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RESEARCH ARTICLE

Developmental Trajectories of Gut Microbiota and Meat Metabolome Reveal the Gut-Muscle Axis in Wuding Broilers

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ABSTRACT

Gut microbiota plays a crucial role in maintaining health and improving meat quality in broilers. In this study, an approach integrating quantitative metabolomics and metatranscriptomics was applied to investigate meat quality and gut microbiota in 90-day-old, 165-day-old, and 240-day-old Wuding chickens. The breast of 90-day-old broilers contained higher levels of amino acids (AAs) and small peptides, especially those contributing to umami flavor. Furthermore, multiple flavor substances that provide sweet flavor were observed to be more abundant in the breast of 90-day-old chicks. In contrast, 240-day-old broilers had higher contents of DPA and DHA in the breast. According to the analysis of meta-transcriptomics, the richness of active gut microbiota was found to be increased with the developmental trajectory, resulting in more complexity and stability of active gut microbiota in mature broilers. Comparison of the gene expression profiles in breast muscle indicated significant up-regulated genes related to the biosynthesis of AAs in 90-day-old chicks, which could contribute to higher levels of AAs and small peptides. Short-chain fatty acids were produced by active gut microbiota in mature broilers, which are associated with the more abundance of DPA and DHA in breast meat. This study represents a comprehensive effort to optimize broiler production through understanding the complex interplay between gut microbiology and meat quality.

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INTRODUCTION

Gut microbiota acts as a vital functional ecosystem in livestock and poultry, enhancing host immunity and thereby regulating growth performance and health (Zeng *et al*., 2024). A complex bidirectional regulatory network exists between muscle quality and gut microbial communities and is often conceptualized as the gut-muscle axis (Grosicki *et al*., 2018). Gut microbes directly impact muscle energy metabolism and protein synthesis (Li *et al*., 2024a). Furthermore, metabolites produced by muscle activity, such as lactic acid and ammonia, provide feedback that regulates the gut microbiota composition (Li *et al*., 2024b). The mechanisms underlying this gut-muscle crosstalk involve microbial metabolites (e.g., AAs or SCFAs), modulation of lipid metabolism enzymes, systemic inflammation, nutrient metabolism, and hormonal signaling pathways, all contributing to muscle quality regulation (Li *et al*., 2022). Although existing studies have shown that the intestinal-muscle axis plays a significant role in meat quality, few reports have investigated the relationship between microorganisms and muscle development trajectories, particularly from the dual perspective of nutrition and flavor.

Some Chinese indigenous chicken breeds, such as the Wuding broiler (WD, black bone type) exhibit significant advantages in meat quality, offering both nutritional and

health benefits (Xiao *et al*., 2024). Originating from Wuding County (Yunnan Province), Wuding broiler is an outstanding Chinese indigenous breed that is renowned for its tender texture and rich flavor profile (Dou *et al*., 2017). Its muscle tissue is abundant in polyunsaturated fatty acids (PUFAs), umami precursors (e.g., glutamic acid and aspartic acid), and umami peptides, which collectively contribute to its distinctive mellow savory taste and nutritional value (Xiao *et al*., 2024). Due to their distinctive growth performance and body composition, Wuding broilers serve as an excellent model for studying biological mechanisms underlying broiler chicken growth and muscle development (Hu *et al*., 2024a).

In this study, Wuding broilers of different ages were investigated using a multi-omics strategy, integrating quantitative muscle tissue metabolomics and gut microbiota meta-transcriptomics with the muscle transcriptomics as a link. Variations in several muscle quality indices, including AAs, peptides, FAs, organic acids, and nucleotides, during the development of Wuding broilers were explored, and their associations with the variations in gene expression in the breast tissues were also analyzed. Furthermore, the gut microbial activities of Wuding broilers throughout their development were uncovered to identify potential correlations with the muscle quality. This study enables us to comprehensively grasp the dynamic changes in muscle quality and their underlying mechanisms during the development of Wuding broilers. Accordingly, this can provide guidance for the scientific breeding of Wuding chickens and the production of higher-quality products.

MATERIALS AND METHODS

Experiment design and sample collection: The experiment in this study was approved by the Animal Care and Use Committee of the Yunan Agricultural University (No. 202311007). A total of 2,400 male Wuding broilers (Zhenji Agricultural Science and Technology Development Co., Ltd.) were raised in a single pen using a conventional method of stocking and feeding. All experimental broilers were provided with feed and water *ad-libitum* under consistent environmental conditions. The basic diets were formulated according to the "Nutrient requirements of yellow chickens (NYT3645-2020)", and their nutritional compositions are shown in Table S1. From this group, eight broilers per group (the same age with similar body weights) were selected for slaughter. The pectoralis breasts and cecal digesta were then collected for analysis. The obtained breast and cecal digesta samples were stored at -80°C for further analysis.

Quantitative metabolomics (targeted 700 metabolites: T700) for breasts: The meat AAs, peptides, AA derivatives, organic acids, nucleotides and other substances were detected by T700 method. All authentic metabolite standards were obtained from Metware Biotechnology Co., Ltd. (Wuhan, China). Quantitative metabolomics was performed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) using a QTRAP 6500+ system (AB SCIEX Corp, Boston, MA, USA) at the Wuhan Metware Co., Ltd. (Wuhan, China).

Determination of free fatty acids (FFA) in breasts: The FFA analysis was performed by Gas Chromatography (GC)-MS/MS using an Agilent 8890-7000E system at the Wuhan Metware Co., Ltd. (Wuhan, China). Breast sample processing was performed according to Marvell's FFA standard procedures. The operating procedure was carried out in accordance with Sun *et al*. (2022).

Measurements of flavor substances in breasts: The flavor substances in the breasts were also measured at the Wuhan Metware Co., Ltd. by GC-MS/MS using the Agilent 8890-7000D system. The operating procedure was carried out in accordance with Zhang *et al*. (2025). The raw mass spectrometry data were processed using the MassHunter software to identify flavor substances, and their contents were calculated using an internal standard semi-quantitative method according to a previous study (Huang *et al*., 2024).

Transcriptomics sequencing for breasts: Total RNA of breast tissues was extracted using the TRIzol reagent kit (Invitrogen, Carlsbad, CA, USA). After check, sequencing libraries (paired-end 150 bp) were constructed using the TruSeqTM RNA sample preparation Kit (Illumina, San Diego, CA, USA) and sequenced on an Illumina NovaSeq 6000 platform at Shanghai BIOZERON Co., Ltd (Shanghai, China). Bioinformatics analysis methods were performed according to Hu *et al*. (2024b).

Targeted metabolomics for SCFAs in cecal digesta: An ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS) system (ExionLC™ AD UHPLC-QTRAP 6500+, AB SCIEX Corp.) was used to quantify SCFAs and AAs. The operating procedure was carried out in accordance with Hu *et al*. (2024c).

Metatranscriptomics sequencing of cecal digesta: Total RNA from the cecal digesta was extracted using the TRIzol Reagent (Invitrogen). After quality check, the meta-transcriptome libraries (450 bp) were constructed using the TruSeqTM Stranded Total RNA Sample Preparation Kit (Illumina) and sequenced by Illumina NovaSeq 6000 platform with a paired-end 150 bp strategy. Bioinformatics analysis including transcripts per million (TPM), the abundance table of the active gut microbiota, the functional genes related to SCFA synthesis, and KEGG were performed by Shanghai BIOZERON Co., Ltd.

Statistical analysis: Variations in the contents of metabolites identified in the breasts of the Wuding broilers at different developmental stages were tested using Duncan's multiple range tests in one-way ANOVA with SPSS 18.0. The gut SCFAs and microbiota were analyzed using R v4.2.2 software, and the differences were tested using Dunn's test. A $P<0.05$ was considered statistically significant. The correlations between the key indices of active gut microbiota and the contents of breast differentially abundant metabolites were analyzed using Spearman's correlation method ("psych" package) to explore potential gut-muscle interactions.

