0.038
HSL	79.03 ± 2.40	61.91 ± 3.17	0.003
FAS	20.39 ± 1.33	13.79 ± 0.61	0.002
TSH	5.31 ± 0.20	3.18 ± 0.38	0.003
LEP	5.15 ± 0.12	4.67 ± 0.82	0.011
ADPN	7.17 ± 0.27	8.10 ± 0.14	0.023

Table 4
Effect of different rearing systems on meat quality.

Indicator		Breast muscle		P-value	Leg muscle		P-value
		CR	FR		CR	FR	
Flesh color	L*	53.43 ± 1.02	56.90 ± 1.36	0.051	48.87 ± 0.90	52.51 ± 1.47	0.040
	a*	2.13 ± 0.30	2.92 ± 0.27	0.047	7.69 ± 0.54	7.83 ± 0.79	0.88
	b*	8.62 ± 0.46	9.08 ± 0.76	0.607	14.68 ± 0.60	12.05 ± 0.58	0.038
pH	pH45min	6.79 ± 0.10	7.03 ± 0.13	0.136	7.08 ± 0.10	7.08 ± 0.08	0.995
	pH24h	6.51 ± 0.03	6.54 ± 0.03	0.495	6.98 ± 0.10	7.16 ± 0.09	0.178
Drip loss (%)		2.81 ± 0.35	2.60 ± 0.43	0.700	2.08 ± 0.25	1.98 ± 0.26	0.769
Shear force (N/Kg)		31.33 ± 3.79	35.72 ± 1.82	0.308	29.45 ± 1.16	39.66 ± 2.65	0.002

Note: L*, brightness value; a*, redness value; b*, yellowness value.

lightness value L* significantly decreased ($P < 0.05$), and the shear force exhibited an extremely significant reduction ($P < 0.01$) compared with the FR group.

Effect of different rearing systems on muscle flavor substances

Effect of different rearing systems on the IMP content

The IMP contents of the two experimental groups are reported in Table 5. In the breast muscle, the IMP content in the CR group significantly decreased ($P < 0.05$) compared with the FR group.

Effect of different rearing systems on muscle AA content

The AA contents were presented in Table 6. In the breast muscle, compared with the FR group, the level of Thr in the CR group significantly increased ($P < 0.05$), while the level of Glu significantly decreased ($P < 0.05$). In the leg muscle, compared with the FR group, the levels of His, Gly, Ala, Pro, and Phe in the CR group significantly decreased ($P < 0.05$).

Effect of different rearing systems on muscle FA content

The FA contents were presented in Table 7. In the breast muscle, compared with the FR group, the levels of C18:1, n9t ($P < 0.05$) and C18:3, n3 ($P < 0.01$) in the CR group significantly decreased, while the levels of C17:1,1n7 ($P < 0.05$), C18:2, n6c ($P < 0.05$) and C15:0 ($P < 0.01$) significantly increased. In the leg muscle, compared with the FR group, the levels of C14:0 ($P < 0.05$) and C23:0 ($P < 0.05$) in the CR group significantly decreased C23:0 ($P < 0.05$), while the levels of MUFA ($P < 0.05$) significantly increased C23:0 ($P < 0.05$).

Table 5
Effects of different rearing systems on IMP content.

Indicator	Breast muscle		P-value	Leg muscle		P-value
	CR	FR		CR	FR	
IMP (mg/g)	1.26 ± 0.38	1.73 ± 0.27	0.042	1.66 ± 0.10	1.82 ± 0.26	0.214

Table 6
Effects of different rearing systems on muscle AA content.

Indicator (%)	Breast muscle		P-value	Leg muscle		P-value
	CR	FR		CR	FR	
● Lys	2.30 ± 0.02	2.31 ± 0.05	0.500	2.19 ± 0.02	2.23 ± 0.04	0.362
His	1.24 ± 0.05	1.26 ± 0.07	0.163	0.38 ± 0.02	0.50 ± 0.05	0.007
Arg	0.18 ± 0.02	0.17 ± 0.02	0.764	0.19 ± 0.01	0.18 ± 0.01	0.738
▲ Gly	0.37 ± 0.02	0.29 ± 0.03	0.314	1.19 ± 0.05	0.84 ± 0.05	0.003
Ser	0.56 ± 0.06	0.60 ± 0.07	0.338	1.19 ± 0.07	1.33 ± 0.14	0.289
▲ Ala	2.22 ± 0.25	1.63 ± 0.13	0.179	2.11 ± 0.10	2.51 ± 0.08	0.048
▲ Asp	2.81 ± 0.06	3.58 ± 0.05	0.636	2.94 ± 0.05	3.39 ± 0.06	0.549
● Thr	1.07 ± 0.13	0.72 ± 0.11	0.035	0.66 ± 0.11	0.57 ± 0.10	0.663
▲ Glu	4.32 ± 0.38	6.43 ± 1.21	0.047	4.28 ± 0.29	4.70 ± 0.66	0.398
Pro	0.28 ± 0.03	0.25 ± 0.01	0.837	0.17 ± 0.01	0.20 ± 0.02	0.043
● Val	5.36 ± 0.18	4.39 ± 0.34	0.259	6.02 ± 0.03	5.94 ± 0.40	0.793
Met	0.36 ± 0.06	0.33 ± 0.03	0.936	0.15 ± 0.01	0.17 ± 0.02	0.337
● Ile	0.48 ± 0.03	0.43 ± 0.05	0.894	0.21 ± 0.02	0.19 ± 0.02	0.553
● Leu	0.31 ± 0.03	0.29 ± 0.03	0.828	0.12 ± 0.01	0.12 ± 0.01	0.633
▲ Tyr	0.40 ± 0.05	0.33 ± 0.04	0.551	0.11 ± 0.01	0.11 ± 0.01	0.729
▲● Phe	0.30 ± 0.03	0.32 ± 0.03	0.208	0.14 ± 0.01	0.16 ± 0.02	0.026
Essential amino acids	9.82 ± 0.39	8.46 ± 0.28	0.425	9.34 ± 0.46	9.21 ± 0.32	0.734
Flavor amino acids	10.43 ± 0.79	12.58 ± 0.72	0.086	11.83 ± 0.53	11.31 ± 0.58	0.526
Total amino acids	22.56 ± 0.45	23.32 ± 0.34	0.253	22.84 ± 0.26	22.35 ± 0.38	0.769

Note: ● indicates essential amino acids, ▲ indicates flavor amino acids.

Electronic nose analysis

Fig. 1A, B presents the PCA plots for the experimental and control groups of white-cut and salt-baked Wenchang chickens, respectively. In Fig. 1A, PC1 contributed to 61.2106 % of the variance, and PC2 contributed to 29.8679 %, with a cumulative contribution rate of 91.0785 %. There was a clear distinction without any overlap between the experimental and control groups, indicating significant differences between the two groups and distinct differences in flavor profiles. Conversely, in Fig. 1B, PC1 accounted for 68.1691 % of the variance, while PC2 accounted for 15.8484 %, resulting in a cumulative contribution rate of 84.0175 %. Some overlap was observed between the experimental and control groups, suggesting a degree of similarity in flavor between the two groups.

Transcriptome analysis

Transcriptome data and quality control

The three samples in the FR group obtained 6.19 Gb, 6.53 Gb, and

Table 7
Effects of different rearing systems on muscle fatty acid content.

