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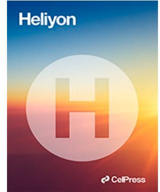

Research article

Transcriptome analysis reveals the synergistic involvement of *MGLL* and *LPIN1* in fatty acid synthesis in broiler pectoral muscles

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ABSTRACT

Fatty acids (**FAs**) are one of the most important bioactive compounds affecting the quality of meat. In this study, we compared the expression profiles of genes involved in FA production in the breast muscle of Jingxing Yellow chickens at different days of age determined by transcriptomic analysis to identify key genes and pathways regulating the FA composition of the breast muscle. Through clustering analysis of gene expression data, the growth process of broiler chickens can be divided into two stages, namely the growth and development stage at the 35th and 63rd days of age (D35, D63), and the mature stage at the 119th day of age (D119). The content of some important unsaturated fatty acids (**UFAs**), such as C18:2n6c, C20:4n6, and C22:6n3, in the pectoral muscles, differed significantly between these two stages ($p < 0.05$). Therefore, we compared the gene expression profiles at D35 and D63 with those at D119, and identified differentially expressed genes (**DEGs**). The gene modules related to the five UFAs with significant changes were identified by weighted gene co-expression network analysis (**WGCNA**), and then 150 crossover genes were identified by crossover analysis of the detected DEGs and WGCNA. The results of the pathway enrichment analysis revealed the glycerolipid metabolism pathway related to lipid metabolism, in which the *MGLL* and *LPIN1* genes were particularly enriched. In this study, the expression levels of *MGLL* and *LPIN1* showed an increasing trend during the growth process of broilers, with a negative regulatory effect on the significantly reduced content of C18:2n6c in the pectoral muscle, and a positive regulatory effect on the significantly increased content of C20:4n6. These findings indicated that *MGLL* and *LPIN1* synergistically promote the deposition of FAs, which may further promote the conversion of linoleic acid (C18:2n6c) to arachidonic acid (C20:4n6). Therefore, screening and identifying FA production-related functional genes are key to elucidate the regulatory molecular mechanism of production of FAs in chicken muscle, aiming to provide a theoretical basis for improving chicken meat quality.

1. Introduction

Chicken is the most consumed meat product in the world and an important source of animal protein for people globally [1].

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However, in recent years, due to the one-sided pursuit of high growth rate and high feed conversion rate of broilers, the quality of chicken meat products has become increasingly prominent, making it difficult to meet the growing consumer demand for high quality livestock products [2,3]. Intramuscular fat (**IMF**) is closely related to the tenderness, flavor, and the nutritional value of meat, and fatty acids (**FAs**) are important components of IMF [4–7]. The influence of FA content and composition of muscle on the quality of chicken meat has become one of the hot research topics in poultry farming and meat science today. Therefore, studying the regulatory mechanism of production of FAs can provide a theoretical basis for improving the quality of chicken products, and has important practical significance for improving the quality and efficiency of broiler meat production and meeting the actual needs of consumers.

The types and composition of muscle FAs are not only important factors affecting the physicochemical properties of adipose tissue and meat flavor, but also important indicators for evaluating the nutritional value of meat [8–10]. Based on their saturation degree, FAs are usually divided into saturated FAs (**SFAs**) and unsaturated FAs (**UFAs**). UFAs are further divided into monounsaturated FAs (**MUFAs**) and polyunsaturated FAs (**PUFAs**) based on the number of double bonds between carbon atoms. Due to their different structures, their functions also vary [11]. The different FA composition patterns among livestock have a high heritability, usually ranging from 0.60 to 0.63 for the FA composition of bovine muscle [12]; and 0.90, 0.73, and 0.40 for production of intramuscular SFAs, MUFAs, and PUFAs in sheep, respectively [13]. These data suggest that it is feasible to genetically modify the FA composition of muscle. However, comparing with other livestock, there is still a lack of research on the genetic regulation of FA composition in chicken muscle. Chicken contains higher levels of PUFAs and lower levels of SFAs, and the content of UFAs is proportional to the concentration of aroma compounds. Zhao et al. [14] found that increased PUFAs were associated with the properties of volatile compounds, which produced desirable roasted meat flavors. In addition, PUFAs have a regulatory effect on lipid metabolism in animals and are key bioactive compounds that influence lipid metabolism in animal tissues [15–17]. There are fewer reports related to MUFAs, and it is only known that they have some nutritional value and can lower human blood cholesterol, triglyceride (**TG**), and low-density lipoprotein cholesterol (**LDL-C**), which can contribute to the prevention of cardiovascular diseases, such as atherosclerosis, as well as reduce the risk of cancer to a certain extent [18,19]. Some studies have shown that growth age is an important factor leading to differences in FA content in poultry meat during the production process of broilers [20]. Dal et al. [21] showed that chickens with higher growth age had higher levels of PUFAs, and Zhu et al. [22] showed that there were significant differences in FA types and contents in Wuding chickens at 150 and 230 days of age. Therefore, it is feasible to elaborate on the finding of genetically regulated genes through the differences in FA composition of broiler chickens at different stages of growth and development.

In recent years, with the rapid development of high-throughput sequencing technology, weighted gene co-expression network analysis (**WGCNA**) has been successfully applied to identify key gene expression networks and biomarkers in a variety of biological fields, and also plays an important role in the analysis of transcriptome data of diverse organisms [23–25]. Based on the differential changes in FA composition and content in the pectoral muscles at different ages, this study divided the growth of chickens into two stages: developmental stage (35, 63 days old) and mature stage (119 days old). Transcriptome sequencing technology was used to analyze the differences in gene expression levels among different age groups. Then, a module of co-expressed genes was constructed using WGCNA, and the genes were analyzed by correlating them with the FA data in the breast muscles, so as to find the key genes related to FA metabolism and determine their regulatory pathways, providing the theoretical bases for the improvement of FA compositions in the muscles of livestock and poultry and enhancement of their meat quality and nutritive values.