RESULTS

FAs of breast meat in Wuding broilers: Compared with 240-day-old broilers, 90-day-old broilers exhibited higher levels of tridecanoic acid, palmitoleic acid, gamma-linolenic acid, and homo-gamma-linolenic acid in breast ($P<0.05$; Table 1). Conversely, some ω-3 PUFAs, including docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA), were more abundant in breast of 165-day-old and 240-day-old broilers ($P<0.05$; Table 1).

AAs, peptides, and AA derivatives of breast meat in Wuding broilers: Compared with 165-day-old and 240-day-old broilers, the 90-day-old broilers had higher levels of most AAs in the breast, including Ala, Ser, Arg, Tyr, L-Homocitrulline, Pro, Thr, Asp, Asn, and Leu ($P<0.05$; Table 2). Conversely, L-norvaline was present at significantly higher levels in 240-day-old broilers compared with 90-day-old and 165-day-old individuals. (Table 2). Furthermore, the contents of Homo-L-arginine and Gly were significantly higher in 90-day-old broilers compared with those of 240-day-old broilers ($P<0.05$; Table 2). Similarly, most peptides were also found to be continuously reduced throughout the developmental trajectory (Table S2). Only few histidine-related peptides (His-Ala and His-Ser) exhibited higher contents in samples from old broilers compared to those from 90-day-old chicks ($P<0.05$; Table S2).

Nucleotides of breast meat in Wuding broilers: Cytosine-associated substances, such as cytosine, isocytosine and 5-methylcytosine, were found to be more abundant in the breast of 90-day-old broilers compared with those of 165-day-old and 240-day-old broilers

Table 1: Contents of fatty acids in breast of Wuding broilers (μg/g)

Index	Compounds	90 days	165 days	240 days
C13:0	Tridecanoic acid	0.21±0.01^{a}	0.19±0.01^{ab}	0.17±0.01^{b}
C14:0	Tetradecanoic acid	4.56±0.43	5.08±1.33	2.65±0.18
C15:0	Pentadecanoic acid	4.07±0.28	3.96±0.77	3.01±0.13
C16:0	Hexadecanoic acid	224.66±19.87	307.89±61.34	225.35±13.73
C17:0	Heptadecanoic acid	4.03±0.22	4.47±1.04	3.34±0.18
C18:0	Octadecanoic acid	147.28±15.48	185.17±41.86	158.72±9.31
C19:0	Nonadecanoic acid	0.17±0.01	0.20±0.07	0.15±0.02
C20:0	Eicosanoic acid	2.16±0.14	2.07±0.49	1.63±0.12
C22:0	Docosanoic acid	0.47±0.06	0.47±0.10	0.33±0.02
C14:1n5c	Myristoleic acid	0.95±0.05	0.99±0.09	0.81±0.05
C15:1n5c	Cis-10-pentadecenoic acid	47.28±6.64	56.36±2.19	61.10±4.15
C16:1n7c	Palmitoleic acid	12.73±1.81^{a}	11.90±2.93^{a}	5.24±0.32^{b}
C18:1n9c	Oleic acid	236.90±20.36	328.58±67.2	198.82±20.42
C18:1n9t	Trans-oleic acid	36.48±3.43	54.43±9.65	38.50±3.12
C18:2n6c	Linoleic acid	403.23±34.48	417.56±87.88	276.33±26.38
C18:3n3c	Alpha-linolenic acid	14.70±1.29	15.32±5.18	7.12±0.52
C18:3n6c	Gamma-linolenic acid	0.95±0.05^{a}	0.91±0.11^{ab}	0.69±0.09^{b}
C20-1n9c	Gondoic acid	5.89±0.48	5.87±1.55	3.28±0.26
C20:2n6c	Homo-gamma-linoleic acid	19.59±2.97	17.61±5.50	10.45±1.19
C20:3n6c	Homo-gamma-linolenic acid	108.51±8.52^{a}	81.48±14.77^{ab}	55.37±3.24^{b}
C20:4n6c	Arachidonic acid	579.46±46.25	737.93±180.72	628.72±47.94
C22:4n6c	Adrenic acid	30.78±2.67	38.72±6.68	33.17±2.18
C22:5n3c	Docosapentaenoic acid (DPA)	30.16±3.01^{b}	48.40±8.11^{a}	48.89±4.75^{a}
C22:6n3c	Docosahexaenoic acid (DHA)	39.89±5.07^{b}	70.75±12.60^{a}	73.30±7.49^{a}

Different lowercases letters represent significant difference ($P<0.05$).

Table 2: Contents of amino acids in breast of Wuding broilers (ng/g)

Index	90 days	165 days	240 days
Ala	796872.51±36800.99^{a}	620046.21±31320.58^{b}	662753.69±33275.18^{b}
L-citrulline	10527.77±1615.32	11276.75±6150.4	7808.74±1536.29
L-Norvaline	153466.31±6619.05^{b}	150792.98±6169.96^{b}	170317.26±2526.92^{a}
Cys	16228.99±2578.35^{ab}	15160.63±3562.23^{b}	26388.12±4116.7^{a}
Homo-L-arginine	13829.72±2687.21^{a}	10067.87±862.61^{ab}	8340.15±862.55^{b}
Met	260876.83±11024.42	232822.04±15731.93	238208.89±5892.21
Phe	256615.83±11093.73	250191.48±7152.33	264781.43±4648.52
Ile	234836.73±17954.09	213131.58±18502.26	226214.57±8257.04
Ser	482495.59±20741.82^{a}	404912.75±17459.7^{b}	434981.06±14692.93^{b}
Arg	473006.95±30005.09^{a}	365548.2±26556.48^{b}	443589.03±14102.13^{a}
His	51416.2±3289.9	58177.7±2169.19	55793.62±3653.88
Tyr	475899.54±15229.71^{a}	406287.48±17565.17^{b}	428158.33±11859.61^{b}
Lys	400638.57±25470.7	350577.67±17153.13	395017.66±11356.51
Gly	135910.05±8390.55^{a}	114395.24±3775.86^{ab}	119623.59±5297.19^{b}
L-Homocitrulline	2309.69±323.6^{a}	643.9±133.56^{b}	605.1±45.96^{b}
Pro	142172.41±11770.32^{a}	112230.02±6308.7^{b}	123336.58±5471.8^{ab}
L-Alloisoleucine	203502.33±12272.96	188037.17±12824.57	194782.63±6050.37
Gln	389963.52±36228.28	331772.13±18322.91	376182.12±30753.22
L-Glutamic acid	75836.64±642.84	75844.2±1492.18	75897.05±725.03
Thr	1322955.2±123481.88^{a}	925028.63±65318.2^{b}	1235642.31±85557.24^{a}
Asp	910161.6±61458.81^{a}	661788.8±68292.87^{b}	739223.63±30596.87^{b}
Val	509912.7±33668.58	439278.29±31829.5	518006.93±14025.17
Asn	80107.77±4684.23^{a}	61485.29±5268.97^{b}	86425.5±6197.74^{a}
Leu	345268.03±15863.38^{a}	185229.9±56475.79^{b}	76656.16±50193.68^{b}

Different lowercases letters represent significant difference ($P<0.05$).

(P<0.05, Table S3). Conversely, guanosine-related compounds, including N2, N2, 7-trimethylguanosine and N, N-dimethylguanosine, were significantly enriched in breast of 240-day-old broilers compared with those of 90-day-old broilers (P<0.05, Table S3). Compared with 165-day-old broilers, the levels of inosine, 5'-deoxy-5'-methylthioadenosine, and ATP were significantly lower in the breast of 90-day-old broilers (P<0.05; Table S3).

