Transcriptome analysis identifies signaling pathways related to meat quality in broiler chickens – the ECM receptor interaction signaling pathway

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Availability of data and material

39 Upon reasonable request, the datasets of this study can be available from the corresponding author.

40 **Authors' contributions**

41 Conceptualization: Jiancheng Yang, Jianmin Hu.

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48 **Ethics approval and consent to participate**

49 The animal welfare committee of the College of Animal Science and Veterinary Medicine of Shenyang Agricultural
50 University approved the experimental procedures, which were performed according to the Regulations for the
51 Administration of Affairs Concerning Experimental Animals (China, 1988) and EU Directive 2010/63/EU for animal
52 experiments. Surgeries were performed according to recommendations proposed by the European Commission
53 (1997). All efforts were made to minimize the animals' suffering.

54 **Abstract**

55 **Background** Meat quality characteristics, including juiciness, flavor, and tenderness,
56 can be mostly attributed to the total muscle fat content, intramuscular fat (IMF), and
57 the composition of its fatty acids (FAs), which are regulated by the balance between
58 lipid uptake, transport, synthesis, and subsequent metabolism, involving many genes
59 and pathways. However, the detailed molecular mechanisms remain unclear.

60 **Results** The present study reports the RNA-sequencing (RNA-seq) analysis of chest
61 muscle and leg muscle tissue of the Zhuanghe dagu chicken (ZD chicken) and the
62 Arbor Acres Broiler chicken (AA chicken). We identified certain differentially
63 expressed genes that affect IMF deposition, such as *EHHADH*, *TECRL*, *NDUFAB1*,
64 *PCCB*, and *HIBCH*, which were upregulated in ZD chickens, and *GCDH*, *TPH1*,
65 *ABHD13*, *PSMCI*, *MYST2*, and *FBXO11*, which were upregulated in AA chickens.
66 Pathway analysis using the Kyoto Encyclopedia of Genes and Genomes indicated that
67 the extracellular matrix (ECM)–receptor interaction pathway is co-enriched in both

68 tissues, and forms a sub-pathway of other enriched pathways. Intriguingly, the
 69 ECM–receptor interaction pathway genes are regulated differently in different gene
 70 pools. Collagens, which are main ECM constituents, and laminin and integrin β 1
 71 transmembrane receptors were significantly downregulated in both tissues of the AA
 72 chicken.

73 **Conclusions** RNA-seq analysis of two tissues from two breeds of chicken hinted that
 74 interactions between transmembrane receptors and ECM components of fat cells
 75 affect breed-specific adipogenesis, which would affect quality and flavor of chicken
 76 meat. These findings will contribute to improving the quality attributes of chicken
 77 meat.

78 **Keyword:** Zhuanghe dagu chicken, Arbor Acres Broiler chicken, IMF deposition,
 79 ECM receptor interaction signaling pathway

80 Abbreviations

EHHADH	enoyl-CoA hydratase and 3-hydroxyacyl CoA dehydrogenase
TECRL	trans-2,3-enoyl-CoA reductase like
NDUFAB1	NADH:ubiquinone oxidoreductase subunit AB1
PCCB	propionyl-CoA carboxylase subunit beta
HIBCH	3-hydroxyisobutyryl-CoA hydrolase
GCDH	glutaryl-CoA dehydrogenase
TPI1	triosephosphate isomerase 1
ABHD13	abhydrolase domain containing 13
PSMC1	proteasome 26S subunit, ATPase 1
MYST2	Histone acetyltransferase MYST2
FBXO11	F-box protein 11
BAP1	BRCA1 associated protein 1
SETD2	SET domain containing 2, histone lysine methyltransferase
MCM2	minichromosome maintenance complex component 2
ACTR3B	actin related protein 3B
ARPC5	actin related protein 2/3 complex subunit 5

81 **Background**

82 Products based on chicken meat are important components of human nutrition. In
83 recent decades, genetic selection for growth rate and yield have improved the amount
84 of available meat in chickens. However, the higher the growth rate, the larger the fiber
85 diameter, the higher the proportion of glycolytic fiber, and the lower the fat content in
86 muscle, which negatively affect the quality of the meat [1-3]. Improving meat quality
87 while maintaining the growth rate remains a challenge in the poultry industry.
88 Recently, there has been increased consumer demand for meat sourced from local or
89 indigenous birds because of its rich flavor, unique taste, and firm texture. Furthermore,
90 there is a perception among consumers that these birds are produced naturally using
91 extensive farming. The growing awareness among consumers regarding poultry health
92 and nutrition has encouraged specialty markets for local poultry varieties produced
93 using extensive farming systems [4, 5]