2. Materials and methods

2.1. Animals and sample collection

Jingxing yellow chickens were obtained from the Institute of Animal Sciences, Chinese Academy of Agricultural Sciences (Beijing, China). Five chickens with similar body weights were randomly selected to be slaughtered at the ages of 35, 63, and 119 days, and pectoral muscle samples were collected and stored at −80 °C for subsequent analyses. All broilers had *ad libitum* access to feed and water, and were immunized accordingly under the same feeding conditions. This study was approved by the Animal Ethics Committee of the Institute of Animal Sciences, Chinese Academy of Agricultural Sciences (permit number: No. IAS2021-66).

2.2. Determination of free fatty acids

Pectoral muscle samples were thawed at room temperature, stripped of visible fat, muscle membranes, and connective tissue, and then minced with a meat grinder. After freeze-drying 6.0 g of chicken breast meat and grinding it into powder, the FA composition of chest muscles was determined using gas chromatography. Subsequently, a 1.0 mg/mL solution of methyl undecanoate was added as the internal standard, detected by gas chromatography using an Agilent Gas Chromatography System (Agilent Technologies Inc., Santa Clara, CA, USA). Based on the chromatogram, the FA composition of the samples was determined by comparing the peak time of the standard sample, and the percentage content of each FA was calculated using the peak area normalization method [26].

2.3. Transcriptome profiling and quality control

After extracting total RNA from 15 pectoral muscle tissue samples as described above, the concentration and integrity of the RNA samples were assessed by measuring the absorbance ratio at A260/A280 by spectrophotometry on a Nanodrop ND-2000 spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) and micro-capillary based electrophoresis on an Agilent Bioanalyzer 2100 System (Agilent Technologies Inc.). After mRNA enrichment using oligo (dt) magnetic beads, the library was constructed. Non-

strand specific RNA sequencing libraries were sequenced on the Illumina HiSeq 2500 platform (Illumina Inc., San Diego, CA, USA). Library construction and sequencing were performed by Guoke Biotechnology Co., Ltd. (Beijing, China). The obtained raw read sequencing data were subjected to filtering steps, such as removing reads with adapters, removing reads with an N (N means that base information could not be determined) ratio greater than 10 %, and removing low-quality reads (reads in which the number of bases with a Qphred $\leq$ 20 accounts for more than 50 % of the total length of the read), *etc*., to obtain high-quality clean reads. The Hisat2 (v2.1.0) software [27] was used to compare clean reads to the chicken reference genome (GRCg6a). The results were quickly assembled using the StringTie (v2.1.2) software [28], and eventually, the featureCounts (v2.0.1) [29] software was used to obtain the raw count of gene expression data, while the normalized gene expression abundance was calculated using the Fragments Per Kilobase of transcript per Million mapped reads (**FPKM**) method.

2.4. Weighted gene co-expression network analysis

In order to obtain the gene modules related to different FA compositions in the pectoral muscle of chickens, the “WGCNA” package in R (v4.3.2) was used to construct a weighted gene co-expression network for gene expression. First, the correlation between any two genes was calculated by Pearson correlation analysis, and the Pearson correlation matrix was constructed. Then, after calculating the optimal soft threshold (β) using the “pickSoftThreshold” function, the weighted neighbor-joining was calculated and converted into topological overlap matrices (**TOM**) and used as a hierarchical clustering matrix for each of the topological overlap matrices. Ultimately, based on the TOM, the genes were clustered using dissimilarity between genes, and the clustering tree was cut into different gene modules using the dynamic cutting method, and each module was set to contain at least 100 genes (minModuleSize = 100). The highly similar modules were identified using class clustering to merge the modules using a highly sheared threshold value of 0.4 as a criterion.

Table 1
Phenotypes of Jingxing yellow chickens in different stages (%).

Phenotypes Age	D35	D63	D119
C6:0	0.09 ± 0.01	0.08 ± 0.01	0.11 ± 0.01
C8:0	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
C10:0	0.00 ± 0.00	0.01 ± 0.01	0.01 ± 0.01
C12:0	0.07 ± 0.00	0.07 ± 0.01	0.07 ± 0.02
C14:0	0.31 ± 0.01	0.35 ± 0.03	0.32 ± 0.02
C14:1	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.07
C15:0	0.07 ± 0.00	0.08 ± 0.01	0.06 ± 0.01
C16:0	23.52 ± 0.12	23.50 ± 0.17	23.89 ± 0.30
C16:1	1.38 ± 0.12	1.35 ± 0.20	1.01 ± 0.12
C17:0	0.13 ± 0.01	0.14 ± 0.01	0.14 ± 0.02
C18:0	13.88 ± 0.09	13.05 ± 0.63	13.66 ± 0.53
C18:1n9c	21.79 ± 0.60b	23.86 ± 1.55ab	26.59 ± 1.61a
C18:2n6c	23.22 ± 0.56a	23.12 ± 0.73a	16.23 ± 1.05b
C18:3n3	0.69 ± 0.08ab	0.89 ± 0.09a	0.58 ± 0.03b
C20:0	0.22 ± 0.02a	0.18 ± 0.01b	0.15 ± 0.01b
C20:1	0.37 ± 0.03a	0.31 ± 0.02a	0.22 ± 0.01b
C20:3n3	0.11 ± 0.02a	0.07 ± 0.00b	0.05 ± 0.00b
C20:3n6	1.90 ± 0.11a	1.47 ± 0.08b	0.91 ± 0.06c
C20:4n6	8.16 ± 0.56b	8.23 ± 0.88b	12.54 ± 0.81a
C20:5n3	0.31 ± 0.03a	0.24 ± 0.02b	0.11 ± 0.01c
C21:0	1.33 ± 0.09a	1.14 ± 0.05b	0.65 ± 0.02c
C22:0	0.24 ± 0.02a	0.18 ± 0.02ab	0.18 ± 0.02b
C22:1n9	0.14 ± 0.03a	0.07 ± 0.00b	0.07 ± 0.00b
C22:2	0.02 ± 0.01b	0.03 ± 0.01b	0.05 ± 0.00a
C22:6n3	1.09 ± 0.10b	0.95 ± 0.16b	1.90 ± 0.31a
C23:0	0.18 ± 0.04a	0.10 ± 0.00b	0.07 ± 0.01b
C24:0	0.25 ± 0.03a	0.16 ± 0.01b	0.17 ± 0.03b
C24:1	0.47 ± 0.04a	0.32 ± 0.03b	0.21 ± 0.02c
SFAs	40.32 ± 0.29	39.06 ± 0.60	39.51 ± 0.60
UFAs	59.67 ± 0.29	60.94 ± 0.61	60.49 ± 0.60
PUFAs	35.50 ± 0.65	35.00 ± 1.19	32.38 ± 1.13
MUFAs	24.17 ± 0.71	25.94 ± 1.72	28.11 ± 1.69
n-6 PUFAs	33.28 ± 0.59a	32.82 ± 1.18ab	29.69 ± 1.19b
n-3 PUFAs	2.20 ± 0.10	2.15 ± 0.09	2.64 ± 0.30
n-6/n-3	15.26 ± 0.66a	15.32 ± 0.76a	11.83 ± 1.44b