Indicator (%)	Breast muscle		P-value	Leg muscle		P-value
	CR	FR		CR	FR	
C6:0	0.28 ± 0.13	0.55 ± 0.13	0.532	0.2 ± 0.12	0.12 ± 0.06	0.529
C11:0	0.29 ± 0.05	0.19 ± 0.09	0.404	0.09 ± 0.04	0.08 ± 0.03	0.865
C12:0	0.61 ± 0.24	0.32 ± 0.04	0.315	0.38 ± 0.18	0.24 ± 0.01	0.496
C13:0	0.59 ± 0.27	0.38 ± 0.17	0.517	0.2 ± 0.07	0.09 ± 0.05	0.242
C14:0	0.99 ± 0.18	0.98 ± 0.03	0.941	0.89 ± 0.05	1.11 ± 0.06	0.025
C15:0	1.32 ± 0.06	0.34 ± 0.23	0.008	0.3 ± 0.18	0.14 ± 0.04	0.434
C16:0	26.68 ± 0.95	26.71 ± 1.13	0.984	27.38 ± 2.19	26.85 ± 0.47	0.827
C17:0	0.39 ± 0.09	0.3 ± 0.13	0.618	0.25 ± 0.1	0.17 ± 0.01	0.505
C18:0	7.73 ± 0.57	7.89 ± 0.35	0.8	6.84 ± 0.44	6.82 ± 0.36	0.982
C23:0	2.33 ± 0.24	2.05 ± 0.44	0.627	0.48 ± 0.14	1.12 ± 0.23	0.045
∑SFA	41.21 ± 0.58	39.71 ± 0.87	0.837	37.01 ± 1.22	36.74 ± 0.85	0.856
C14:1, n-9	0.68 ± 0.32	0.24 ± 0.1	0.186	0.19 ± 0.02	0.17 ± 0.01	0.397
C16:1, 1n7	3.81 ± 0.19	3.49 ± 0.32	0.449	5.51 ± 0.74	4.65 ± 0.33	0.294
C17:1, 1n7	0.41 ± 0.13	0.19 ± 0.08	0.042	0.18 ± 0.05	0.14 ± 0.03	0.476
C18:1, n9t	0.56 ± 0.54	1.76 ± 0.39	0.032	0.66 ± 0.49	0.56 ± 0.42	0.885
C18:1, n9c	30.85 ± 0.46	31.56 ± 2.63	0.819	37.56 ± 3.35	34.1 ± 1.03	0.31
C20:1	0.39 ± 0.07	0.59 ± 0.28	0.545	0.98 ± 0.04	1.13 ± 0.27	0.657
∑MUFA	36.07 ± 0.32	37.83 ± 1.94	0.802	45.08 ± 2.24	40.75 ± 0.67	0.034
C18:2, n6t	3.43 ± 0.83	3.87 ± 2.63	0.889	1.21 ± 0.67	0.13 ± 0.06	0.208
C18:2, n6c	16.99 ± 0.32	14.76 ± 1.19	0.047	14.07 ± 4.7	20.09 ± 0.32	0.291
C18:3, n6	0.33 ± 0.06	0.27 ± 0.1	0.625	0.2 ± 0.02	0.37 ± 0.14	0.345
C18:3, n3	0.74 ± 0.05	1.21 ± 0.1	0.006	0.17 ± 0.08	0.4 ± 0.29	0.507
C20:3, n6	0.6 ± 0.1	0.67 ± 0.38	0.881	0.56 ± 0.24	0.41 ± 0.19	0.646
C22:6, n3	0.74 ± 0.13	0.91 ± 0.41	0.734	0.64 ± 0.25	0.44 ± 0.21	0.559
∑EFA	17.73 ± 0.26	15.97 ± 1.13	0.065	14.24 ± 4.34	20.49 ± 0.22	0.129
∑PUFA	22.83 ± 0.23	21.69 ± 1.27	0.784	16.85 ± 4.10	21.84 ± 0.25	0.098

Note: SFA, saturated FAs (C6:0, C11:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, and C23:0); MUFA, monounsaturated FAs (C14:1, n-9, C16:1, 1n7, C17:1, 1n7, C18:1, n9t, C18:1, n9c and, C20:1); EFA, essential FAs (C18:2, n6c, and C18:3, n3); PUFA, polyunsaturated FAs (C18:2, n6t, C18:2, n6c, C18:3, n6, C18:3, n3, C20:3, n6, and C22:6, n3).

6.08 Gb of raw data and 6.00 Gb, 6.32 Gb, and 5.89 Gb of valid data, respectively. The three samples in the CR group obtained 6.54 Gb, 6.42 Gb, and 6.05 Gb of raw data and 6.33 Gb, 6.22 Gb, and 5.87 Gb of valid data, respectively. All six samples in this sequencing exhibited Q30 values (%) > 97.11 %. Therefore, the transcriptome libraries of the six Wenchang chicken breast muscle tissues constructed in this experiment could be used for subsequent analysis.

Identification of DEGs

In CR versus FR, a total of 252 differential genes were detected, with 68 up-regulated and 184 down-regulated genes (Fig. 2A and C).

Functional enrichment analyses of DEGs

GO and KEGG enrichment analyses were conducted on the identified DEGs. A total of 312 GO terms and nine pathways were significantly enriched. The top 20 enriched GO terms of the DEGs were shown in Fig. 3A. These include "regulation of lipid metabolic process" (three enriched genes; *NR1D1, NR1D2*, and *BBS4*), "cholesterol homeostasis" (four enriched genes; *PCSK9, NR1D1, INSIG1*, and *APOA4*), and "amino acid transport" (two enriched genes; *SLC6A20* and *SFXN2*). The top 20 enriched KEGG pathways of DEGs were shown in Fig. 3B. Significantly enriched KEGG pathways include "ECM-receptor interaction" (five enriched genes; *LAMB1, COL4A1, COL4A2, CHAD*, and *COL4A4*) and "linoleic acid metabolism" (two enriched genes; *CYP2C18* and *CYP3A5*). Tables 8 and 9 reported the significantly enriched KEGG pathways and GO terms, respectively, both of which may be potentially related to changes in muscle quality or flavor.

Untargeted metabolomics and differential metabolite analyses

Quality control of metabolomic data results

Quality control analysis indicated that the quantity and quality of metabolome assay data were sufficient for subsequent metabolite expression analysis (Fig. 4A, B). The OPLSDA revealed significant differences between the CR and FR groups (Fig. 4C, D).

Metabolite identification and correlation analysis

A total of 545 metabolites were identified (Fig. 4E), with lipids and lipid-like molecules (35.96 %), organic acids and derivatives (26.61 %), and organoheterocyclic compounds (8.44 %) as the dominant categories. Lipids and lipid-like molecules constituted the highest proportion, of which 54.08 % were glycerophospholipids and 36.22 % were fatty acyls.

DMs screening and identification

The fold-change was obtained through univariate analysis. T-tests were used for statistical testing, q-values were determined using the BH correction, and the variable importance in projection (VIP) values were obtained through multivariate statistical analysis of the PLS-DA results. Based on the above data, differential metabolite ions were screened using the following criteria: 1) ratio ≥ 2 or ratio ≤ 0.5; 2) q-value < 0.05; and 3) VIP ≥ 1. Through screening, 475 differential metabolic ions were identified in the comparison group (CR versus FR), including 166 in positive ion mode (61 up-regulated and 105 down-regulated) and 309 in negative ion mode (139 up-regulated and 170 down-regulated) (Fig. 5A). The screened differential metabolite ions yielded 34 DMs through the identification of secondary metabolites and annotations from the HMDB database. Compared with the FR group, there were 18 DMs up-regulated and 16 down-regulated in the CR group (Supplementary Table 1). Among them, the DMs related to meat flavor include (R)-3-hydroxybutyric acid, phenol sulfate, 3-indoleacrylic acid, palmitelaidic acid, palmitic acid, linoleic acid, oleic acid, PC 21:1, PC(6:0/15:1), acylcarnitine 12:1, lauroyl-L-carnitine, acylcarnitine 16:1, 3-hydroxyhexadecanoylcarnitine, acylcarnitine 18:3, oleoyl-L-carnitine, 3-hydroxyoleylcarnitine, lysoPC 20:4, DL-arginine, phosphocholine, Ala-Gln, pantothenic acid, Ala-Phe, acylcarnitine 5:0, Ala-Arg, nicotinamide riboside cation, D-(+)-pantothenic acid, Ala-Trp, honyucitrin, 3-dehydroxycarnitine, and lysine.