94 The Zhuanghe Dagu chicken (ZD chicken), also called the Zhuanghe chicken,
95 originated in Zhuanghe, Liaoning province, China and is mainly bred in parts of
96 Northeast China, Hebei, and Inner Mongolia. The ZD chicken has many advantages,
97 such as a big body, superb meat quality, a tall trunk, large red eggs, adaptability of
98 rough forage, cold resistance, and high disease resistance, leading it to be known as
99 the king of northeast chickens [6]. As one of the most valuable materials for poultry
100 breeding in China, the Chinese government listed the ZD chicken as a nationally
101 protected domestic animal in 2000 [7]. In the present study, the chest muscles and leg
102 muscles of the ZD chicken and the Arbor Acres broiler chicken (AA chicken) were

103 used as test materials.

104 Meat quality characteristics, such as juiciness, flavor, and tenderness, are attributed
105 mainly to the total muscle fat content, the intramuscular fat (IMF), and its fatty acid
106 (FA) composition [8]. IMF comprises the amount of fat in muscles, including that in
107 the outer layer, perimuscular region, and the endometrium [9]. In contrast to adipose
108 tissue, in which triglyceride, as the major lipid category, accounts for more than 90%
109 of lipids, IMF includes a significant proportion of phospholipids. Muscle FAs are
110 rich in polyunsaturated fatty acids (PUFA), including arachidonic acid (20:4n-6),
111 linoleic acid (18:2n-6), and α -linolenic acid (18:3n-3) [10]. Heating of PUFAs results
112 in their oxidation to produce volatile components, e.g., 2, 4-sebacal, which improve
113 meat flavor [11]. Studies have demonstrated that juiciness, flavor, and tenderness
114 correlate positively with the muscle total fat content [8, 9, 12-16]. Previous studies
115 used microarray technology to analyze chicken breast muscle [17] and liver [18],
116 which revealed certain potential candidate genes and pathways that might influence
117 chicken meat flavor; however, no further validation has been performed.

118 The commonly accepted method of comparing meat production is to use the
119 chronological time (age). However, growth comprises both mass and time
120 components, either of which could be used effectively to compare growth traits [19].
121 It has been proposed that physiological time might be a better measure than
122 chronological time when comparing growth and meat quality traits between fast
123 growing broilers and slow growing native chickens, particularly when their market
124 ages differ [20-23]. Therefore, the present study performed RNA-sequencing

125 (RNA-seq) analyses of breast and leg muscle samples of 6-month-old ZD chickens
126 and 6-week-old AA chickens.

127 **Materials and Methods**

128 **Ethics statement**

129 The animal welfare committee of the College of Animal Science and Veterinary
130 Medicine of Shenyang Agricultural University approved the experimental procedures,
131 which were performed according to the Regulations for the Administration of Affairs
132 Concerning Experimental Animals (China, 1988) and EU Directive 2010/63/EU for
133 animal experiments. Surgeries were performed according to recommendations
134 proposed by the European Commission (1997). All efforts were made to minimize the
135 animals' suffering.

136 **Animals and tissues**

137 The chickens used in this study comprised ZD chickens (24 weeks old) and AA
138 chickens (6 weeks old). Feeding management was carried out according to the
139 standard routine. The daily food comprised a whole diet and feed, and its nutritional
140 components could satisfy the growth and development demands of chickens. Selected
141 chickens (50% male: 50% female) were killed by cutting their neck arteries and veins
142 to allow exsanguination. Then, the chest and leg muscles were excised immediately,
143 frozen in liquid nitrogen, and stored at -80 °C until use in RNA extraction.

144 **Total RNA extraction and the construction of the RNA-Seq library**

145 RNA-seq analysis was carried out on samples from 12 individuals from the two
146 groups. An Animal tissue total RNA extraction kit (Beijing Tiangen biochemical
147 technology co. LTD, Beijing, China) was used to isolate total RNA from the muscle
148 tissues, following the manufacturer's instructions. Agarose gel electrophoresis and a
149 NanoDrop 8000 spectrophotometer (NanoDrop, Thermo Scientific, Waltham, MA,
150 USA) were used to assess the concentration and purity of the RNA, respectively. A
151 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) was used to
152 determine the RNA integrity. To minimize the effect of transcriptome variation
153 among individuals, we pooled equal amounts of the RNA samples from six
154 individuals in each group to form one mixed sample for each group. Subsequently, a
155 complementary DNA (cDNA) library was constructed using the mixed RNA samples.
156 A TruSeq RNA Sample Preparation Kit (Illumina, Inc., San Diego, CA, USA) was
157 then used to construct mRNA libraries in accordance with the TruSeq protocol.
158 Finally, an Illumina HiSeq 2000 instrument at the Huada gene technology co. LTD
159 (Shenzhen, China) was used to sequence the libraries.