Organic acids of breast meat in Wuding broilers: Regarding organic acids, most of them also exhibited higher contents in the 90-day-old broilers, similar to the results of AAs and their derivatives. This was particularly for N-acetylneuraminic acid, N-acetylputrescine, guanidinoacetic acid, and 4-guanidinobutanoic acid (P<0.05; Table S4). Furthermore, 2-Hydroxyglutaric acid and creatinine were more abundant in the breast of 240-day-old broilers compared with those of 90-day-old broilers (P<0.05; Table S4).

The other substances of breast meat in Wuding broilers: Some carnitine substances were exhibited higher concentrations in the breast of 90-day-old broilers, including 2-methylbutyroylcarnitine, propionyl-L-carnitine, and isovaleryl-carnitine (P<0.05; Table S5). Conversely, the contents of DL-carnitine and acetyl-carnitine were higher in the breast of 240-day-old broilers (P<0.05; Table S5). Regarding indole substances, the concentrations of beta-indole-3-acetic acid, indolelactic acid, and 5-hydroxyindoleacetic acid were significantly higher in the breast of 90-day-old broilers than in 165-day-old and 240-day-old broilers (P<0.05; Table S6). Most amines, including sphinganine, trimethylamine-N-oxide, and 1-methylhistamine, exhibited higher concentrations in the breast of 90-day-old chickens (P<0.05; Table S7). Regarding vitamins, trigonelline were more abundant in the breast of 90-day-old broilers (P<0.05; Table S8).

Flavor substances of breasts: Based on the identification of flavor substances, aldehydes, ketones, and esters represented the dominant class (30.86%) in the breasts of the Wuding broilers throughout the developmental trajectory, followed by hydrocarbons (25.15%), then alcohol and amines (15.67%) (Fig. 1a). According to the rOVA, six substances consistently contributed to the main flavor characteristics of breast meat of the Wuding broilers across all three developmental stages (Fig. 1b). These were 2-methoxy-3-(2-methylpropyl)-pyrazine (pea taste), dihydro-2-methyl-3(2H)-furanone, 4-methoxy-benzaldehyde, beta-lonone (sweet taste), dimethyl trisulfur compounds (meaty taste), and trans-beta-lonone (floral taste) (Fig. 1b). Orthogonal partial least squares discriminant analysis (OPLS-DA) showed obvious differences in the composition of flavor substances in the breast of Wuding broilers at different growth stages, particularly between 90 and 240 days (Fig. 1c). Flavor substances with different contents among samples at different developmental stages were identified (Fig. 1d).

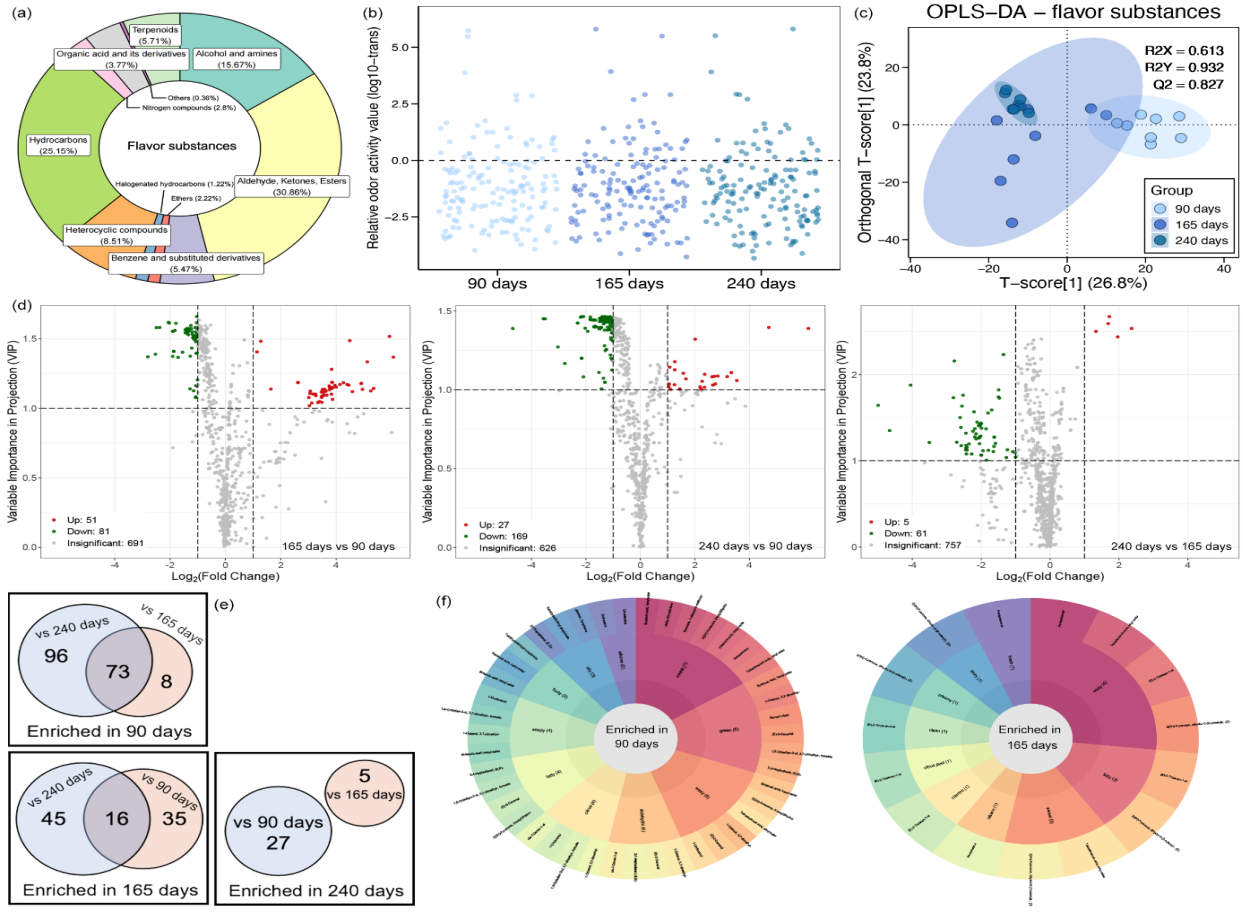

Fig. 1: Flavor substances in the breast of 90-day-old, 165-day-old, and 240-day-old Wuding broilers. (a) Compositions of flavor substances. (b) The relative odor activity value of flavor substances. (c) Orthogonal partial least squares discriminant analysis for flavor substances. (d) Volcano plots identifying the differentially abundant flavor substances. (e) Venn diagram of flavor substances. (f) The order types of flavor substances.

Among these, substances that were consistently enriched in a single developmental stage were identified using Venn diagrams (Fig. 1e). A total of 73 and 16 flavor substances were found to be enriched in the breast of 90-day-old and 165-day-old broilers (Fig. 1e). The flavor substances enriched in the 90-day-old broiler breasts primarily provided sweet and green notes, whereas those enriched in the 165-day-old broiler breasts were primarily waxy and fatty flavors (Fig. 1f).

Gene expression profiles of breasts: Principal Coordinates Analysis (PCoA) showed separate clusters of the gene expression profiles in the breasts of Wuding broilers across the developmental trajectory (Fig. 2a), and these differences were significant (P<0.05). Genes that were differentially expressed among samples at different developmental stages were identified (Fig. 2b). Subsequently, those that were consistently enriched at a single developmental stage were identified using Venn diagrams (Fig. 2c). A total of 170, 153, and 222 genes were found to be upregulated in the breasts at 90, 165, and 240 days, respectively (Fig. 2c). Further enrichment analysis based on these upregulated DEGs indicated that the pathways for histidine metabolism and amino acid biosynthesis were significantly enriched in the breasts at 90 days (P<0.05, Fig. 2d). Three other pathways: thermogenesis, oxidative phosphorylation, and cardiac muscle contraction, were significantly enriched in the breast of 165-day-old broilers (P<0.05, Fig. 2d).