Cluster and pathway analyses of DMs

The heatmap of the DMs was generated through the cluster analysis of the DMs expression levels between samples (Fig. 5B). The relative expression levels of DMs in the CR and FR groups were well clustered, and the expression levels between the two groups can be clearly differentiated. The KEGG database was utilized to identify the pathways associated with the DMs, and enrichment analysis was subsequently performed. A total of 29 metabolic pathways were enriched. The KEGG enrichment analysis results were presented as scatter plots using the R

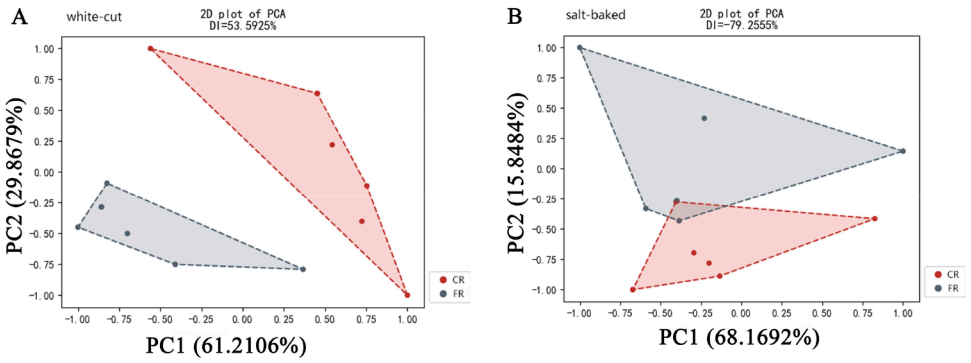

Fig. 1. Electronic nose analysis. (A) PCA analysis of white-cut chickens in experiment and control groups. (B) PCA analysis of salt-baked chickens in experiment and control groups.

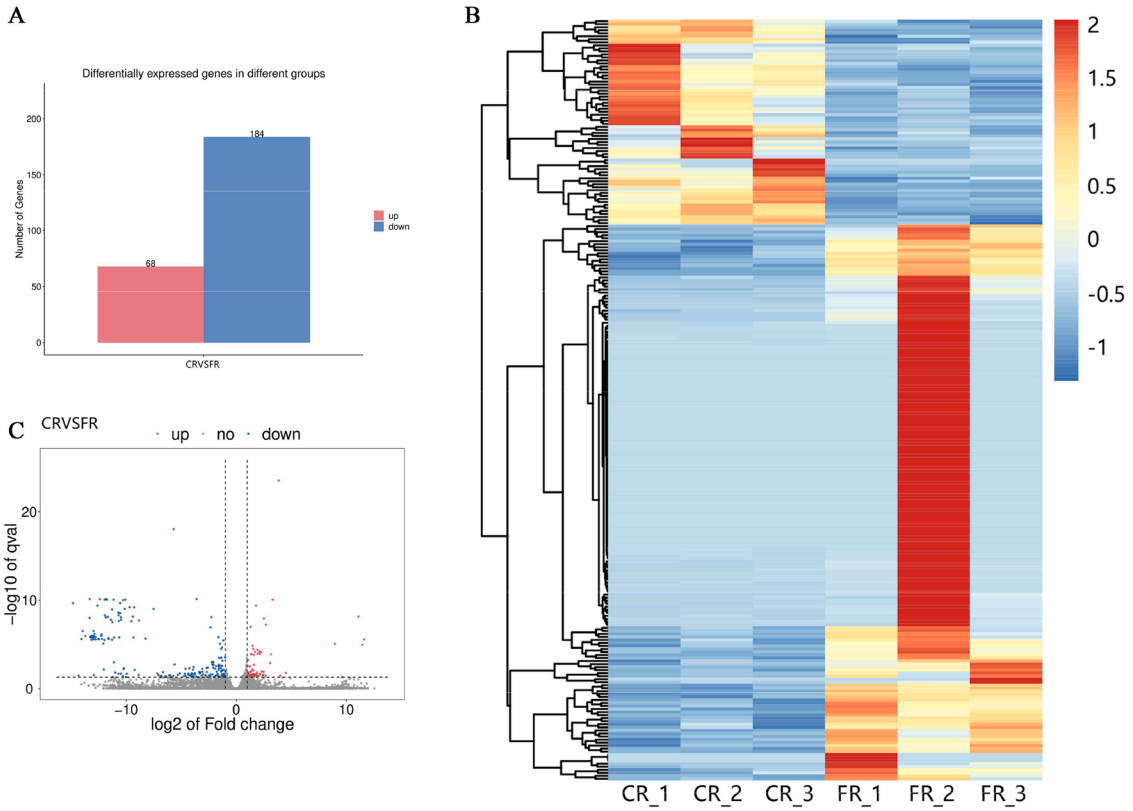

Fig. 2. Statistical analysis of DEGs of Wenchang Chicken breast muscle. (A). Statistics of the frequency of up-down and down-regulation of DEGs. (B). Gene expression clustering heat map under two rearing systems. Each column in the heat map represents the expression of different genes in the same sample, and each row represents the expression of the same gene in different samples. Red indicates up-regulated genes, and blue indicates down-regulated genes in the affected samples. (C). Volcano plot of DEGs. The red dots represent up-regulated genes, and the blue dots represent down-regulated genes.

package ggplot2 (R Core Team) and the bubble plots for the enrichment of DMs pathways were then derived (Fig. 5C). Table 10 reports the significantly enriched KEGG pathways potentially related to flavor compounds and their precursors.

Integrative analysis of the transcriptome and metabolome

The joint analysis revealed eight KEGG pathways shared by DMs and DEGs, including four related to amino acid metabolism (lysine degradation, histidine metabolism, biosynthesis of AAs, and beta-alanine metabolism) and four associated with lipid metabolism (pantothenate and CoA biosynthesis, metabolic pathways, linoleic acid metabolism, and arachidonic acid metabolism) (Fig. 6A, B). The correlation heatmap of the DMs versus the DEGs (Fig. 6C) indicates that several genes potentially linked to meat quality and flavor displayed significant associations with specific metabolites. For example, nicotinamide riboside cation had a strong positive correlation with *NDST4*; lysine was positively correlated with *SLC22A16*; and 4-imidazoleacrylic acid was negatively correlated with *NPR2, NDST4*, and *ST3GAL1*, but positively correlated with *SARDH, CYP3A5*, and *GALNT14*. Pantothenic acid exhibited positive correlations with *ARG2, CRYL1, GADL1*, and CA2; (R)-3-hydroxybutyric acid was negatively correlated with *PIPOX, SLC22A16, CYP2C18, ARG2*, and *CA2*; DL-beta-hydroxybutyric acid exhibited negative correlations with *PIPOX, CYP2C18, FAH*, and *MAT1A*; and palmitic acid was positively correlated with *NDST4* and negatively correlated with *UQCRHL, CYP2C18, FAH*, and *MAT1A*. The association network of the DMs and DEGs (Fig. 6D) suggests that the genes *NDST4* and *CYP2C18* may influence meat flavor, and metabolites