160 **Bioinformatic analysis of the RNA-Seq data**

161 Using transcriptome profiling, the gene expression profiles of the pectorales and
162 crureus muscles of the ZD chicken and AA broiler were acquired and analyzed using
163 GeneSpring7.0 software (<http://www.silicongenetics.com>). We identified the
164 differentially expressed genes (DEGs) of the pectorales and crureus in both chickens
165 as those whose expression was at least two times higher or lower in each comparison.

166 Genes whose expression changed by less than 2-fold, or that did not change, were
167 excluded from further analysis. Then, the resultant DEGs were subjected to gene
168 ontology (GO) functional clustering analysis to identify DEGs involved in meat flavor
169 formation in the chest and leg muscles of ZD and AA chickens
170 (<https://david.ncifcrf.gov/home.jsp>).

171 **Validation using quantitative reverse transcription real-time PCR reverse** 172 **transcription (qRT-PCR)**

173 The reliability of the Illumina analysis was verified using qRT-PCR analysis of 17
174 genes believed to affect the meat quality traits of ZD Chickens or their muscle growth
175 and development. Primer 5.0 was used to design the primers used for qRT-PCR and
176 the 17 pairs of primer are shown in Table S1. Tissues from every three samples were
177 collected as a group and for each sample there were three replicates. The total RNA of
178 both chicken muscle tissues (breast and leg) was then isolated and extracted.

179 A PrimerScript RT Reagent Kit (Takara, Dalian, China) was used to reverse
180 transcribe the total RNA. Subsequently, a Bio-Rad iQ5 Real-time PCR Detection
181 System (BIO-RAD, Hercules, CA, USA) was used to perform the qPCR reactions. A
182 series of 4-fold diluted samples(cDNA products:PCR-water of 1:3) was used to
183 construct standard curves for the different genes. The PCR efficiency and the slopes
184 of the standard curves were calculated for the genes to verify that the qRT-PCR data
185 were reliable and precise. The gene expression levels were verified using three

186 independent biological replicates together with the internal reference gene(β -actin).

187 The $2^{-\Delta\Delta C_t}$ method [24] was used to calculate the fold changes.

188 **Statistical analyses**

189 The data are reported as the mean $\pm$ the standard error of the mean (SEM) based on

190 the results of at least three replicates for each treatment. To determine statistical

191 significance, one-way analysis of variance (ANOVA) was used. Statistical

192 significance was accepted at $P < 0.05$

193 **Results**

194 RNA-seq was employed to acquire the chest muscle transcriptome of ZD and AA

195 chickens of different ages, with the aim of determining the potential molecular

196 mechanism underlying chicken meat quality, flavor, and growth and development. We

197 selected two birds per breed at each age to construct separate cDNA libraries for

198 RNA-seq. For each sample there were over 3 million clean reads, which corresponded

199 to 7,953 to 9,923 expressed genes (Table 1).

200

201

202

203

204 **Table 1. Statistics of the RNA-seq data.**

Samples	Clean reads	Genome mapping rate (%)	Gene mapping rate (%)	Expressed genes
6MDXA	3388751	96.56	41.57	7953
6MDTA	3399815	97.46	43.68	8357
6WAXA	3525200	96.40	46.32	8861
6WATA	3522407	94.45	51.87	9923

205 *4WDXA, Chest muscle of 4-week-old ZD chickens; 4WDTA, leg muscle of 4-week-old*
206 *ZD chickens; 6MDXA, Chest muscle of 6-month-old ZD chickens; 6MDTA, leg muscle*
207 *of 6-month-old ZD chickens; 6WAXA, Chest muscle of 6-week-old AA chickens; 6WDTA,*
208 *leg muscle of 6-week AA chickens*

209 **DEGs Analysis**

210 DEGs were identified as those genes with a false discovery rate (FDR) < 0.05 and at
211 least a two-fold difference in expression. The expression levels were estimated using
212 the RPKM method (Reads per Kb per Million mapped reads) to obtain the up- and
213 downregulated genes (figure 1).