Active gut microbiota: Richness (Chao1) of active gut microbiota in the Wuding broilers increased significantly (P<0.05; Fig. 3a) throughout the developmental trajectory. The diversity (Shannon) of the active gut microbiota was also significantly higher in 165-day-old and 240-day-old broilers compared with that in 90-day-old broilers (P<0.05; Fig. 3a). PCoA further showed distinct compositions of the active gut microbiota between the chicks (90 days) and older boilers (165 and 240 days) (Fig. 3b). Subsequent comparisons of the Bray-Curtis distance indicated that the composition of the active gut microbiota became more stable with the development of Wuding broilers (P<0.05; Fig. 3c).

Firmicutes and Bacteroidota dominated the active gut microbiota of Wuding broilers (Fig. 3d), although their abundances did not show significant difference among samples from different developmental broilers (P>0.05). Moreover, the ratio of Firmicutes and Bacteroidota, an indicator for gut health, also did not vary significantly throughout the developmental trajectory of Wuding broilers (P>0.05; Fig. 3e). At the genus level, some abundant genera, including *Mediterranea*, *Coprenecus*, *Merdivivvens* and *Lawsonibacter*, showed more powerful activity in 165-day-old and 240-day-old broilers compared with that in 90-day-old broilers (P<0.05; Fig. 3f).

The co-occurrence network of active gut microbiota in Wuding broilers was constructed, forming a network structure containing three modules (Fig. 3g). Modules 1 and 3 contained 153 and 187 bacterial species, respectively,

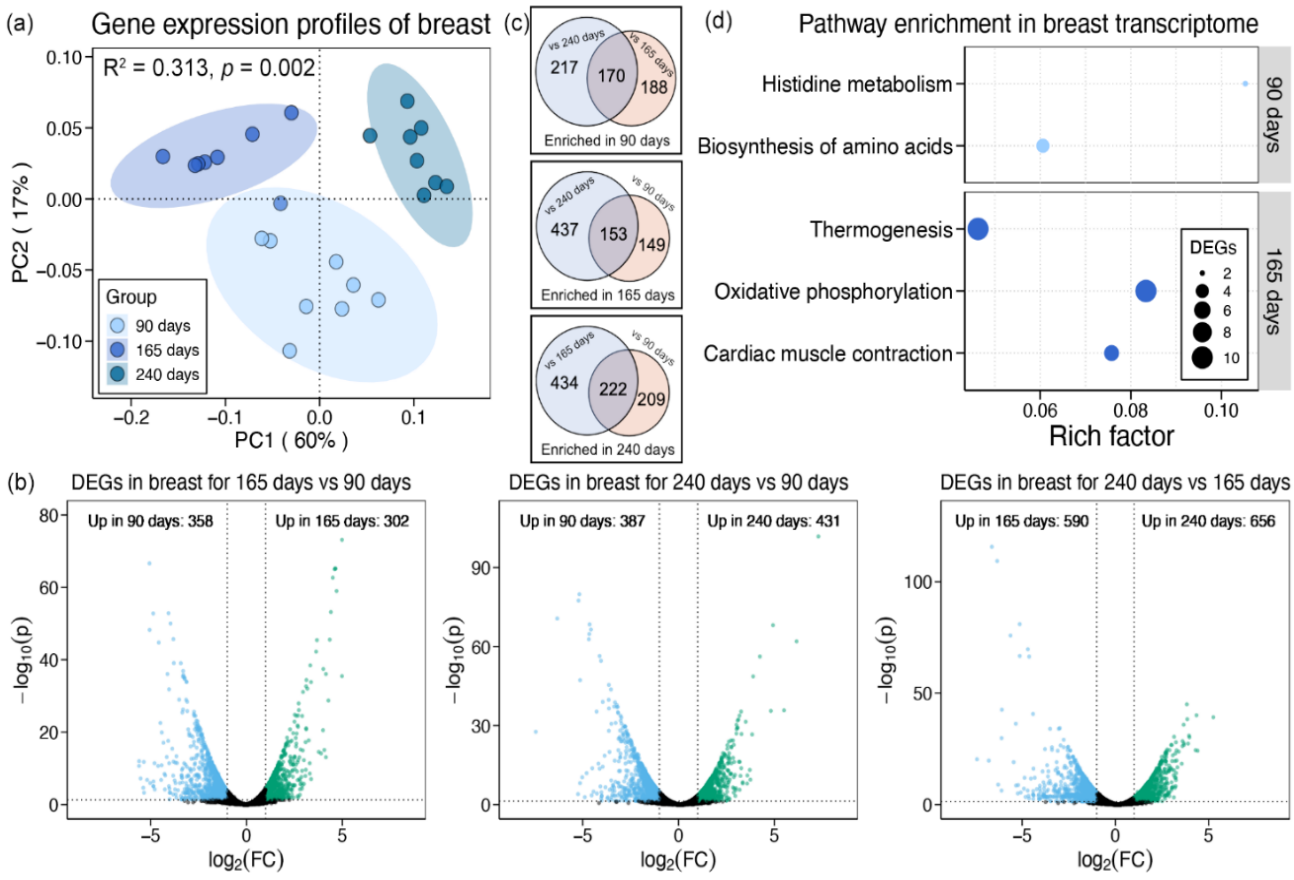

Fig. 2: Gene expression profiles in the breast of 90-day-old, 165-day-old, and 240-day-old Wuding broilers. (a) Principal coordinates analysis and adonis test of gene expression profiles. (b) Volcano plots. (c) Venn diagrams. (d) Pathways that enriched in breasts at different developmental stages based on the up-regulated expressed genes.