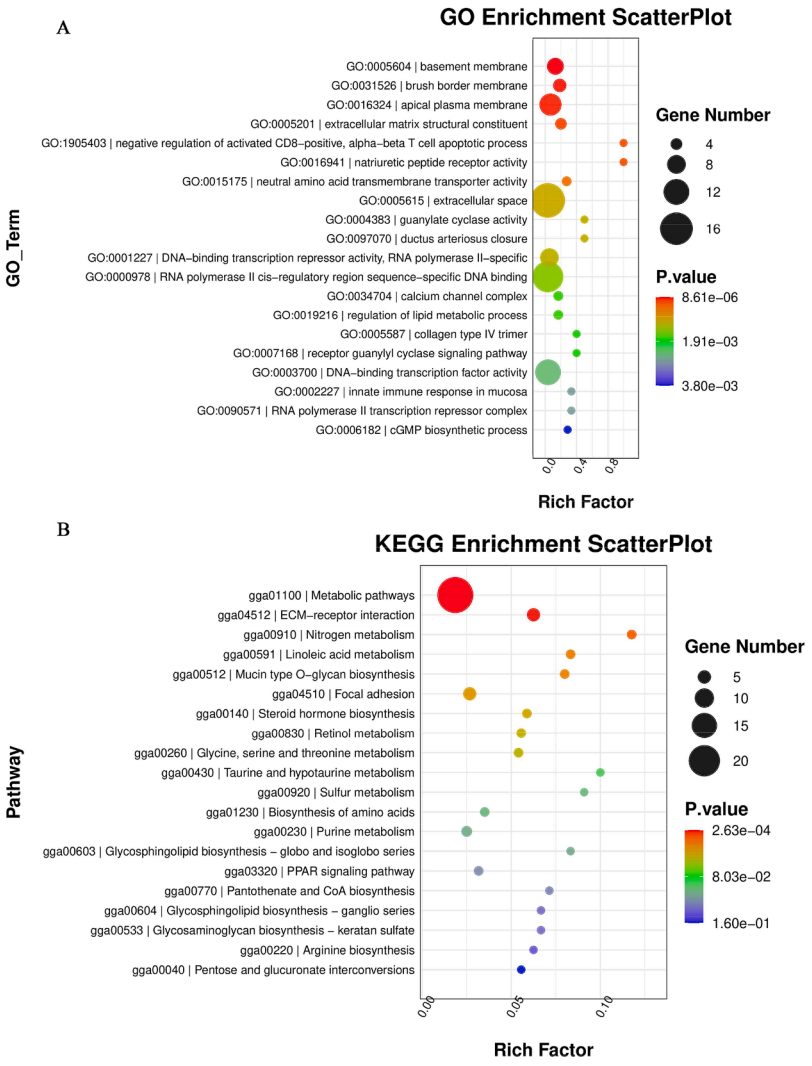

Fig. 3. Functional enrichment analyses of DEGs. (A).The top 20 enriched GO terms of DEGs. The X-axis refers to the rich factor. The Y axis refers to different GO terms. (B). The top 20 enriched KEGG pathways of DEGs. The X-axis refers to the rich factor. The Y axis refers to different KEGG pathways.

Table 8
GO terms potentially related to meat and meat flavor.

Term	Count	Gene list
Extracellular matrix structural constituent	4	*LAMB1, COL4A1, COL3A1, COL4A4*
Cholesterol metabolic process	3	*PCSK9, INSIG1, APOA4*
Fatty acid transport	2	*EXFABP, SLC27A1*
Sarcosine oxidase activity	1	*PIPOX*
Negative regulation of skeletal muscle tissue growth	1	*TLL2*
Amino acid transport	2	*SLC6A20, SFXN2*
Short-chain fatty acid transmembrane transporter activity	1	*SLC5A8*
Acetyl-CoA hydrolase activity	1	*ACOT12*
Regulation of gluconeogenesis	2	*FOXO1, PDK2*
Creatinine metabolic process	1	*MME*
Regulation of lipid metabolic process	3	*NR1D1, NR1D2, BBS4*
Response to lipid hydroperoxide	1	*APOA4*

such as nicotinamide riboside cation, 4-imidazoleacrylic acid, pantothenic acid, (R)-3-hydroxybutyric acid, DL-beta-hydroxybutyric acid, and palmitic acid may play a crucial role in flavor development.

Table 9
Significantly enriched KEGG pathways potentially related to muscle quality and flavor.

Term	Count	Gene list
ECM-receptor interaction	5	*LAMB1, COL4A1, COL4A2, CHAD, COL4A4*
Linoleic acid metabolism	2	*CYP2C18, CYP3A5*
Glycine, serine, and threonine metabolism	2	*SARDH, PIPOX*
Steroid hormone biosynthesis	2	*CYP2C18, CYP3A5*
Nitrogen metabolism	2	*ENSGALG00010024007, CA2*

Discussion

Evaluating growth patterns in Wenchang chickens

Growth performance is a pivotal metric for the evaluation of the productivity and economic viability of livestock and poultry farming (Guo et al., 2021). Body weight serves as a direct indicator of the impact of rearing systems on the production efficiency of these animals. Moreover, body size selection is a critical tool in poultry genetic breeding, enhancing our understanding of the development and growth status of individual birds through direct and indirect measurements

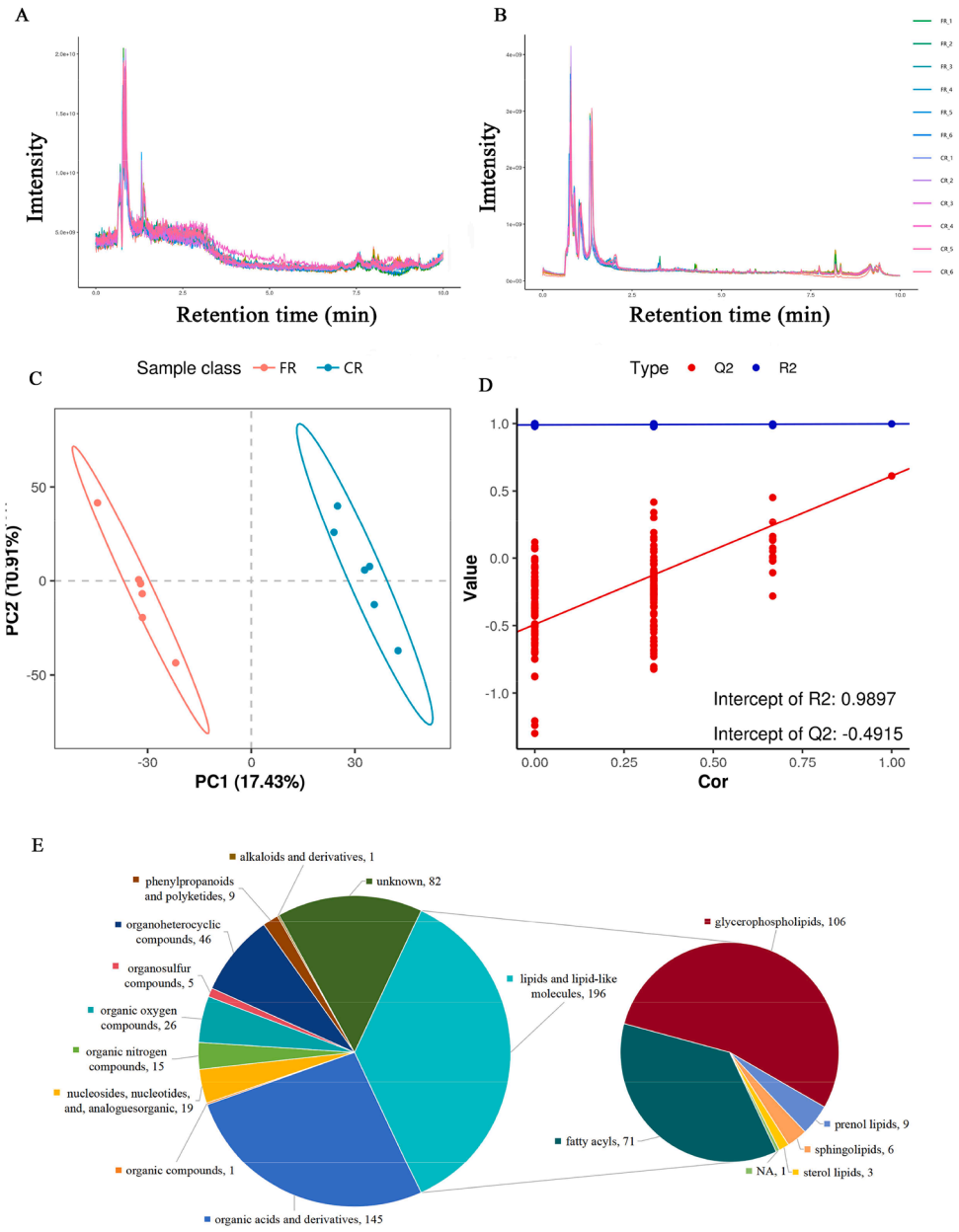

Fig. 4. Metabolite identification and correlation analysis. (A) Positive ion mode total ion chromatogram. (B) Negative ion mode total ion chromatogram. (C) Score plot of PLS-DA analysis of Wenchang Chicken breast muscle sample. (D) PLS-DA replacement test of Wenchang Chicken breast muscle sample. The PLS-DA analysis result were displayed as the two-dimensional principal components of PC1 and PC2. (E) Pie chart of metabolite identification.