Peer data with the same or no letter on the shoulder indicates no significant difference ($p > 0.05$), while different letters on the shoulder indicate significant significant differences ($p < 0.05$).

2.5. Analysis of differentially expressed genes

Based on the phenotypic data, the R (v4.3.2) package "DEGseq2" [30] was used to screen for differentially expressed genes (**DEGs**) at different key growth and developmental ages. Genes with a $|\log2(\text{fold change})| \geq 0.58$ and $p < 0.05$ were considered differentially expressed. Finally, the R package "ggplot2" was used to plot intergroup DEGs volcanoes.

2.6. Functional enrichment analysis of differentially expressed genes

The DEGs and genes screened by WGCNA were intersected and subjected to Gene Ontology (**GO**) term functional enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (**KEGG**) pathway enrichment analysis using the KOBAS online tool (http://bioinfo.org/kobas). KEGG pathways and GO term categories with $p \leq 0.05$ were considered significantly enriched. Bubble plots and histograms were generated using the "ggplot2" package in the R software.

2.7. Statistical analysis

Descriptive statistical data for different types of FAs were generated using the SPSS 19.0 software (IBM, Armonk, NY, USA), and one-way analysis of variance (**ANOVA**) was used for multiple group comparisons. Duncan's method was used for multiple comparisons between groups. The data is expressed as the mean ± standard error, and $p < 0.05$ is considered a statistically significant difference.

3. Results

3.1. Analysis of fatty acid composition within the pectoral muscle at different days of age

A total of 28 types of FAs were detected by analyzing the FA composition data of pectoral muscles of Jingxing yellow chickens of different ages, as shown in Table 1. These results revealed that most SFAs, such as C8:0, C14:0, and C16:0, did not show significant differences at different stages of broiler growth ($p > 0.05$). Therefore, this study focused on analyzing the differences in composition of UFAs in pectoral muscles at different ages.

Linoleic acid (C18:2n6c) and arachidonic acid (C20:4n6), which are highly abundant FAs, showed insignificant differences compared to broilers at D35 and D63 ($p > 0.05$), but at D119 exhibited significant ($p < 0.05$) differences from those at D35 and D63, with decreasing and increasing trends during broiler growth, respectively. At the same time, some important UFAs, such as docosahexaenoic acid (**DHA**, C22:6n3), which are beneficial to human health, also exhibited similar patterns, showing significant differences compared to D119 and D35, D63 ($p < 0.05$).

Due to the varying trends in the content of different FAs during the growth of broilers, the differences in UFAs, PUFAs, and MUFAs at each age of broiler growth are not significant. However, the ratio of n-6 PUFAs to n-3 PUFAs showed a significant decrease ($p < 0.05$) in broilers at D119 compared with those at D35 and D63. Likely due to the significant decrease ($p < 0.05$) in n-6 PUFAs, while the differences in n-3 PUFAs were not significant ($p > 0.05$) across different growth periods.

3.2. Evaluation of sequencing data

Transcriptome sequencing was completed on breast muscle tissue samples from 15 Jingxing Yellow Chickens, and 49,341,246 to 78,667,244 clean reads were obtained, which generated 7.18 Gb or above of clean data for all samples. All clean read data had a Phred-

Table 2
Summary of the sequencing read alignment to the reference genome.

Sample	Clean Reads	Mapped Reads	GC (%)	Q20 (%)	Q30 (%)
D35-2	77,151,672	67,366,020 (87.32 %)	51.77	97.72	93.96
D35-4	73,916,332	64,747,167 (87.60 %)	51.93	97.69	93.87
D35-7	74,946,864	66,606,466 (88.87 %)	51.17	97.92	94.40
D35-9	69,613,436	62,369,345 (89.59 %)	50.92	97.85	94.21
D35-10	77,846,628	69,495,897 (89.27 %)	50.84	97.75	93.99
D63-5	75,556,172	66,449,545 (87.95 %)	51.57	97.67	93.87
D63-6	78,171,502	69,224,561 (88.55 %)	51.29	97.79	94.09
D63-8	78,667,244	69,042,011 (87.76 %)	51.69	97.77	94.10
D63-9	73,266,266	64,806,781 (88.45 %)	51.36	97.68	93.83
D63-10	72,530,030	63,878,445 (88.07 %)	51.57	97.68	93.89
D119-4	50,396,272	42,628,543 (84.59 %)	51.89	96.08	90.57
D119-5	50,598,370	43,162,457 (85.30 %)	51.84	96.97	92.35
D119-6	51,170,122	43,424,224 (84.86 %)	51.88	96.83	92.09
D119-7	49,341,246	42,116,359 (85.36 %)	51.94	96.97	92.28
D119-8	50,054,676	42,520,460 (84.95 %)	51.9	96.88	92.20

Mapped reads means the number of Reads to the reference genome and the percentage of Clean Reads were compared. Q20 means clean data percentage of bases with a quality value of 20 or greater. Q30 means clean data percentage of bases with a quality value of 30 or greater.

like quality score (Q) of 30 > 90.57 %, and the GC content of the clean data was 51.57 %. Sequence alignment was performed between the clean reads of each sample and the designated reference genome, with alignment efficiency ranging from 84.59 to 89.59 %, indicating that the quality of the sequencing data was good. The basic information of the sequencing data is shown in Table 2.