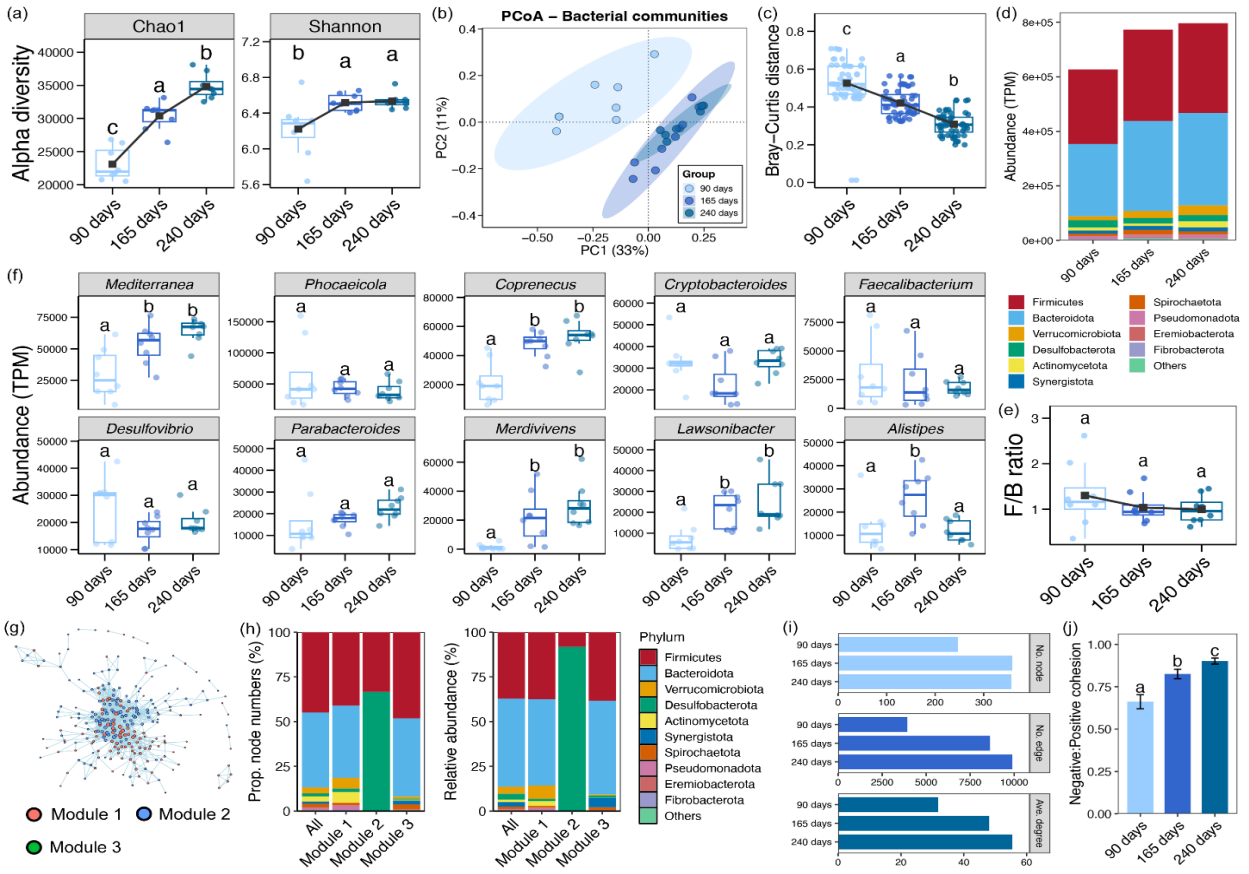

Fig. 3: Active gut microbiota in 90-day-old, 165-day-old, and 240-day-old Wuding broilers. (a) The alpha diversity. (b) Principal coordinates analysis. (c) Variations in the Bray-Curtis distance. (d) Abundance of domain phyla. (e) Variations in the ratio of Firmicutes and Bacteroidota. (f) Variations in the abundance of top 10 abundant active bacterial genera. (g) Co-occurrence network for the active gut microbiota. Spearman's rank correlations among ASVs with a detected rate higher than 60% were calculated. The jcorrelation coefficientj was >0.6 and the p-value was <0.05 was the thresholds for correlation between two ASVs. The modularity and topological parameters of networks were calculated and compared (igraph' package). (h) Proportion of nodes belonged to different bacterial phyla in different modules at the number and abundance levels, respectively. (i) Differences in the topological parameters in the co-occurrence networks of active gut microbiota. (j) Variations in the negative: positive cohesion of the co-occurrence networks of active gut microbiota. Different lowercases letters above each box in the same sub-figure represent significant difference (P<0.05).

and were dominated by Firmicutes and Bacteroidota (Fig. 3h). Conversely, module 2 contained only three bacterial species: two of which belong to Desulfobacterota and the other to Firmicutes (Fig. 3h). As Wuding broilers developed, the complexity of co-occurrence network was enhanced, as indicated by an increase in numbers of nodes and edges as well as the average degree (Fig. 3i). Moreover, the negative-to-positive cohesion of the co-occurrence network also increased significantly throughout the developmental trajectory, suggesting improved stability (P<0.05; Fig. 3j).

SCFAs produced by active gut microbiota: According to the targeted metabolomics, the contents of SCFAs in the cecal digesta of Wuding broilers were obtained. PCoA then showed variations in the compositions of SCFAs throughout the developmental trajectory (Fig. 4a). Specifically, multiple SCFAs, including 2-methylbutyrate, acetic acid, isobutyric acid, isovaleric acid, propionic acid, and valeric acid showed lower contents in chicks (90-d) compared to older broilers (165 and 240 days) (P<0.05; Fig. 4b). SCFAs were produced by the fermentation of the gut microbiota via some AA metabolism pathways. However, no significant variations were observed in the expression levels of genes involved in these pathways throughout the developmental trajectory (P>0.05; Fig. 4c). Focusing on the specific genes that directly synthesize SCFAs, several genes, including *phnA*, *cysK* and *ALDH* for acetate synthesis and *buk*, *atoD*, and *atoA* for butyrate synthesis, were upregulated in the active gut microbiota of Wuding broilers throughout their development (P<0.05; Fig. 4d). Furthermore, two genes for the synthesis of acetate and propionate, *pct* and *acdAB*, were downregulated throughout the developmental trajectory (P<0.05; Fig. 4d). Firmicutes and Bacteroidota were also the dominant active hosts for these SCFAs synthesizing genes (Fig. 4e). The abundance of active SCFA synthesizing genes belonging to Bacteroidota significantly increased in 165-day-old and 240-day-old broilers compared with that in 90-day-old broilers, whereas active Firmicutes showed no significant change. This suggests a reduced Firmicutes/Bacteroidota (F/B) ratio for SCFA synthesis throughout developmental trajectory (Fig. 4f). Furthermore, the abundances of some other active SCFA-synthesizing bacteria, including Pseudomonadota, Elusimicrobiota, and Eremiobacterota, also increased throughout the developmental trajectory (Fig. 4f).

Associations between active gut microbiota and meat quality: Correlation analysis showed significantly positive correlations among the richness of active gut microbiota, the abundance of module 1 gut bacteria, and the abundance of active *Merdivivens* with two important FAs (DPA and