(Anderson & Adams, 1994).

Research on rearing systems indicates that the body weight of CR chickens is generally higher than that of FR chickens, while different rearing systems have minimal impact on body size indicators (Tolon & Yalcin, 1997). In this study, the body weight of the CR group was significantly higher than that of the FR group, while the fossil bone length, pelvis width, and shank length were significantly lower. The results were consistent with previous research findings (Avila et al., 2023; Jin et al., 2019a; Tang et al., 2023) and may be attributed to the limited movement range of the chickens due to the confinement of the cage. This consequently reduces their energy expenditure and thus increases feed conversion efficiency. In addition, the living environment for caged chickens is more stable, allowing them to focus on productive activities. In contrast, FR chickens may experience disruptions in their production progress due to environmental disturbances or the threat of predators. In conclusion, FR may offer benefits in terms of certain body size indicators, while CR can increase body weight.

Interpreting variations in slaughter metrics

Slaughter performance is an important means of evaluating the meat quality of poultry. Poultry with good meat quality should have a slaughter rate of no less than 80 % and a dressing percentage of no less than 60 %. In this experiment, the slaughter rate of Wenchang chicken under different rearing systems was higher than 80 %, and the dressing percentage was higher than 60 %. This indicates that Wenchang chicken has good meat quality. The slaughter performance of poultry is influenced by several factors, such as the nutritional level, disease, environment, and rearing systems (Liang et al., 2022; Trocino et al., 2015; Wang et al., 2024). Scholars have determined the fat level in CR environments to exceed that in FR environments, with significant increases in intramuscular fat rate, the subcutaneous fat rate, and the abdominal

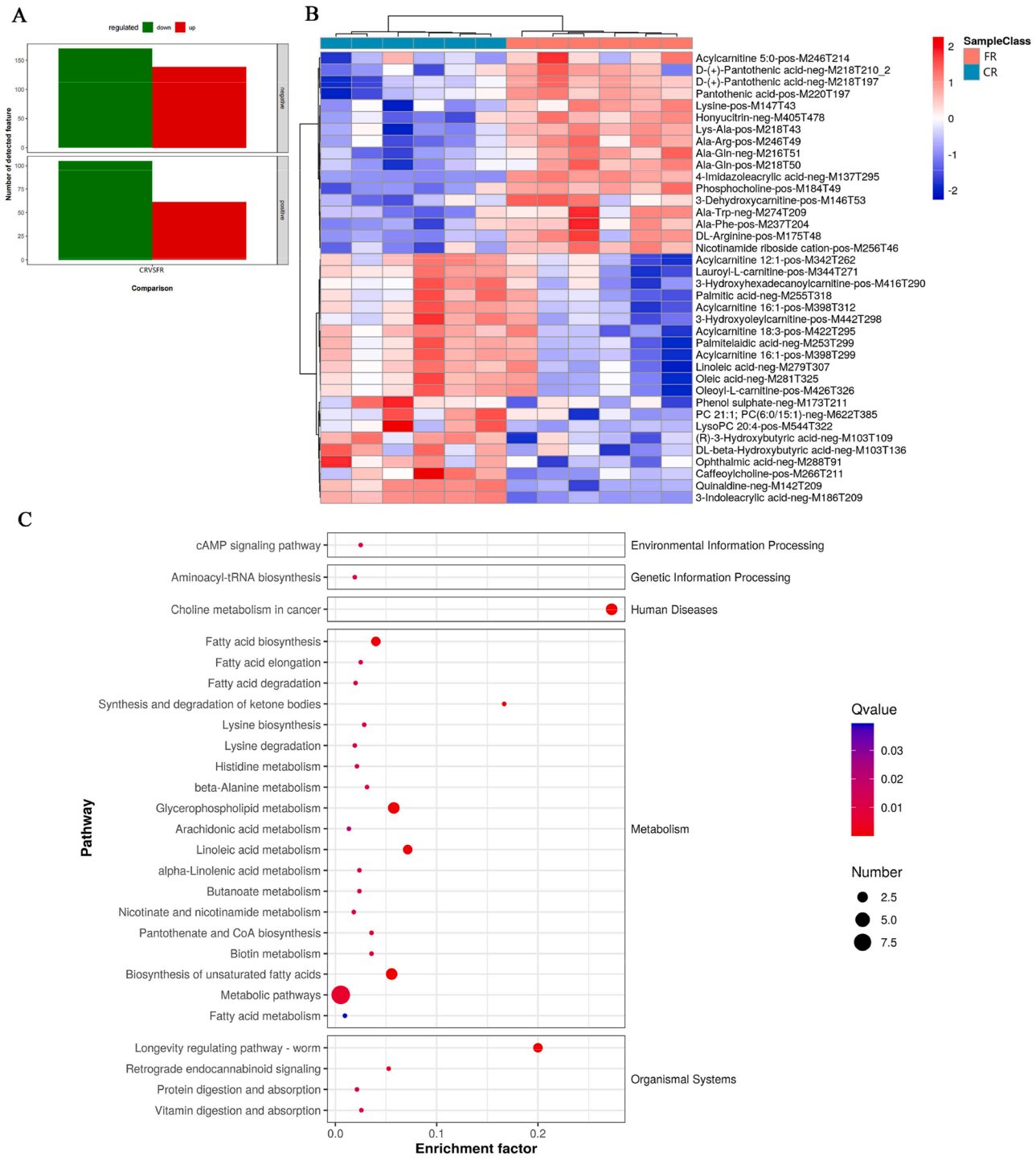

Fig. 5. Cluster analysis and pathway analysis of DMs. (A) Statistical histogram of differential metabolic ions. (B) Cluster analysis of DMs in Wenchang Chicken breast muscle. (C) Bubble map of differential metabolite pathway enrichment in Wenchang Chicken.

fat rate compared to FR (Yamak et al., 2018). This may be because FR chickens have a greater energy expenditure, which is not conducive to fat deposition. In this experiment, the live weight, slaughter weight, eviscerated weight, half-eviscerated weight, breast muscle weight, abdominal fat weight, percentage of abdominal fat, percentage of half-eviscerated yield, and percentage of eviscerated yield of the CR group were significantly higher than those of the FR group. This may be due to the larger activity range of FR chickens, which provides greater stimulation to the development of the breast and leg muscles due to running and jumping. The higher breast muscle weight in the CR group may also be attributed to their larger body weight. Although there were no significant differences in leg muscles between the two groups, the leg muscle percentage was smaller in the CR group. This is consistent with previous research (Bai et al., 2022). In summary, the CR group has an absolute advantage in terms of body weight and slaughter performance, except for the leg muscle rate, which is significantly lower than that of the FR group. This may be related to the longer shank length observed in the FR group.

Table 10
Metabolites potentially related to flavor compounds and their precursors.