A

B

Figure 1. Number of differentially expressed genes (DEGs) between two samples. (A) Numbers of unique or shared DEGs between two samples. (B) Numbers of

220 DEGs showing upregulated or downregulated expression in in each comparison. W, week; M,
221 month; A, AA broiler; D, Zhuanghe Daggu chicken; TA, leg muscle; BA, breast muscle.

222 In the comparison between the pectoral muscle data of the 24-week-old ZD chickens
223 and the 6-week-old AA chickens (6WAXA vs. 6MDXA), there were 155 upregulated
224 genes, including 42 unknown genes, and 488 downregulated genes, among which 143
225 were unknown. While in the comparison of crureus muscle between the chickens
226 (6WATA vs. 6MDTA), there were 399 upregulated genes, of which 123 were
227 unknown, and 981 downregulated genes, of which 281 were unknown.

228 Additionally, in the 6WATA vs. 6MDTA and 6WAXA vs. 6MDXA comparisons, 222
229 DEGs were shared. These DEGs might correlate with the origin of the meat flavor
230 between the breeds. The genes with large differences in expression are shown in table
231 2.

Table 2. Expression levels of differentially expressed genes with the most significant differences

Gene	Raw Intensity -S1	Raw Intensity -S2	TPM -S1	TPM- S2	P-Value	FDR	Symbol	Transcript ID
424877	7	32	1.99	9.41	2.63E-05	0.00015	EHHADH	gi 18094871 ref XM_422690.2
422618	4	45	1.14	13.24	2.10E-10	2.30E-09	TECRL	gi 18090172 ref XM_420576.2
416571	243	967	68.99	284.43	2.13318E-108	1.70918508E-106	NDUFAB1	gi 18097960 ref XM_414872.2
768706	40	83	11.36	24.41	4.22E-05	0.000232	PCCB	gi 18094795 ref XM_001231793.1
423979	171	426	48.55	125.3	2.99E-28	8.08E-27	HIBCH	gi 71895122 ref NM_001031243.1
56507	73	26	20.72	7.65	3.91E-06	2.52E-05	GCDH	gi 18111165 ref XM_001231725.1
396435	4065	624	1154.04	183.54	0	0	TP11	gi 45382060 ref NM_205451.1

232 Validation using qRT- PCR

233 Seventeen genes were selected randomly for qRT-PCR verification of the RNA-seq
234 data. Figure 2 show that in general, the expression patterns of the 17 genes were
235 consistent between the RNA-seq data and the qRT-PCR data, which supported the
236 reliability of the Illumina sequencing data. Any discrepancies regarding ratios could
237 be attributed to the different algorithms and sensitivities of the two techniques.

238
239 Figure 2. QRT-PCR validation of the gene expression profiles.

240 GO classification analysis of the DEGs

241 Figure 3 shows that the DEGs in the ZD and AA chickens influenced the GO terms
242 included in the three secondary classifications of biological process, cellular
243 component, and molecular function.

244

245

Figure. 3 A: Histogram showing the GO annotation of up- and downregulated genes between 6WATA and 6MDTA. B: Histogram showing the GO annotation of up- and downregulated genes between 6WAXA and 6MDXA. Red indicates upregulated genes, and blue represents downregulated genes

In the category of biological process, relatively large effects were observed on cellular process, metabolic process, and single-organism, which were significantly different from the positive and negative controls.

255 In the cellular component category, development process, response to stimulus, and
256 other biological regulatory functions showed large effects. This analysis showed that
257 pathways related to energy metabolism in cells might have a marked impact on the
258 origin of meat quality.

259 The results for the categories of cell components, cell and cell parts changed the
260 most, indicating that the two different chickens had significantly different
261 intracellular components, particularly in the ECM and extracellular structures.

262 The GO categories of extracellular regions, macromolecular complex, membrane,
263 organelle, and organelle part showed large differences, which suggested that the
264 membrane, extramembranous protein, and organelle part might have an impact on
265 the origin of meat quality.

266 In terms of Molecular Function, binding and catalytic activity changed the most,
267 showing that the origin of meat quality might be related to the catalytic oxidation
268 activity of certain enzymes. The structural molecule activity and transporter activity
269 also changed markedly, suggesting that origin of meat quality might be determined
270 by differences in membrane surface transport systems.