3.3. WGCNA identified module genes significantly related to phenotypes

The 21,987 genes obtained from transcriptome sequencing were used to construct a weighted gene co-expression network, and 15 samples were clustered using the Pearson correlation test (Fig. 1A). These results revealed that broilers at D35 and D63 exhibited a clustering trend, while those at D119 were separated from these two age groups, which showed a similar trend to the phenotypic results in terms of day-age variation of FA compositions. Therefore, based on the results of this study the growth process of Jingxing Yellow Chickens can be divided into a vigorous development stage (D35, D63) and mature stage (D119) for subsequent differential gene expression analysis.

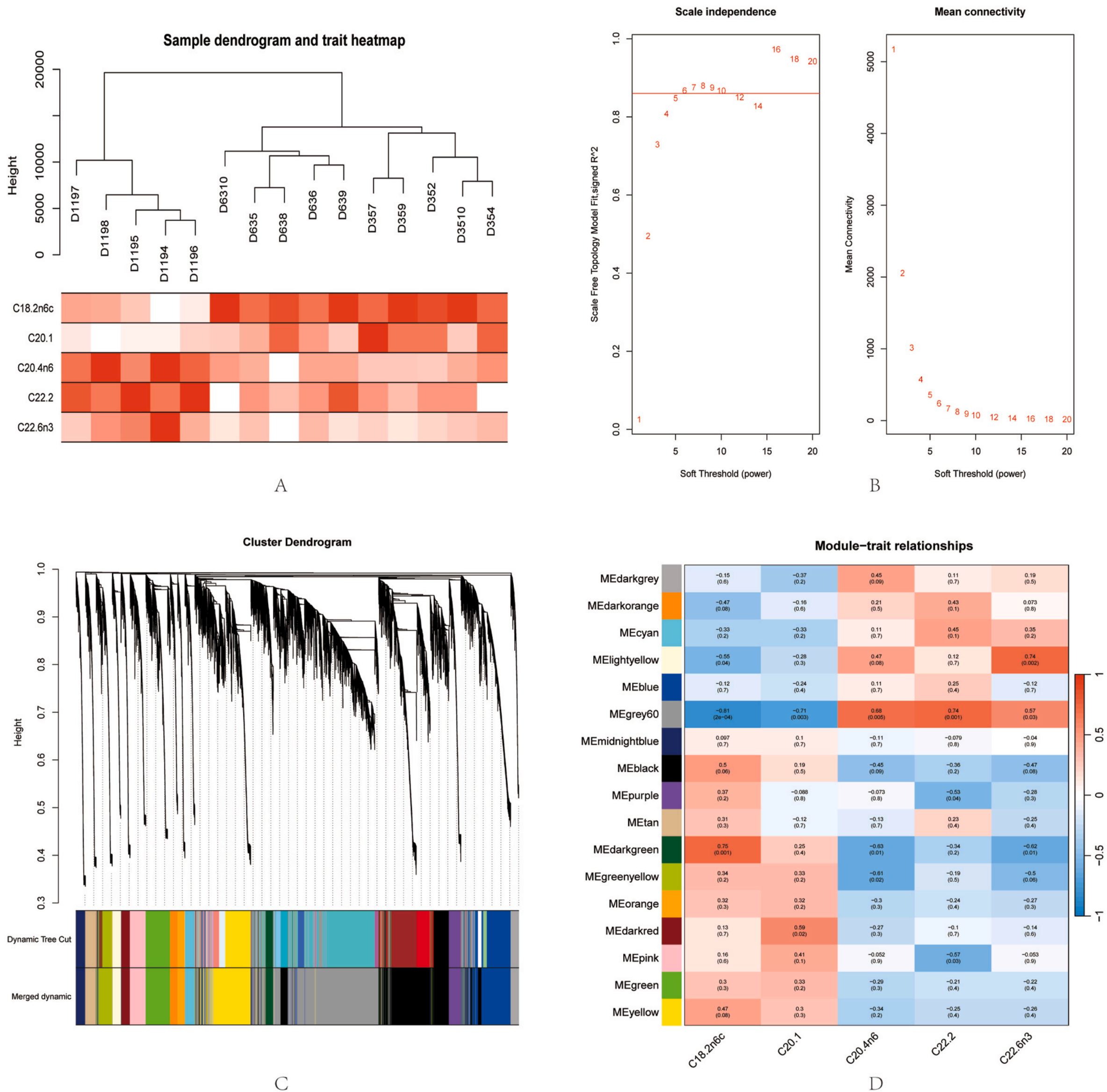

Fig. 1. Co-expression network analysis of fatty acid (FA) composition changes in pectoral muscle at different developmental ages. (A) Clustering of the remaining 15 samples after removing outliers, sample correlations were shown in different colors (the darker the color, the stronger the correlation). (B) Network topology analysis to determine soft thresholds. (C) Clustering of the co-expression modules, genes were clustered into different groups. (D) Module heatmap, the gene modules and trait relationships (red indicates positive correlation; blue indicates negative correlation).

To ensure a scale-free network, β = 6 ($R^2 > 0.86$) was chosen as the soft threshold (Fig. 1B). The dynamic shear method was used to divide the modules and the genes were clustered into 17 modules: the black module with the highest number of gene clusters contained 3866 genes, the orange module with the lowest number of gene clusters contained only 346 genes, and the grey module contained unclustered genes (Fig. 1C). According to the analysis of phenotypic data, 5 types of UFAs showed differential patterns, namely C18:2n6c, C20:1, C20:4n6, C22:2, and C22:6n3, were selected as phenotypes for correlation analysis.