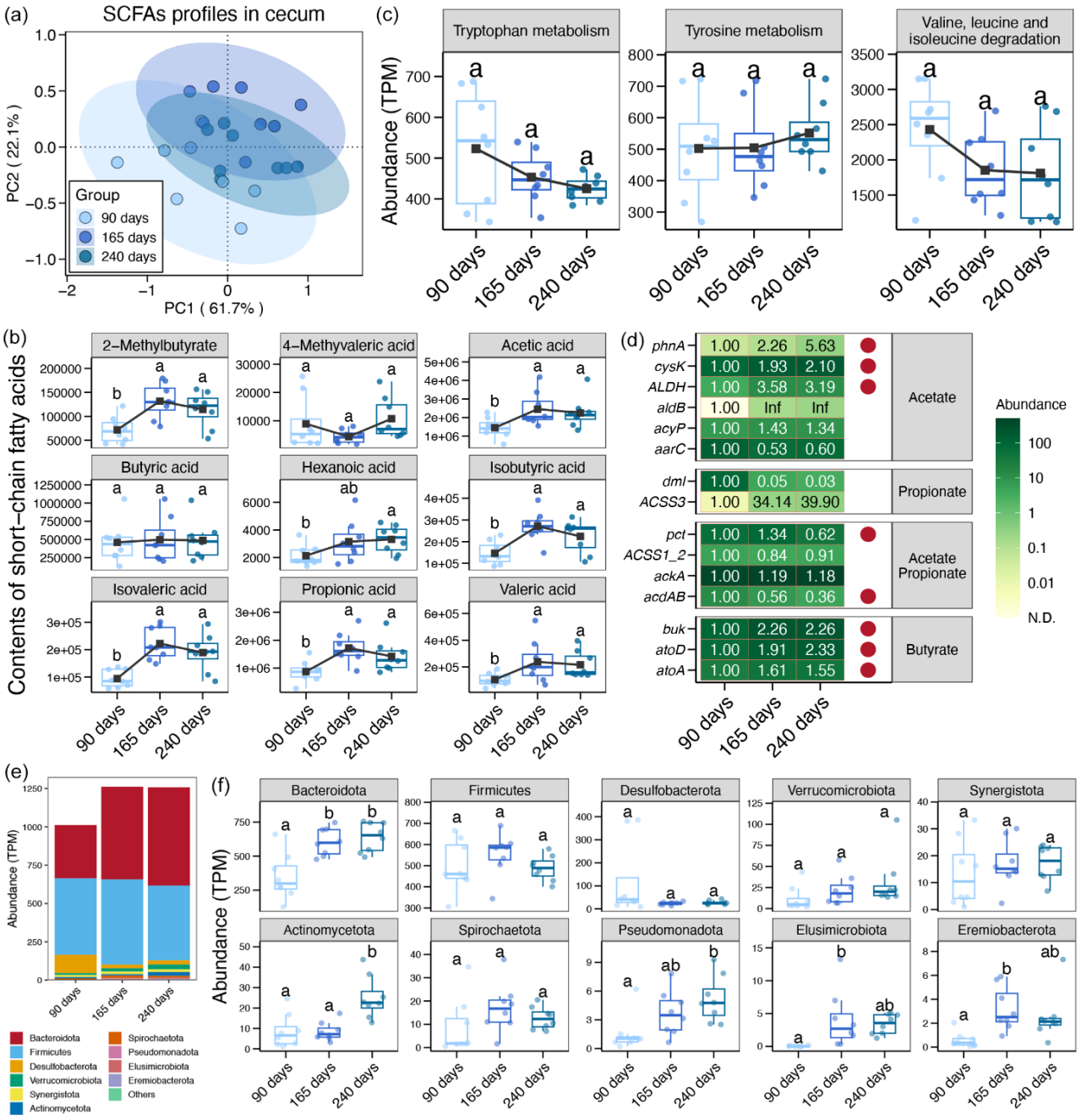

Fig. 4: Gut SCFAs in 90-day-old, 165-day-old, and 240-day-old Wuding broilers. (a) Principal coordinates analysis. (b) Variations in the contents of SCFAs. (c) Variations in the expression levels of SCFA synthesis related pathways in the gut microbiota. (d) Heatmap revealing the variations in the expression levels of genes to synthesize SCFAs. Values in each block represent the fold change of gene expression level compared with 90-day-old broilers and the red point represents the significant difference (Dunn's test, P<0.05). (e) Abundance of domain phyla. (f) Variations in the abundance of top 10 abundant active bacterial phyla that carrying SCFA synthesized genes. Different lowercases letters above each box in the same sub-figure represent significant difference (Dunn's test; P<0.05).

DHA) enriched in the breast of mature broilers (P<0.05; Fig. 5a). Further analysis of the relationship between FAs in the breasts and SCFAs in the cecum suggested significantly positive correlations between the contents of DPA and DHA in the breasts and the contents of acetic acid and propionic acid in the cecal digesta (P<0.05; Fig. 5b). They were also significantly positive correlated with the abundances of key active SCFA-synthesizing gut bacteria (P<0.05; Fig. 5b).

Some key flavor substances in the breasts showed significantly positive correlations with the alpha diversity and the abundances of the main bacterial genera in the active gut microbiota (P<0.05; Fig. 5c), indicating that active gut microbiota may contribute to the flavor of the breast meat in Wuding broilers. Conversely, most AAs and peptides in the breasts showed negative correlations with the active gut microbiota (Fig. 5d and 5e).

Moreover, correlations between the active gut microbiota and other metabolites in the breasts of Wuding broilers were also investigated (Fig. 5f). The contents of inosine showed significantly positive correlations with the abundance of module 3 and *Coprenecus* (P<0.05). Positive correlations were also found between the Chao1 index and module 1 abundance with some other nucleotides and their derivatives, such as thymidine (P<0.05). Conversely, multiple cytosine-related metabolites were negatively correlated with the active gut

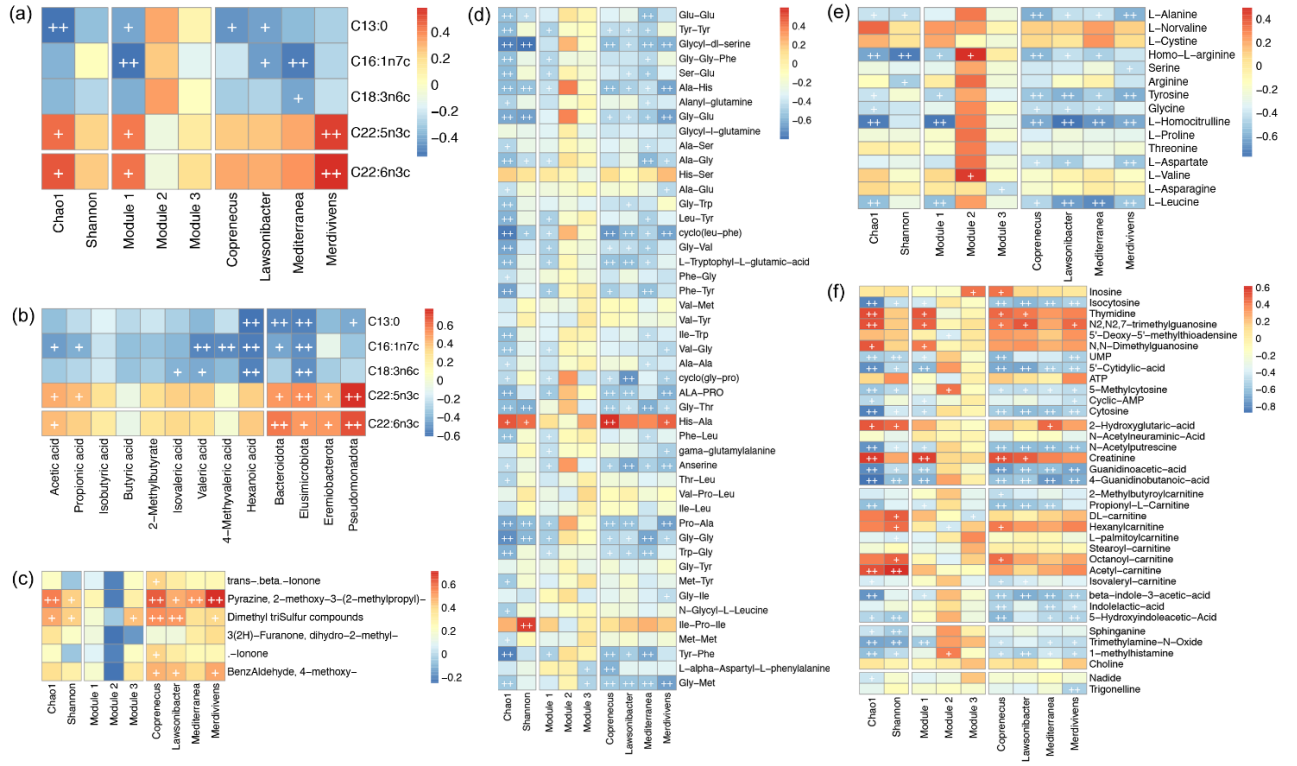

Fig. 5: Correlation analysis. (a) Correlation analysis between active gut microbiota and breast fatty acids. (b) Correlation analysis between breast fatty acids and gut SCFAs as well as active microbiota. (c) Correlation analysis between active gut microbiota and flavor substances. (d) Correlation analysis between active gut microbiota and breast small peptides. (e) Correlation analysis between active gut microbiota and breast AAs. (f) Correlation analysis between active gut microbiota and breast other metabolites.

microbiota. Regarding organic acids, most of them were also negatively correlated with the active gut microbiota, including guanidinoacetic acid. Conversely, the contents of creatinine were positively correlated with the Chao1 index and abundances of module 1, *Coprenecus*, and *Lawsonibacter* ($P<0.05$). Furthermore, most indole derivatives, amines, and vitamins were negatively correlated with the active gut microbiota ($P<0.05$).