271 **Analysis of DEGs using KEGG pathway enrichment**

272 KEGG pathway enrichment analysis suggested that certain pathways in fatty acid
273 metabolism and synthesis might have a significant effect on meat quality. The results
274 of GO and KEGG functional enrichment analysis suggested that the ECM-receptor

275 interaction signaling pathway is closely related to meat quality (figure 4) The
 276 ECM-receptor interaction signaling pathway showed an enrichment rate of 0.6 to 0.8
 277 ($P < 0.05$).

278
 279 Figure 4. Histogram of the KEGG enrichment analysis of the up- and downregulated
 280 genes between 6WATA and 6MDTA. The x-axis shows the name and classification of
 281 the path; the y-axis represents the enrichment rate; * indicates $p < 0.05$. The side-color
 282 gradient represents the increasing P value (faint to dark).

283 Visualization of KEGG pathways associated with the DEGs

284 Using the KEGG database, the 6MD gene was used as a reference. The KEGG
 285 pathways associated with changes in gene expression in the 6WA group were
 286 displayed in a KEGG annotation pathway map. In figure 5, the genes and pathways
 287 associate with ECM-receptor interaction signaling pathway are indicated by a red box
 288 (representing proteins with up-regulated mRNA expression) and a green box
 289 (downregulated). The expression of reduced ECM component mRNA can be

simultaneously seen in the presence of many meat-quality-related genes in the
ECM-receptor interaction signaling pathway.

The ECM-receptor interaction signaling pathway

Figure 5. KEGG annotation pathway map of the downregulated ECM–receptor
interaction signaling pathway. Blue background boxes indicate proteins encoded by
background genes in the chicken transcriptome data. White background boxes
indicate proteins encode by genes from non-chicken species. Boxes with red borders
represent the protein products of upregulated differentially expressed genes. Boxes

299 with green borders represent the protein products of downregulated differentially
300 expressed genes.

301 **Discussion**

302 The Zhuanghe dagu chicken originated from Zhuanghe city, Dalian, China, and is a
303 unique local breed that was bred during a particular historical period and under special
304 geographical and climatic conditions. The ZD chicken has characteristics of rough
305 feeding, cold resistance, and strong adaptability, and is famous for its large body, deep
306 and broad chest, wide and long back, tall and stout legs, and plump belly [25]. The
307 Zhuanghe dagu chicken has strong disease resistance, a high egg yield, and delicious
308 meat taste. It is a famous local fine breed for meat and eggs in China, and was listed
309 as a national livestock and poultry genetic resources protected breed in 2006 [26].

310 With the industrialized production of the Zhuanghe dagu chicken, the product
311 coverage area has gradually expanded and the market share is increasing year by year.
312 The processed products are sold to 12 countries and regions in five continents,
313 including Europe, Asia, America, and Africa [27]. In recent years, researchers have
314 studied the characteristics of big-bone chickens using cytological polymorphisms,
315 protein polymorphisms, DNA polymorphisms, and candidate genes [28, 29]. These
316 studies showed that the meat quality of big-bone chickens is significantly better than
317 that of commercial broiler chickens. Chicken meat contains less fat and a higher
318 proportion of polyunsaturated fatty acids (PUFA) compared with meat from other
319 species, making it a better choice for healthy diets [30]. In western countries, chicken

320 meat is very popular, with an annual per capita consumption of approximately 22 kg
321 in Europe and 38 kg the USA (Eurostat, 2008; USDA, 2010). Consequently,
322 consumers and researchers are paying attention to factors that affect chicken meat
323 quality.

324 Genetic and environmental factors combine to make meat quality a complex trait, and
325 there can be large variations in meat quality within and between animals [31]. One
326 key meat quality trait is IMF, which directly affects meat sensory properties [32, 33],
327 as well as juiciness, flavor, tenderness, and overall acceptability. Consumers consider
328 meat sensory traits as extremely important for meat acceptability. IMF seems to play
329 important roles in eating quality [34, 35]. Therefore, increasing the IMF level to
330 produce poultry meat with high sensory quality without affecting total carcass yields,
331 is desired by consumers and the poultry industry.

332 Low lysine incorporation or reduced protein diets (RPD) [36] in pigs represent a
333 successful nutritional strategy to increase muscle fat accumulation, which does not
334 affect other fat depots like the abdominal or subcutaneous depots (i.e., improved fat
335 partitioning). Furthermore, enhanced pork sensory acceptability might result from the
336 increased IMF induced by RPD [37, 38]. In beef, the analysis muscle
337 growth-associated DEGs [39] suggested that the biological mechanisms controlling
338 fat deposition and muscle growth are different. In ducks, tenderness and flavor were
339 promoted by increasing lipid levels in the breast muscle [13]. Although the IMF is
340 useful in improving meat quality, the mechanism underlying in increased IMF content
341 is unknown [8].