The results of the correlation analysis of each gene co-expression module with the FA composition at different days of age revealed (Fig. 1D) that: C18:2n6c and C20:1 had similar correlation trends; C20:4n6, C22:2, and C22:6n3 had similar trends; the grey60 module showed a high correlation with four UFAs, with significant positive correlations with C20:4n6 and C22:2, with correlation coefficients of 0.68 and 0.74, respectively ($p < 0.01$); a significant negative correlation with C18:2n6c and C20:1, with a correlation coefficient of −0.81 and −0.71 ($p < 0.01$). Ultimately, a total of 6 modules with | Person Coefficient | > 0.68 and $p < 0.05$ were selected, based on the criteria of | GS | > 0.25, | MM | > 0.8, and $p < 0.05$, which identified 1549 genes related to C18:2n6c, 1200 genes related to C20:1, 1135 genes related to C20:4n6, 1277 genes related to C22:2, and 134 genes related to C22:6n3, a total of 1713 genes were screened.

3.4. Identification of DEGs in pectoral muscles from broilers of different ages

In order to identify genes related to FA composition during the growth and development stages, D35 and D63 were selected to be compared separately with D119 for the analysis of DEGs. The DEGs in the two groups were screened using the following criteria: threshold cut-off of | log2(Fold Change) | ≥ 0.58, and $p < 0.05$. At the age of 35–119 days, there were a total of 1459 DEGs, including 379 upregulated genes and 728 downregulated genes (Fig. 2A). At the age of 63–119 days, there were a total of 1194 DEGs, including 207 upregulated genes and 572 downregulated genes (Fig. 2B). The obtained DEGs from the intersection of “D35 *vs*. D119” and “D63 *vs*. D119”, consist of 473 common genes (Fig. 2C), including 418 annotated genes and 55 new genes.

3.5. Identification of candidate genes related to differential changes in fatty acid composition

The DEGs of “D35, D63 *vs*. D119” were separately cross-analyzed with the related genes identified by WGCNA for five FAs, namely C18:2n6c, C20:1, C20:4n6, C22:2 and C22:6n3. Each of them identified 198, 162, 166, 166, and 0 genes. Among them, no genes were

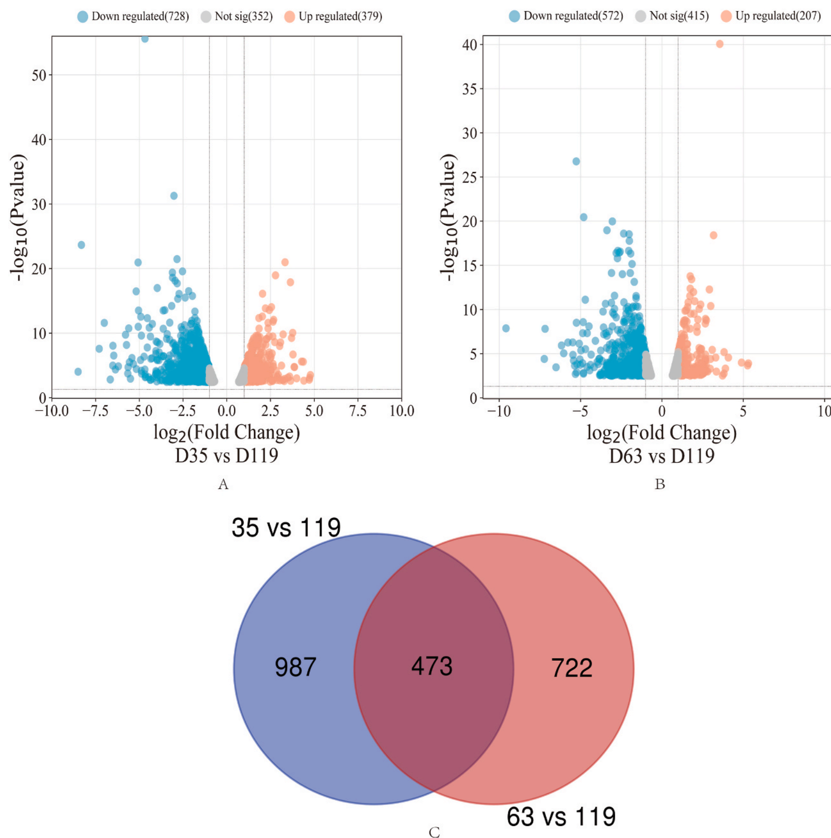

Fig. 2. Results of differentially expressed genes (DEGs) in pectoral muscle FA composition between “D35, D63 vs. D119”. (A) Volcano plot of DEGs in pectoral muscle FA composition between “D35 *vs*. D119”. (B) Volcano plot of DEGs in pectoral muscle FA composition between “D63 *vs*. D119”. (C) Venn diagram of intersection of sets of DEGs at “D35 *vs*. D119” and “D63 *vs*. D119”.

taken for crossover for C22:6n3. The results are presented in the form of a Venn diagram (Fig. 3A–E). The genes associated with the four FAs screened above were then used for intersection analysis, totaling 155 genes, including 140 known genes (Fig. 3F). Among them, genes related to lipid metabolism were identified, such as *MGLL*, *NAPEPLD*, *PLPP7*, *RXRA*, *LPIN1*.

3.6. Results of GO term functional and KEGG pathway enrichment analyses

Through GO term functional enrichment and KEGG pathway enrichment analyses of 155 genes screened for co-expression in four FAs, the KEGG pathway enrichment analysis results showed that 13 pathways were significantly enriched ($p < 0.05$), including, as shown in Fig. 4A, glycerolipid metabolism, which deserved attention. Other pathways closely related to lipid metabolism included mTOR signaling pathway, MAPK signaling pathway, *etc*.