Pathway	Metabolite	Pathway ID
Biosynthesis of amino acids	L-lysine	map01230
Fatty acid metabolism	Hexadecanoic acid	map01212
Biosynthesis of unsaturated fatty acids	Hexadecanoic acid;(9Z)-octadecenoic acid;linoleate	map01040
Glycerophospholipid metabolism	Phosphatidylcholine;choline phosphate;1-Acyl-sn-glycero-3-phosphocholine	map00564
Fatty acid biosynthesis	Hexadecanoic acid;(9Z)-octadecenoic acid	map00061
Linoleic acid metabolism	Phosphatidylcholine;linoleate	map00591
alpha-Linolenic acid metabolism	Phosphatidylcholine	map00592
Arachidonic acid metabolism	Phosphatidylcholine	map00590
Fatty acid degradation	Hexadecanoic acid	map00071
Synthesis and degradation of ketone bodies	(R)-3-hydroxybutanoate	map00072
Biotin metabolism	L-lysine	map00780
Nicotinate and nicotinamide metabolism	Nicotinamide-beta-riboside	map00760
Pantothenate and CoA biosynthesis	Pantothenate	map00770
Beta-alanine metabolism	Pantothenate	map00410
Protein digestion and absorption	L-lysine	map04974
Vitamin digestion and absorption	Pantothenate	map04977
Metabolic pathways	L-lysine;phosphatidylcholine; hexadecanoic acid;choline phosphate; urocanate;pantothenate;(R)-3-hydroxybutanoate;linoleate; nicotinamide-beta-riboside	map01100

Insights into fat metabolism and serum indicators

TG is a major component of vegetable oils and animal fats (Gupta et al., 2003). The TG in the feed can be broken down into glycerol and free FAs in the intestine to then enter the bloodstream. Once in the blood, free FAs were transported to adipose cells by lipoproteins, where they can be used to re-synthesize TG for storage (Lee et al., 2022). Scholars have shown that chickens raised in FR systems tend to have lower levels of TG and TC in their blood compared to chickens in other rearing systems (Krawczyk et al., 2011). In this experiment, the serum TG and TC levels in the FR group were significantly lower than those in the CR group, consistent with the aforementioned findings and indicating that the CR system is more conducive to fat deposition. HDL transports cholesterol from aging cell membranes and plasma to the liver for metabolism, while LDL transports endogenous cholesterol synthesized by the liver to various tissues (Lewis & Rader, 2005; Rosendorff, 2002). HDL reduces the accumulation of cholesterol on the blood vessel walls, while LDL has the opposite effect. Previous research found that the HDL content in chicken serum increases with the number of days spent in an FR system, with no effects observed for LDL levels (Jin et al., 2019b). In this experiment, there were no significant differences in the levels of HDL and LDL between the two rearing systems, which may be due to the relatively short duration of the rearing systems of Wenchang chicken. FAS, a key enzyme in fatty acid synthesis, consists of multiple enzymes that catalyze intermediate reactions in fatty acid synthesis (Liu et al., 2010). In this experiment, the levels of LPL, HSL, and FAS in the serum of the CR group were significantly higher than those in the FR group, suggesting that the adipose tissue of the CR group was more developed and had a stronger capacity for fat mobilization, which also confirmed the higher TG levels in the serum of the CR group. TSH maintains the body's energy balance and can regulate lipid metabolism. Studies have shown that an increase in TSH levels can accelerate fat breakdown in mice. In this experiment, the TSH levels in the serum of the CR group were significantly higher than those in the FR group, which may be due to the greater energy expenditure and lower efficiency of fat deposition in the FR chickens during the rearing process. LEP in poultry can indirectly regulate the body's energy balance by controlling appetite through the sympathetic nervous system. Scholars have reported that the levels of LEP are higher in obese individuals, which is considered to be a phenomenon of LEP resistance, where the continuously high concentration of LEP in adipose tissue reduces the body's sensitivity to LEP (Zhao et al., 2019). Therefore, LEP can be used as a marker of body fat in animals. In this experiment, the LEP levels were higher in the CR group with more fat content, which aligns with the aforementioned findings in the literature. ADPN is a secretory protein derived from adipose tissue. Although it originates from fat tissue, the circulating levels of ADPN were reported to be inversely correlated to the degree of obesity (Arita et al., 1999). ADPN can promote lipid metabolism and accelerate energy supply(Cai et al., 2021). In this experiment, the levels of ADPN in the serum of the caged group were significantly lower than those in the FR group. This may indicate that FR chickens have a higher level of activity, requiring more energy, which in turn increases the expression of ADPN and its receptors.

Understanding meat quality determinants

Meat quality indicators were an important basis for evaluating the quality of livestock and poultry meat. Most research suggests that although FR rearing has a negative impact on the slaughter weight of poultry, it has a positive effect on meat quality (Almasi et al., 2015; Chen et al., 2018). Meat color is a key factor influencing consumer purchasing decisions. Factors that affect changes in chicken meat color include the age of the chicken, genetics, muscle glycogen storage, and the stress state of the broiler before slaughter (Baéza et al., 2022). Ponte et al. (2008) found that FR poultry has higher a* value than caged poultry. Castellini et al. (2002) suggested that the higher a* value in the muscles of FR broilers may be attributed to their higher level of activity. In this experiment, the a* value of the pectoral muscle of the FR group was significantly higher than that of the CR group. This is consistent with the aforementioned studies and indicates that the FR can improve the meat color of broilers. The muscle pH value is closely related to other meat quality indicators, and extensive research has proven that the extent of glycolysis directly affects muscle pH (Listrat et al., 2016). Semwogerere et al. (2018) reported that the rearing method does not affect the pH value of meat. Similarly, we did not observe any significant differences in the muscle pH between the CR and FR groups. Drip loss is an important indicator of the water-holding capacity of chickens. A poor water-holding capacity can affect the sensory characteristics of muscles. Baeza et al. (2002) found that as the slaughter age of poultry increases, drip loss gradually decreases due to the potentially lower water content in the muscles of older poultry. Endo (Endo, 2015) showed that FR chickens have more hemoglobin in their muscles and firmer muscles than caged chickens, indicating that greater physical activity can increase the muscle water-holding capacity and reduce drip loss. In this experiment, there were no significant differences in drip loss between the CR and FR groups, yet the drip loss of the FR group was less than that of the CR group. This may be due to the relatively mature development of the muscles of Wenchang chicken before the application of the two rearing systems. Shear force is an indicator of muscle tenderness, and the lower the shear force, the higher the tenderness of the muscle. The magnitude of the shear force can reflect characteristics such as the proportion of muscle fibers and connective tissue and the content, distribution, and structure of intramuscular fat in poultry muscles. Studies have shown that exercise can affect the proportion of muscle fibers, increasing the number of myoglobin. This is particularly true for explosive movements such as running and jumping. In this experiment, the shear force of the leg muscles in the FR group was significantly higher than that in the CR group. FR chickens have larger muscle fiber diameters and higher shear forces than caged chickens. This may be