DISCUSSION

Poultry with a slow growth rate (a rearing cycle exceeding 90 days) are increasingly favored by consumers, whereas fast-growing broilers with a rearing period of only 35 to 42 days face considerable doubts in the Chinese market (Yue *et al*., 2024). Wuding chicken, a famous chicken breed in China, is renowned for its good meat quality. Notably, slaughter age stands as a pivotal factor governing broiler meat quality (Dou *et al*., 2017). The profiles of AAs, peptides, and other key metabolites (contributors to umami and flavor) differ with age, and chicks tend to have higher concentrations of certain flavor-related compounds (Mikulski *et al*., 2011). In the current study, significantly higher contents of AAs and peptides were observed in 90-day-old chicks, including those that contribute to umami taste, such as aspartate, alanine, glycine, and anserine. The higher expression of genes involved in AA biosynthesis was also uncovered in the breasts of chicks. Moreover, multiple flavor substances that provided a sweet taste were found to be enriched in the breast of 90-day-old Wuding broilers, whereas few flavor substances were enriched in mature broilers. Conversely, broilers at 165 and 240 days of age contained higher levels of DHA and DPA, which is consistent with the deposition pattern of PUFA in chickens. In response to diverse consumer needs, broilers of different ages can be selected, thereby promoting the further refinement of the chicken market.

Emerging research indicates a notable connection between the gut microbiota and the development of meat flavor in poultry (Ruan *et al*., 2024; Xu *et al*., 2024). Many studies have shown that chickens with certain beneficial gut bacteria have meat with more desirable sensory attributes, particularly in the early stages of growth (Liu *et al*., 2023a). The results of the present study indicated significant correlations between the active gut microbiota and the dominant flavor substances to impart meaty taste to the breast of Wuding broilers. Meaty flavor in chicken breast is largely impacted by a complex set of volatile organic compounds, including sulfur-containing compounds, nitrogen-containing volatiles (pyrazines), and aldehydes (Jayasena *et al*., 2013). Although the direct impact of the gut microbiota on these flavor substances in chicken breast has not been conclusively investigated, it is plausible that gut microbial composition affects nutrient and metabolite profiles that serve as flavor precursors in the muscle (Chang *et al*., 2023). Regarding umami metabolites that were more abundant in chicks, such as AAs and peptides, most of them were negatively correlated with the active gut microbiota. This negative correlation could represent a trade-off where enhanced muscle amino acid and peptide content is associated with a less diverse or different gut microbiota profile (Chang *et al*., 2023). However, detailed mechanistic studies should continue to

explore how gut microbiota influences muscle metabolism and vice versa.

In the current study, the results showed that the active microbiota of Wuding broilers became progressively more complex, exhibiting higher richness, diversity, and interconnected co-occurrence networks, while concurrently becoming more stable during ontogeny. A progressive increase in species richness with age has also been found in some previous studies on both broilers and layer hens (Feng *et al*., 2023; Bajagai *et al*., 2024). As complexity grows, the gut ecosystem becomes more resilient and consistent across individuals. Comparative longitudinal studies demonstrate a reduced inter-individual variation in cecal microbiota profiles of adult layers compared to those of chicks, indicating greater community stability in older birds (Glendinning *et al*., 2019). A similar phenomenon has also been observed in this study, which exhibited a lower Bray-Curtis distance of active gut microbiota during the developmental trajectory. At a deeper level, early gut colonization is influenced heavily by stochastic events, whereas later stages are impacted by deterministic factors, yielding a more reproducible microbiota composition among mature chickens (Feng *et al*., 2023). From the host development perspective, this trajectory could be driven by host immune maturation, gut physiological changes, and metabolite-mediated microbial interactions. Intestinal morphological development, including small intestinal elongation, cecal crypt deepening and increased mucus secretion, shapes a unique microenvironment, promotes the assembly of segment-specific microbial communities, and maintains the structural stability of mature gut microbiota. (Bajagai *et al*., 2024). Moreover, production of SCFAs by early colonizers enhances gut barrier integrity and modulates immune signaling, further selecting for SCFA-producing taxa and strengthening co-occurrence networks (Zhu *et al*., 2023). Understanding these mechanisms provides avenues to optimize poultry health and productivity through targeted dietary interventions and microbial management.

The enrichment of omega-3 long-chain PUFAs, particularly DPA and DHA, in broiler breast meat is a long-standing goal for both human nutrition and poultry value-addition. The results of this study indicated that there were more DPA and DHA in the breast of Wuding broilers at 240 days, and the enrichment of these PUFAs could be associated with the SCFAs produced by gut microbiota. An increase in the production of SCFAs in the digestive system of broilers has been proven to be linked to an optimized gut microbiota (Gu *et al*., 2025). A similar result was also found in the present study; the abundances of some active gut bacteria for SCFA production were found to be increased in samples with more SCFAs, and they were significantly correlated with the DPA and DHA contents in the breasts. The supplementation of FAs in the diets has been reported to increase the content of PUFAs, including DPA and DHA, in the breast meat of broilers (Makiš *et al*., 2024). Animals receiving protected SCFAs showed a proportional rise in hepatic acetyl-CoA carboxylase (ACC) phosphorylation (AMP-activated protein kinase target) together with fatty acid desaturase 2 (FADS2) and elongation of very long chain fatty acids protein 5 (ELOVL5) mRNA upregulation (Weitkunat *et al*., 2015). FADS2 is the rate-limiting enzyme for converting α-linolenic acid to stearidonic acid and further to DPA, whereas ELOVL5 catalyzes the elongation of FAs from C20 to C22 (Liu *et al*., 2023b). Once elongation to C22 occurs, Δ6-desaturation and β-oxidation in peroxisomes rapidly convert FAs to DPA and DHA. SCFAs accelerate both steps via AMPK-mediated peroxisomal biogenesis (Ding *et al*., 2024; Witt *et al*., 2023). Moreover, SCFAs can upregulate CD36 and FATP4 in enterocytes, increasing the selective uptake of pre-formed DHA/DPA when present in the lumen (Melaku *et al*., 2024). Taken together, when integrated into balanced formulations that supply adequate n-3 precursors, protected butyrate emerges as a practical, antibiotic-free strategy to enhance the health value of poultry products while simultaneously promoting gut integrity and growth performance. However, current evidence is butyrate-centric, while the results of the present study showed significant correlations with acetate and propionate; comparative propionate/acetate trials should be further performed.

Multiple studies have demonstrated that altering the gut microbiota can change the muscle-flavor precursor profiles (Ruan *et al*., 2024). Some gut bacteria, particularly in genera like *Bacteroides*, *Lactobacillus*, *Alistipes*, and *Eubacterium*, have been shown to be closely linked to enhancing broiler meat flavor (Yi *et al*., 2024). In the present study, the key substances contributing to the flavor of chicken breast were found to be significantly positively correlated with active gut microbiota. These substances are primarily responsible for the aroma flavor (Hao *et al*., 2023), which may also explain why older chickens that have been raised for a longer period have a meaty aroma. Conversely, flavor substances that impart an umami taste, including AAs, peptides, as well as some nucleotides, were more abundant in chicks and negatively correlated with the active gut microbiota. It should be noted that the main active gut bacterial genera were not proven to be associated with the production of muscle-flavor precursors. These findings indicate that the intestinal environment might gradually select for active microorganisms related to growth and energy metabolism as chickens develop and mature (Bajagai *et al*., 2024). Modifying the broiler gut microbiota through alteration of diets or supplementation of probiotics could strategically enhance meat flavor by elevating umami nucleotides, essential and flavor AAs, and PUFA contents, as well as by increasing the formation of desirable volatile compounds (Zheng *et al*., 2014). In the future, attention may need to be paid to how to improve the diet to enhance the umami taste of mature Wuding chickens.