In particular, both *MGLL* and *LPIN1* were enriched in the glycerolipid metabolism pathway, and only these two genes were enriched in this pathway. In the glycerolipid metabolism pathway, monoglyceride is converted into FAs under the action of *MGLL*, entering the fatty acids degradation pathway, while another component is converted into glycerol. Under the action of various enzymes, including

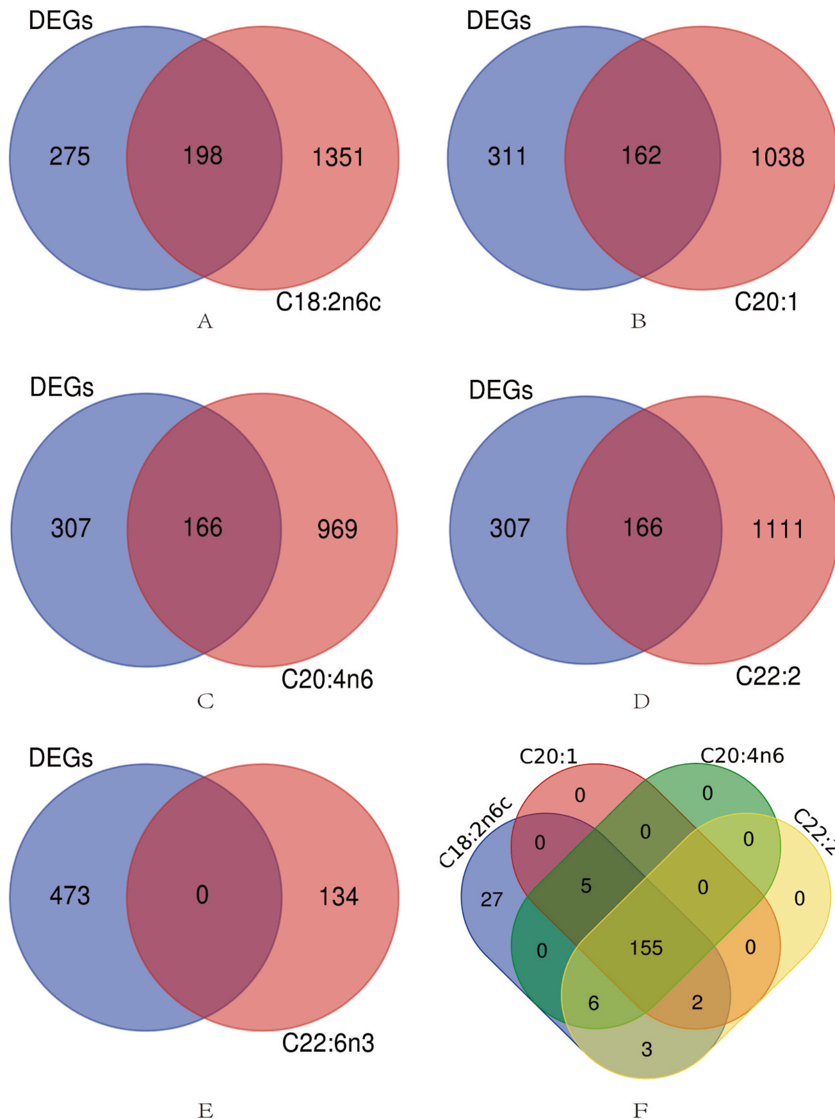

Fig. 3. Venn diagram of intersection of DEGs at different developmental stages and genes associated with five FAs in the WGCNA results. (A) Venn diagram of intersection of DEGs at different developmental stages and genes associated with C18:2n6c in the WGCNA results. (B) Venn diagram of intersection of DEGs at different developmental stages and genes associated with C20:1 in the WGCNA results. (C) Venn diagram of intersection of DEGs at different developmental stages and genes associated with C20:4n6 in the WGCNA results. (D) Venn diagram of intersection of DEGs at different developmental stages and genes associated with C22:2 in the WGCNA results. (E) Venn diagram of intersection of DEGs at different developmental stages and genes associated with C22:6n3 in the WGCNA results. (F) Venn diagram of intersection genes associated with four FAs.

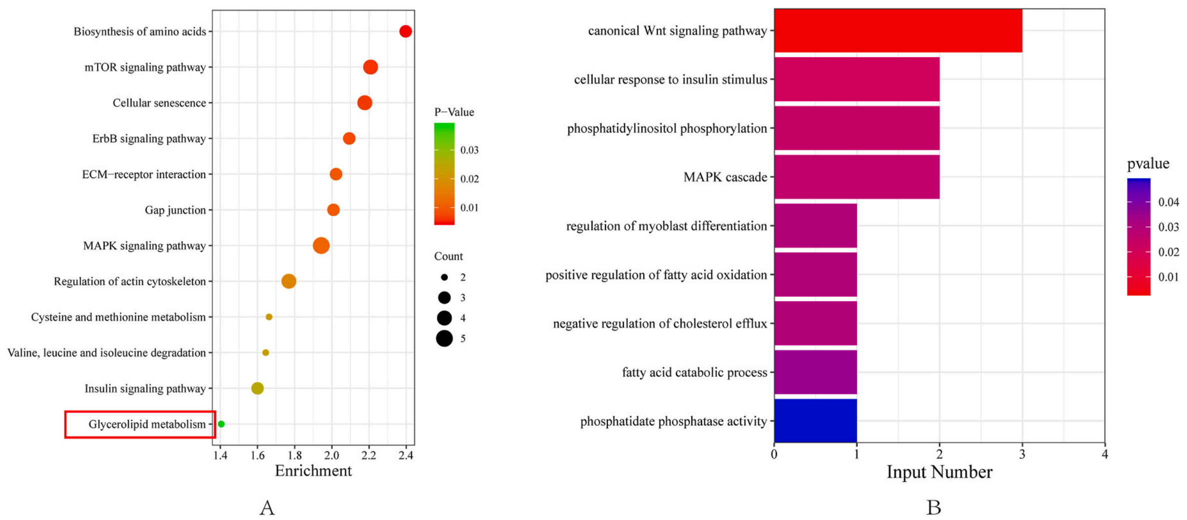

Fig. 4. Gene ontology (GO) term functional enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of intersecting genes. (A) KEGG pathway enrichment analysis of intersecting genes associated with four FAs ($p < 0.05$). (B) GO term functional enrichment analysis of intersecting genes associated with four FAs ($p < 0.05$).

GK and AGPAT, phosphatidate is formed from glycerol 3-phosphate and two fatty acyl CoAs, which is then converted into 1,2-diacyl-sn-glycerol by *LPIN1*, some into triacylglycerol, and others into monoglyceride, forming a circle (Fig. 5A). In this study, the expression levels of *MGLL* and *LPIN1* were found to be significantly increased ($p < 0.05$) with the growth and development of Jingxing yellow chickens (Fig. 5B).