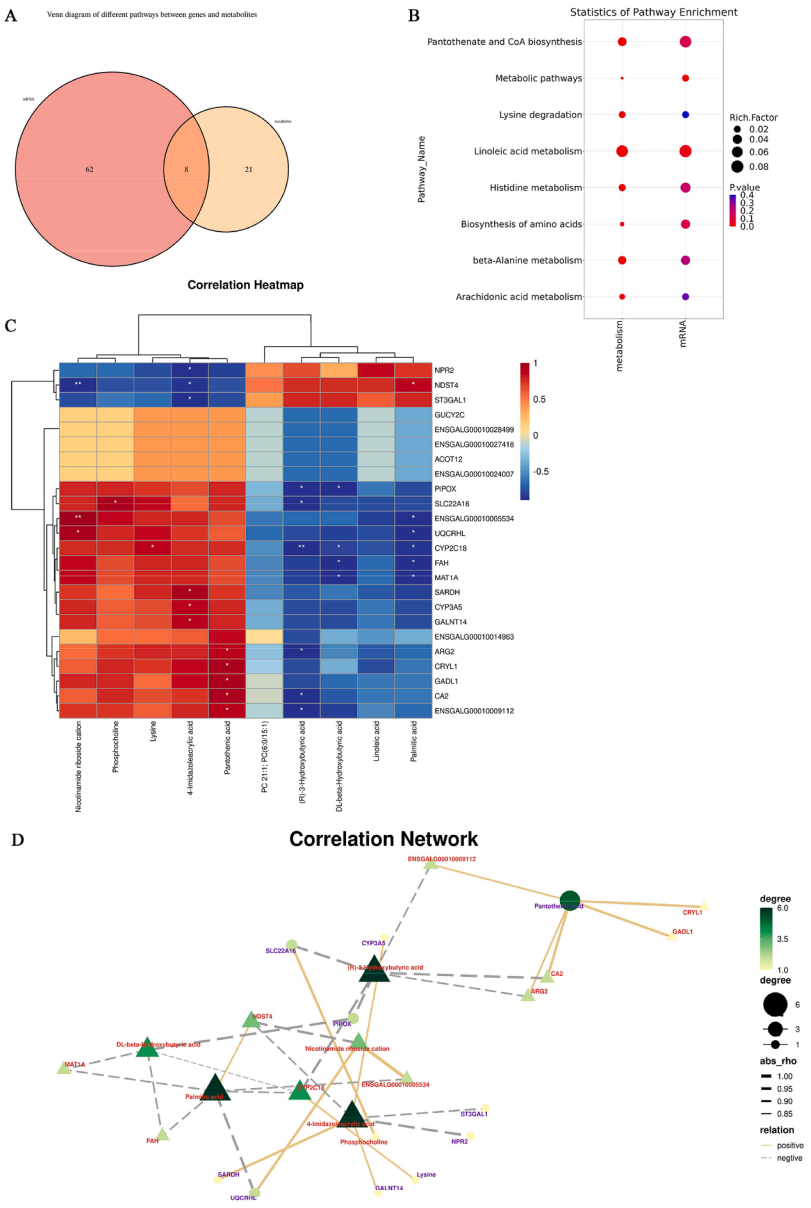

Fig. 6. Integrative analysis of the transcriptome and metabolome. (A) Venn diagram of different pathways between genes and metabolites. (B) Bubble map of the common pathways of DMs and differential genes. (C) Correlation heat map of DMs with DEGs. (D) Association network diagram of DMs with DEGs.

linked to the ability of exercise to promote the development of muscle fiber tissue.

Flavor profiles and their underlying factors

IMP affects the volatile aroma of chicken meat, primarily contributing to its umami taste. The IMP content in chicken is mainly influenced by factors such as the genetics, age, nutritional level, additives, rearing systems, and post-slaughter storage conditions of the chicken. Scholars generally consider the flavor of chicken from FR environments to be superior to chicken from CR environments. Our results revealed that the IMP content in the breast muscle of the FR group was significantly higher than that of the CR group, while there were no significant differences between the FR and CR groups in the leg muscle.

AAs were also closely related to the umami taste of meat. The type and proportion of AAs in meat proteins can result in different flavors (Long et al., 2016). AAs that affect flavor include Gly, Ala, Asp, Glu, Tyr, and Phe. The combination of these AAs can also produce different tastes, with Glu, Ala, Asp, and Gly identified as main contributors to the umami taste of chicken, while combinations of Tyr and Phe can produce bitterness, and combinations of His, Asp, and Glu can produce sourness (Rabie et al., 2014). In this experiment, the Glu content in the breast muscle of the CR group was significantly lower than that of the FR group, and the contents of Gly, Ala, and Phe in the leg muscle were significantly lower than that of the FR group. However, the two groups had no significant differences in the contents of FAA and EAA. These results suggest that the deposition effect of some flavor AAs in FR chickens is stronger compared to CR chickens, yet the overall differences between the two groups were not significant. The contents of the essential AAs Thr, Pro, and His were significantly different in the leg and breast muscles, which merits further consideration. Thr plays a crucial role in promoting antibody production and enhancing the immune system, while Pro aids in the synthesis of collagen, elastin, and muscle tissue, helping to maintain the strength and flexibility of connective tissue and muscles. His functions as a cofactor for certain enzymes and is involved in neurotransmission and acid-base balance. The differences in these essential AAs may be related to the varying disease resistance of chickens under different rearing conditions.

The composition and content of FAs were important indicators of meat quality (Skrivan et al., 2000). Scholars have reported that the main FAs present in meat include saturated FAs such as C14:0, C16:0, and C18:0, monounsaturated FAs such as C16:1 and C18:1, and polyunsaturated FAs such as C18:2, C18:3, and C20:4 (Agostoni & Bruzzese, 1992). The content of unsaturated FAs has the greatest impact on the formation of meat flavor. For example, unsaturated FAs such as oleic acid and linoleic acid interact or break down during the heating process of meat, ultimately forming aldehydes, esters, alcohols, ketones, carboxylic acids, and hydrocarbons, which give meat a rich aroma (Choe & Min, 2007). In this experiment, four differential unsaturated FAs were detected in the breast muscle, with the contents of C17:1,1n7 and C18:2, n6c (C18:1, n9t and C18:3, n3) determined to be higher (lower) in the CR group than in the FR group. No differential unsaturated FAs were detected in the leg muscle, while the content of MUFAs in the CR group was higher than in the FR group. MUFAs are typically liquid at room temperature and are beneficial for heart health as they can increase HDL levels while lowering LDL levels. Unsaturated FAs are generally considered healthier than saturated fats as they help improve lipid profiles and reduce the risk of cardiovascular diseases. The CR group exhibited a higher content of unsaturated FAs. However, it is important to note that the excessive intake of any type of fat can lead to weight gain and health issues. The higher content of unsaturated FAs in the CR group may be attributed to the richer fat deposition in this group, which is more conducive to the production of unsaturated FAs. This is consistent with the results of the serum lipid metabolism measurements. In summary, the CR and FR systems have beneficial impacts on the flavor of Wenchang chicken muscle. The FR system is more conducive to the increase in IMP content in the breast muscle and the deposition of some flavor AAs. The overall content of flavor AAs in the CR group is not significantly different from that in the FR group, and the deposition of unsaturated FAs is richer in the former.

Electronic nose analysis

Electronic nose technology uses a chemical sensor system to analyze odors and is capable of detecting various types of gases and complex scents (Wojnowski et al., 2017). The electronic nose plays a crucial role in assessing volatile compounds in the food industry and the olfactory quality of meat substances (Ghasemi-Varnamkhasti et al., 2009). Studies have shown that the flavor of poultry meat is positively correlated with fat content as the oxidation of fat produces a variety of flavor substances (Chartrin et al., 2006; Chen et al., 2021). Therefore, poultry meats with significant differences in fat content and fatty acid composition also exhibit differences in the volatile substances produced after cooking (Xia et al., 2021). In this experiment, there was a clear distinction between the CR and FR white-cut chicken, indicating that CR and FR significantly alter the muscle flavor of white-cut Wenchang chicken. There was some overlap between the CR and FR groups in terms of the salt-baked chicken flavor (Jiang et al., 2022). Many FAs, particularly unsaturated FAs, can degrade at high temperatures. During the processing of salt-baked chicken, the temperature exceeds that of white-cut chicken, which likely leads to the degradation of the different FAs produced under various rearing environments.

Transcriptomic and untargeted metabolomic analysis

Chicken flavor primarily depends on the content of IMP, AAs, and FAs (Ferrini et al., 2008). Both transcriptomic and metabolomic data indicate that lipids, FAs, free AAs, sugars, and nucleotides were influenced by the rearing system. In this experiment, the FA content and fat deposition capacity of Wenchang chicken in the CR group was significantly higher compared to the FR group. Thus, genes and metabolites related to fat deposition and FA metabolism are of particular interest.