Conclusions: Young broiler breast contained higher levels of amino acids, peptides and sweet-tasting flavor compounds, exhibiting better flavor characteristics than mature broiler breast. These features of young chicks may be attributed to the high expression levels of AA-synthesizing genes and may be closely associated with gut microbiota. Conversely, ω-3 PUFAs (DPA and DHA) were more abundant in chicken breast muscle at 165 and 240 days of age. Mature broilers harbor a more diverse and stable gut microbiota, which contributes to higher SCFA production. Higher contents of SCFAs may induce the accumulation of DPA and DHA in the breast meat of mature broilers. The present study suggested that intestinal

microbiota are critical to the formation and modulation of broiler meat quality.

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REFERENCES

Bajagai YS, Van TTH, Joat N, *et al*., 2024. Layer chicken microbiota: a comprehensive analysis of spatial and temporal dynamics across all major gut sections. Journal of Animal Science and Biotechnology 15:20.

Chang C, Zhang QQ, Wang HH, *et al*., 2023. Dietary metabolizable energy and crude protein levels affect pectoral muscle composition and gut microbiota in native growing chickens. Poultry Science 102:102353.

Ding J, Liu J, Chen J, *et al*., 2024. Sodium butyrate alleviates free fatty acid-induced steatosis in primary chicken hepatocytes via the AMPK/PPARα pathway. Poultry Science 103:103482.

Dou T, Zhao S, Rong H, *et al*., 2017. Biological mechanisms discriminating growth rate and adult body weight phenotypes in two Chinese indigenous chicken breeds. BMC Genomics 18:469.

Feng Y, Zhang M, Liu Y, *et al*., 2023. Quantitative microbiome profiling reveals the developmental trajectory of the chicken gut microbiota and its connection to host metabolism. Imeta 2:e105.

Glendinning L, Watson KA and Watson M, 2019. Development of the duodenal, ileal, jejunal and caecal microbiota in chickens. Animal Microbiome 1:17.

Grosicki GJ, Fielding RA and Lustgarten MS, 2018. Gut microbiota contributes to age-related changes in skeletal muscle size, composition, and function: biological basis for a gut-muscle axis. Calcified Tissue International 102:433-42.

Gu Z, Xu W, Gu T, *et al*., 2025. Exploring the dietary strategies of coated sodium butyrate: Improving antioxidant capacity, meat quality, fatty acid composition, and gut health in broilers. Genes (Basel) 16:433.

Hao M, Wang W, Zhang J, *et al*., 2023. Flavour characteristics of fermented meat products in China: a review. Fermentation (Basel) 9:830.

Hu H, Huang Y, Shi C, *et al*., 2024a. Comparison of Chinese medicine additives for antibiotic resistance risks in the Wuding chickens under high stocking density: Advantages and drawbacks. Journal of Clean Production 469:143152.

Hu H, Huang Y, Li A, *et al*., 2024b. Effects of different energy levels in low-protein diet on liver lipid metabolism in the late-phase laying hens through the gut-liver axis. Journal of Animal Science and Biotechnology 15:98.

Hu H, Li A, Shi C, *et al*., 2024c. Mulberry branch fiber improved lipid metabolism and egg yolk fatty acid composition of laying hens via the enterohepatic axis. Microbiome 12:73.

Huang D, Chen X, Tan R, *et al*., 2024. A comprehensive metabolomics analysis of volatile and non-volatile compounds in matcha processed from different tea varieties. Food Chemistry X 21:101234.

Jayasena DD, Ahn DU, Nam KC, *et al*., 2013. Flavour chemistry of chicken meat: A review. Asian Australasian Journal of Animals 26:732.

Li G, Jin B and Fan Z, 2022. Mechanisms involved in gut microbiota regulation of skeletal muscle. Oxidative Medicine and Cell Longevity 2022:2151191.

Li W, Sheng RW, Cao MM, *et al*., 2024a. Exploring the relationship between gut microbiota and sarcopenia based on gut–muscle axis. Food Science and Nutrition 12:8779-92.

Li T, Yin D and Shi R, 2024b. Gut-muscle axis mechanism of exercise prevention of sarcopenia. Frontiers in Nutrition 11:1418778.

Liu X, Ma A, Zhi T, *et al*., 2023a. Dietary effect of *Brevibacillus laterosporus* S62-9 on chicken meat quality, amino acid profile, and volatile compounds. Foods 12:288.

Liu Y, Sun D, Li X, *et al*., 2023b. Identification of core promoter region of the polyunsaturated fatty acid synthesis-related gene family in chicken. Poultry Science 102:102857.

Makiš A, Čertík M, Klempová T, *et al*., 2024. Fermented products enriched with polyunsaturated fatty acids in broiler chicken nutrition and fat quality of produced meat. Applied Science 14:4327.

Melaku M, Su D, Zhao H, *et al*., 2024. The new buffer salt-protected sodium butyrate promotes growth performance by improving intestinal histomorphology, barrier function, antioxidative capacity, and microbiota community of broilers. Biology (Basel) 13:317.

Mikulski D, Celej J, Jankowski J, *et al*., 2011. Growth performance, carcass traits and meat quality of slower-growing and fast-growing chickens raised with and without outdoor access. Asian Australasian Journal of Animals 24:1407-16.

Ruan D, Jiang J, Huang W, *et al*., 2024. Integrated metabolomics and microbiome analysis reveal blended oil diet improves meat quality of broiler chickens by modulating flavor and gut microbiota. Animal Nutrition 19:453-65.

Sun SY, Yang WY, Tan Z, *et al*., 2022. Serum levels of free fatty acids in obese mice and their associations with routine lipid profiles. Diabetese Metabolic Syndrome and Obesity 15:331-43.

Weitkunat K, Schumann S, Petzke KJ, *et al*., 2015. Effects of dietary inulin on bacterial growth, short-chain fatty acid production and hepatic lipid metabolism in gnotobiotic mice. Journal of Nutrition and Biochemistry 26:929-37.

Witt A, Mateska I, Palladini A, *et al*., 2023. Fatty acid desaturase 2 determines the lipidomic landscape and steroidogenic function of the adrenal gland. Science Advance 9:eadf6710.

Xiao L, Qi L, Fu R, *et al*., 2024. A large-scale comparison of the meat quality characteristics of different chicken breeds in South China. Poultry Science 103:103740.

Xu L, Mao T, Xia M, *et al*., 2024. New evidence for gut-muscle axis: Lactic acid bacteria-induced gut microbiota regulates duck meat flavor. Food Chemistry 450:139354.

Yi W, Liu Y, Fu S, *et al*., 2024. Dietary novel alkaline protease from *Bacillus licheniformis* improves broiler meat nutritional value and modulates intestinal microbiota and metabolites. Animal Microbiome 6:1.

Yue K, Cao QQ, Shaukat A, *et al*., 2024. Insights into the evaluation, influential factors and improvement strategies for poultry meat quality: A review. NPJ Science of Food 8:62.

Zeng T, Cao Y, Yin J, *et al*., 2024. Unraveling the gut microbiota of Tibetan chickens: insights into highland adaptation and ecological advantages. Microbiology Spectrum 12:e00519-24.

Zheng A, Luo J, Meng K, *et al*., 2014. Proteome changes underpin improved meat quality and yield of chickens (*Gallus gallus*) fed the probiotic *Enterococcus faecium*. BMC Genomics 15:1167.

Zhu La ALT, Feng Y, Hu D, *et al*., 2023. Enzymatically prepared alginate oligosaccharides improve broiler chicken growth performance by modulating the gut microbiota and growth hormone signals. Journal of Animal Science and Biotechnology 14:96.

Zhang X, Kong Y, Chen J, *et al*., 2025. Comprehensive genetic map of muscle lipidome reveals novel insights into flavor variation in ruminant meat. Advanced Science 12:e06900.