GO term enrichment analysis showed that 187 GO terms were significantly enriched ($p < 0.05$), including 107 GO terms enriched in biological processes related to fatty acid catabolic process, positive regulation of fatty acid oxidation, *etc*. Another 41 GO terms were enriched in cellular components, such as actin cytoskeleton, actomyosin, etc. The remaining 39 GO terms were enriched in the molecular function category, which were related to phosphatidate phosphatase activity, Wnt-protein binding, *etc*. Ten of these terms were shown in Fig. 4B. Among them, *LPIN1* was enriched in multiple pathways, including phosphatidate phosphatase activity, fatty acid

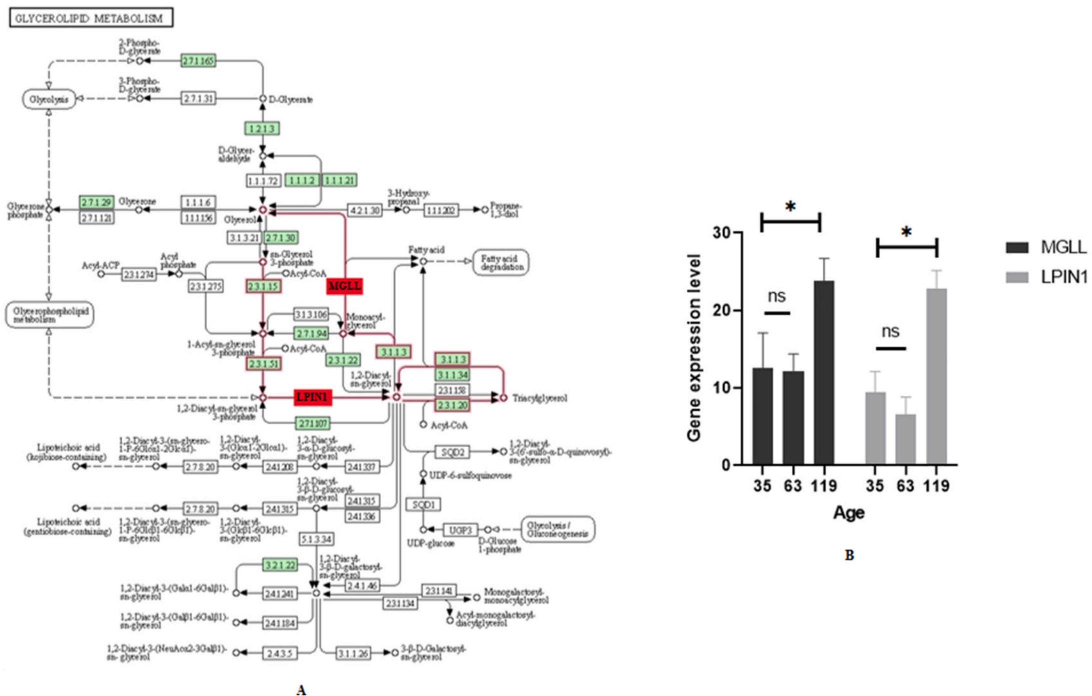

Fig. 5. The roles and expression levels of key candidate genes. (A) Glycerolipid metabolism pathway. (B) Expression levels of *MGLL* and *LPIN1* at 35, 63, 119 days of age.

catabolic process, and cellular response to insulin stimulus, which was closely related to sugar and lipid metabolism; *MGLL* was enriched in the acylglycerol lipase activity pathway and mainly participates in the glyceride hydrolysis reaction in lipid metabolism, breaking down glycerides into glycerol and FA.

4. Discussion

The growth age of livestock and poultry affects the changes in the levels of different types of FAs in the animal body, which is one of the main means by which age affects the flavor and nutritional value of meat products [31]. To date, there have been many reports on the changes in FA composition of livestock and poultry at different ages. For instance, Dal et al. [21] showed that older chickens have higher levels of long-chain PUFAs and total PUFAs; Jiang [32] found that, as the growth cycle increased, the contents of PUFAs and essential FAs in the muscles of Beijing-you chickens were higher than those of other breeds of broilers, which may explain their high nutritional value and excellent flavor quality; Popova et al. [33] showed that with age the ratio of n-6/n-3 PUFAs decreased in chickens. However, all the above studies were limited to the determination and analysis of the phenotypes of broilers at different days of age, and lacked the mining of genetic regulation of genes related to FA composition of chicken muscle. Therefore, in this study, we sequenced the transcriptome of a representative breed of China's high-quality yellow-feathered broilers, namely Jingxing yellow chickens, during the growth and development process at D36, D63 and D119, and concurrently determined the FA composition and other phenotypic data to analyze the dynamic gene expression process at different stages of broiler growth, identified the candidate genes related to the generation of lipids, and provided a theoretical basis for the genetic breeding of high-quality broilers through the study of the regulatory mechanism of production of FAs in muscle.

The main n-3 PUFAs required by the human body include eicosapentaenoic acid (**EPA**, C20:5n-3), DHA (C22:6n-3), and linolenic acid (**LNA**, C18:3n-3). The intake of n-3 PUFAs, especially EPA and DHA, is beneficial to reduce the incidence rate of heart disease [34] and delay the decline of immunity [35], they are necessary for the normal development of fetal brain and vision [36]. Instead, n-6 PUFAs have an anti-inflammatory effect, and excessive intake will cause chronic diseases, such as hyperlipidemia and atherosclerosis [37]. High n-6/n-3 PUFAs ratio is one of the major factors inducing cardiovascular diseases, inflammation and metabolic disorders [38]. Therefore, the World Health Organization and the Food and Agriculture Organization (**FAO-WHO**, 2010) of the United Nations recommend a 5 to 10 ratio as an appropriate ratio of n-6/n-3 PUFAs [39], while the Chinese Nutrition Society proposed a 4 to 6 ratio as an appropriate ratio of n-6/n-3 PUFAs in the Chinese dietary reference intakes [40]. In this study, as the growth age of Jingxing yellow chickens increased, the n-6/n-3 PUFAs ratio showed a downward trend, and at the age of 119 days before and after the listing date, it approached the appropriate ratio requirement of n-6/n-3 PUFAs proposed by FAO-WHO. According to the age of listing, yellow feather broilers can be divided into three categories: fast large (before 60 days old), medium speed (after 60–100 days old), and slow speed (also known as high-quality, after 100 days old). The slower the growth rate, the more flavor, delicate taste, and better nutrients are developed in the body of yellow feather broilers [41]. Considering whether these excellent characteristics are achieved by gradually reaching the suitable n-6/n-3 PUFAs ratio in muscles with increasing age, it is of critical to appropriately increase the proportion of n-3 PUFAs beneficial to human health in livestock and poultry production, so that broilers can maintain a nutrition diet suitable FA composition for humans at the age of market.