The transcriptomic data resulting from GO enrichment and KEGG pathway analysis identified a series of genes involved in lipid deposition and FA metabolism, including *PCSK9* and *SLC27A1*. *PCSK9* (Proprotein Convertase Subtilisin/Kexin Type 9) is a protease involved in cholesterol metabolism, regulating the degradation of the low-density lipoprotein receptor (LDLR) in the liver (Wu & Ballantyne, 2017). In this experiment, the DEG *PCSK9* was downregulated in the CR group compared to the FR group, indicating that the CR group has a richer content of FAs and stronger fat deposition capacity. The down-regulation of *PCSK9* can lead to an increase in LDLR, allowing LDL cholesterol in the plasma to bind to the *LDLR*, and it is subsequently absorbed by cells. This facilitates the deposition of fat in adipose tissue (Stoekenbroek et al., 2018), which is consistent with the aforementioned findings.

The DMs and KEGG pathway results of Wenchang chicken muscles indicate that the impact of different rearing systems on muscle metabolism in Wenchang chicken is mainly observed in lipid metabolism. 3-Hydroxybutyric acid, a degradation product of branched-chain AAs released from muscles, can provide acetyl-CoA for the synthesis of cholesterol, FAs, and complex lipids (Innis, 2016). The upregulation of 3-hydroxybutyric acid content in the CR group suggests that these chickens have more vigorous fat metabolism (Tokiwa & Ugwu, 2007). Palmitoleic acid is a trans-fatty acid that can alter the phenotypic changes of plasma lipids and lipoproteins, but the mechanisms involved remain unclear. Linoleic acid is a polyunsaturated fatty acid involved in the metabolism of alpha-linolenic acid. Linoleic acid cannot be synthesized in the body and must be obtained from the diet. The upregulation of linoleic acid content in the meat of caged chickens indicates higher nutritional value. Oleic acid is the most abundant fatty acid in adipose tissue, and some studies suggest that consuming oleic acid can increase HDL and decrease LDL levels (Ali Abd El-Aal et al., 2019). Lysophosphatidylcholine (lysoPC) is a monoacylglycerophospholipid. LysoPC with different lengths and saturations of fatty acids linked at the C-1 position—especially lysoPC (20:4 (5Z, 8Z, 11Z, 14Z)) composed of the arachidonic acid chain at the C-1 position—can transmit lipid signals by binding to their receptors (Oishi et al., 1988). In this experiment, lipid substances such as 3-hydroxybutyric acid, linoleic acid, and oleic acid were generally upregulated in the CR group. This indicates that chickens in the CR group exhibit a greater fat deposition.

In this study, we found that the transcriptional levels of *FAH, CYP2C18*, and *UQCRHL* were significantly negatively correlated with palmitic acid. Previous research has shown that FAH plays a role in the metabolism of the tyrosine (Yang et al., 2019). The enzyme encoded by the *CYP2C18* gene is involved in the metabolism of various endogenous and exogenous compounds (Lofgren et al., 2009). The protein encoded by *UQCRHL* is a component of the mitochondrial electron transport chain, specifically part of ubiquinol-cytochrome c reductase (Complex III). *UQCRHL* is involved in transferring electrons from ubiquinol to cytochrome c, a process that is crucial for cellular energy (ATP) production (Park et al., 2017). In this experiment, compared to the FR group, the transcriptional levels of *FAH, CYP2C18*, and *UQCRHL* were downregulated in the CR group, while the content of palmitic acid was upregulated. We speculate that under the regulation of *FAH, CYP2C18*, and *UQCRHL*, the increased content of palmitic acid contributed to the substantial accumulation of fat in the CR group. Our results suggest that these genes may also be involved in fat metabolism, and their specific regulatory roles need to be confirmed through further research. Integrative analysis of the eight significantly enriched KEGG pathways from DEGs and DMs revealed four pathways to be related to amino acid metabolism and the other four to fatty acid metabolism. Currently, the pathways “pantothenate and CoA biosynthesis,” “metabolic pathways,” “lysine degradation,” “linoleic acid metabolism,” “histidine metabolism,” “biosynthesis of AAs,” “beta-alanine metabolism,” and “arachidonic acid metabolism” have been confirmed to play important roles in regulating the flavor of chicken (Gai et al., 2023), duck (Luo et al., 2023; Weng et al., 2022), goose (Cao et al., 2024; Hong et al., 2024), and pig (Wang et al., 2022) meat. These pathways are also enriched in the analysis. The CR and FR groups influence the quality of Wenchang chicken meat by modulating lipid and amino acid metabolic pathways.

We observed that the lipid metabolites involved in the network (linoleic acid, phosphocholine, PC 21:1; PC(6:0/15:1), (R)-3-hydroxybutyric acid, DL-beta-hydroxybutyric acid, linoleic acid, and palmitic acid) were all upregulated in the CR group. These results show that the CR group induced the activity and expression of enzymes and genes related to fatty acid transport and metabolism. This promoted the synthesis and storage process of lipid compounds such as linoleic acid and palmitic acid, thereby increasing fat deposition and the content of unsaturated fatty acids in Wenchang chicken.

The association network diagram of DMs with DEGs reveals that *NDST4* and *CYP2C18* may serve as key genes influencing meat flavor. Specifically, *NDST4* is suggested to positively regulate nicotinamide riboside cation and negatively regulate 4-lmidazoleacrylic acid. Conversely, *CYP2C18* is associated with the negative regulation of metabolites such as (R)-3-hydroxybutyric acid, palmitic acid, and DL-beta-hydroxybutyric acid. These metabolites play crucial roles in various metabolic pathways, including pantothenate and CoA biosynthesis, linoleic acid metabolism, and arachidonic acid metabolism, thereby regulating fat deposition. In addition, they are involved in pathways such as lysine degradation, histidine metabolism, amino acid biosynthesis, and beta-alanine metabolism, which collectively contribute to the modulation of meat quality and flavor.

Conclusions

This study highlights the effects of rearing systems on Wenchang chickens. The CR system significantly increases body weight and enhances slaughter performance, while promoting fat deposition and metabolism. In contrast, the FR system improves skeletal development and enhances meat quality with higher levels of IMP and flavor-related amino acids. Transcriptomic and metabolomic analyses indicate that the main differences in muscle composition are linked to unsaturated fatty acid biosynthesis and glycerophospholipid metabolism. These findings provide insights into optimizing rearing methods for improved production and meat quality.

Funding

This work was supported by the Key R&D Projects of Hainan Province (ZDYF2024XDNY274, ZDYF2023XDNY036, ZDYF2024XDNY271) and National Key R&D Project (2021YFD1300105, 2021YFD1300100).

Data availability statement

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Ethics statement

The animal study protocol was approved by the Institutional Animal Care and Use Committee at the Experimental Animal Center of Hainan Academy of Agricultural Science (HNXMSY-20221316).

Glossary

ADPN: Adiponectin
Ala: Alanine
AAs: Amino Acids
Arg: Arginine
Asp: Aspartic Acid
DEGs: Differentially Expressed Genes
DMs: Differential Metabolites
EAA: Essential Amino Acids
FAs: Fatty Acids
FAS: Fatty Acid Synthase
FAA: Flavor Amino Acids
Glu: Glutamic Acid
GO: Gene Ontology
Gly: Glycine
HDL: High-Density Lipoprotein
His: Histidine
HSL: Hormone-Sensitive Lipase
IMP: Inosine Monophosphate
Ile: Isoleucine
LPL: Lipoprotein Lipase
Leu: Leucine
LEP: Leptin
Lys: Lysine
Met: Methionine
Phe: Phenylalanine
Pro: Proline
Ser: Serine
TC: Total Cholesterol
TG: Triglycerides
Thr: Threonine
TSH: Thyroid-Stimulating Hormone
Tyr: Tyrosine
Val: Valine
VIP: Variable Importance in Projection

CRediT authorship contribution statement

Tieshan Xu: Conceptualization, Methodology, Writing – review & editing. **Qicheng Jiang:** Writing – original draft. **Chaohua Xu:** Data curation, Writing – original draft. **Zhepeng Xiao:** Formal analysis, Software. **Xinli Zheng:** Investigation, Resources. **Lihong Gu:** Investigation, Funding acquisition, Supervision.

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psj.2025.105043.

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