342 In this study, breast and leg muscle transcriptomes of ZD and AA chickens with
 343 different genetic backgrounds were compared and analyzed. Many DEGs that might
 344 affect IMF deposition were identified between the breeds. The DEGs *EHHADH*,
 345 *TECRL*, *NDUFAB1*, *PCCB*, and *HIBCH*, were upregulated in ZD chickens, and
 346 *GCDH*, *TPII*, *ABHD13*, *PSMC1*, *MYST2*, and *FBXO11* were upregulated in AA
 347 chickens (table 2). Then, we verified the accuracy of the transcriptome sequencing by
 348 qRT-PCR technology. Using KEGG enrichment analysis, we found that the ECM
 349 receptor interaction pathway was a significantly enriched IMF-related pathway. The
 350 expression of reduced ECM component mRNA can be simultaneously seen in the
 351 presence of many meat-quality-related genes in the ECM-receptor interaction
 352 signaling pathway. We believe that ECM mediates a mechanism involved in the
 353 increasing of the IMF.

354 Reports suggest that IMF differs from other fats in three ways: Metabolic activities,
 355 adipocyte size, and developmental timing [40-42]. In cattle and pigs, non-muscular
 356 adipocytes are larger than intramuscular adipocytes [40, 43]. Based on the DEGs'
 357 functional annotation analysis in the two tissues, we hypothesized that the
 358 ECM-receptor interaction pathway is involved in tissue-specific variations in IMF
 359 levels. The ECM is a crucial component of tissue architecture and has an important
 360 function in adipogenesis[44]. However, the ECM in IMF tissue has received little
 361 research the analysis of ECM components is difficult in this tissue.

362 Fat cells are surrounded by the basement membrane (a thick ECM), of which type IV
 363 collagen is the main component [45]. The basement membrane of adipose tissue is

364 necessary for the survival of adipocytes. The lipid and cytoplasm within adipocytes
365 are separated by a single molecular layer of lipid, which can be easily damaged by
366 mechanical stress.

367 In 1974, Green and Meuth proposed that for differentiation and fat storage, collagen
368 synthesis is a prerequisite [61]. In 1990, Nandan et al. found that fat cell
369 differentiation was required for collagen synthesis [63]. In 2004, Bouwman et al.
370 confirmed used stable isotope labeling to study the protein dynamics of mature
371 non-dividing 3T3-L1 fat cells, which demonstrated that the ECM is important for the
372 function and survival of fat cells [65].

373 The ECM of fat cells has the same components as other cell types; however, the
374 number of components determines the cellular specificity of the ECM. [44] Among
375 them, type VI collagen is the most specific and is widely enriched in fat cells [58, 59].
376 Type VI collagen consists of three subunits: alpha 1 (VI), alpha 2 (VI), and alpha 3
377 (VI). [60] Aratani and Kitagawa [46] observed marked upregulation of collagen IV,
378 nidogen-1 (entactin), and various laminin complexes during adipocyte differentiation.
379 Wang et al. [68] found that when the net cell triglyceride content was reduced, cell
380 shrinkage, and decreased expression of genes encoding matrix proteins and various
381 processing enzymes occurred, causing a slowdown in the accumulation of the ECM.

382 Transmembrane molecules such as $\beta 1$ mediate specific interactions between the ECM
383 and cells. Our results showed that different members of the ECM collagen proteins,
384 such as laminin and transmembrane molecule $\beta 1$ were downregulated in both tissues
385 of the AA chicken. Liu J et al. have reported that changes in the expression of

transmembrane molecules are associated with adipocyte differentiation [75]. The regulation of other genes involved in the ECM–receptor interaction might be chicken breed dependent. According to the results of the comparative transcriptome analysis of the two tissues, the interaction between transmembrane receptors and ECM components of the cell might affect tissue specific adipogenesis [76], which would affect the quality and flavor of chicken meat.

Conclusion

In summary, the ECM-receptor interaction might affecting the differentiation of intramuscular adipocytes, and lipid synthesis and metabolism, thereby changing the IMF content, which would affect the meat flavor of broilers. These results will contribute to improving the quality and flavor of broiler chickens.

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