The deposited FAs in chicken are mainly derived from the endogenous synthesis in the liver or from dietary intake. Some PUFAs, such as linoleic acid (C18:2n6c) and LNA (C18:3n3), are essential FAs and cannot be synthesized in the liver, they can only be deposited in chicken through dietary intake [42]. The synthesis and metabolism of FAs mainly involve the synthesis of saturated FAs, carbon chain extension, and the synthesis of UFAs [43]. Many important physiological functions in the animal body require the essential FAs LNA (ω-3 series) and linoleic acid (ω-6 series) for further synthesis of arachidonic acid (20:4n-6), EPA (C20:5n3) and DHA (C22:6n3), *etc*., through a series of desaturation (addition of a double bond between two carbon atoms) and elongation (addition of two carbon atoms) reactions. In this study, with the growth and development of Jingxing yellow chickens, the content of linoleic acid significantly decreased, while the content of arachidonic acid significantly increased, indicating that in the mature stage of broiler chickens, most of the linoleic acid has been converted into arachidonic acid. Arachidonic acid, as a structural lipid component present in phospholipids of cell membrane, plays an important regulatory role in health and can prevent cardiovascular and cerebrovascular diseases in humans. In addition, arachidonic acid has an improving effect on the strength and freshness of meat flavor [44]. Therefore, the increase in arachidonic acid content in market aged chicken to a certain extent has important production significance. However, there is a limitation in this study due to the lack of elucidation and understanding of the relationship between the changes in intermediate conversion products, such as C18:3n6 and C20:3n6.

For the results of the KEGG pathway enrichment analysis, the focus is on the *MGLL* and *LPIN1* genes that are simultaneously enriched in the glycerolipid metabolism pathway. MGLL catalyzes the conversion of monoacylglycerols to free FAs and glycerol, Xu et al. [45] found a correlation between *MGLL* and FA metabolism through mining and analysis of transcriptome data of follicles from Zhedong white geese. Expression of *LPIN1* is required for adipocyte differentiation and it also functions as a nuclear transcriptional coactivator with some peroxisome proliferator-activated receptors to modulate expression of other genes involved in lipid metabolism. Yang et al. [46] found that *LPIN1* is highly expressed in chicken liver and ovaries, and lipid synthesis is significantly increased during the peak egg laying period. The expression level of *LPIN1* is also significantly higher than that in early development, suggesting that it may be involved in the regulation of chicken reproduction and muscle development, but there is a lack of studies on *LPIN1* expression in broiler muscle. In this study, the WGCNA results indicate that both genes play a role in co-expression in the grey60 module, with a negative regulatory effect on C18:2n6c, C20:1, and a positive regulatory effect on C20:4n6 and C22:2 deposition. In addition, compared with the developmental and mature stages of Jingxing yellow chickens, the content of C18:2n6c and C20:1 significantly decreased, while the content of C20:4n6 and C22:2 significantly increased, confirming the promoting effect of MGLL and LPIN1 on FA

production. Moreover, in the glycerolipid metabolism pathway, it can be inferred that the enzymes encoded by these two genes work together to catalyze the conversion of monoglycerides into FAs. This also makes the deposition of C18:2n6c lower than its degradation in the mature stage of broilers, while the deposition of C20:4n6 is substantial, leading to the transformation of linoleic acid into arachidonic acid. However, there are few studies on the joint effects of MGLL and LPIN1, Yousuf et al. [47] identified genes affecting FA metabolism disorder in breast cancer subtypes, and found that *MGLL* and *LPIN1* expression was downregulated. These findings are consistent with the results of this study, but there have been no reports of the synergistic effect of these two genes on FA production in broilers, and further validation of this finding at the cellular level *in vitro* or *in vivo* is needed.

5. Conclusion

In summary, this study elucidated the phenotypic and genetic differences in FA composition of the pectoral muscles of Jingxing Yellow Chicken at different ages. The key genes *MGLL* and *LPIN1*, which regulate FA metabolism in chicken muscle at different development stages, were identified and found to be involved in the glycerolipid metabolism pathway. It is hypothesized that these two genes may work together to degrade C18:2n6c, and convert it into C20:4n6 for deposition. The results of this study also provide insights to understand the genetic regulation of FA composition in chicken breast muscles, which is beneficial to improve the quality of meat products.

Data availability

Data will be made available on request. The RNA sequencing raw data reported in this paper have been deposited in the Genome Sequence Archive (Genomics, Proteomics & Bioinformatics 2021) in National Genomics Data Center (Nucleic Acids Res 2022), China National Center for Bioinformation/Beijing Institute of Genomics, Chinese Academy of Sciences (GSA: CRA011927) that are publicly accessible at https://ngdc.cncb.ac.cn/gsa.

CRediT authorship contribution statement

Yanji Chen: Writing – original draft, Data curation. **Yongli Wang:** Writing – original draft, Data curation. **Xiaojing Liu:** Writing – original draft. **Yanke Wang:** Writing – original draft. **Jie Wen:** Writing – review & editing. **Guiping Zhao:** Writing – review & editing. **Huanxian Cui:** Writing – review & editing.

Declaration of competing interest

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

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