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

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**Research**

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

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

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## Declaration

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**Competing interests**

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**Availability of data and material**

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

**Authors' contributions**

Conceptualization: Jiancheng Yang, Jianmin Hu.

Data curation: Rifeng Xu.

Formal analysis: RIfeng Xu.

Methodology: Gaofeng Wu.

Validation: Yanting Du.

Writing - original draft: Jishuang San.

Writing - review & editing: Yanting Du, Jishuang San

**Ethics approval and consent to participate**

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


## Abstract

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

**Results** The present study reports the RNA-sequencing (RNA-seq) analysis of chest muscle and leg muscle tissue of the Zhuanghe dagu chicken (ZD chicken) and the Arbor Acres Broiler chicken (AA chicken). We identified certain differentially expressed genes that affect IMF deposition, such as *EHHADH*, *TECRL*, *NDUFAB1*, *PCCB*, and *HIBCH*, which were upregulated in ZD chickens, and *GCDH*, *TPI1*, *ABHD13*, *PSMC1*, *MYST2*, and *FBXO11*, which were upregulated in AA chickens. Pathway analysis using the Kyoto Encyclopedia of Genes and Genomes indicated that the extracellular matrix (ECM)–receptor interaction pathway is co-enriched in both

tissues, and forms a sub-pathway of other enriched pathways. Intriguingly, the ECM–receptor interaction pathway genes are regulated differently in different gene pools. Collagens, which are main ECM constituents, and laminin and integrinβ1 transmembrane receptors were significantly downregulated in both tissues of the AA chicken.

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

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

## 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** |

## Background

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

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

used as test materials.

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

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

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

## Materials and Methods

### Ethics statement

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

### Animals and tissues

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

### Total RNA extraction and the construction of the RNA-Seq library

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

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

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

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

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

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

A PrimerScript RT Reagent Kit (Takara, Dalian, China) was used to reverse transcribe the total RNA. Subsequently, a Bio-Rad iQ5 Real-time PCR Detection System (BIO-RAD, Hercules, CA, USA) was used to perform the qPCR reactions. A series of 4-fold diluted samples(cDNA products:PCR-water of 1:3) was used to construct standard curves for the different genes. The PCR efficiency and the slopes of the standard curves were calculated for the genes to verify that the qRT-PCR data 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 $2^{-\Delta\Delta Ct}$ method [24]was used to calculate the fold changes.

188 **Statistical analyses**

189 The data are reported as the mean ± 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).

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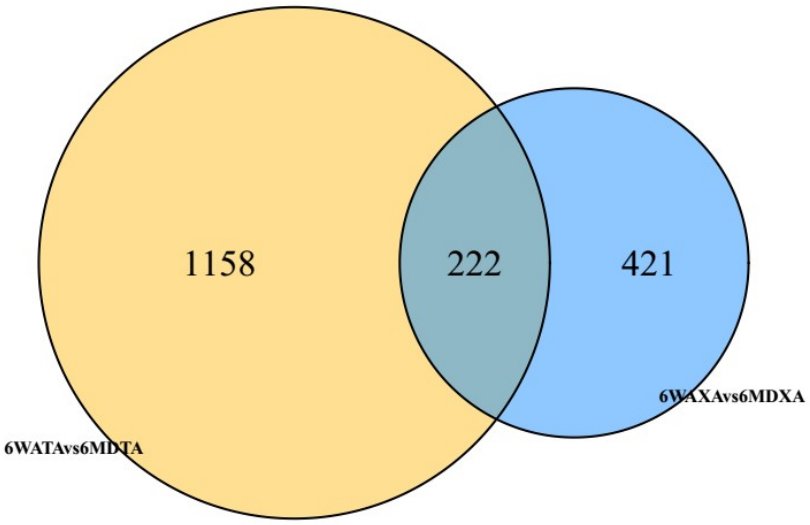
**Table 1. Statistics of the RNA-seq data.**

| 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** |

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

## DEGs Analysis

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

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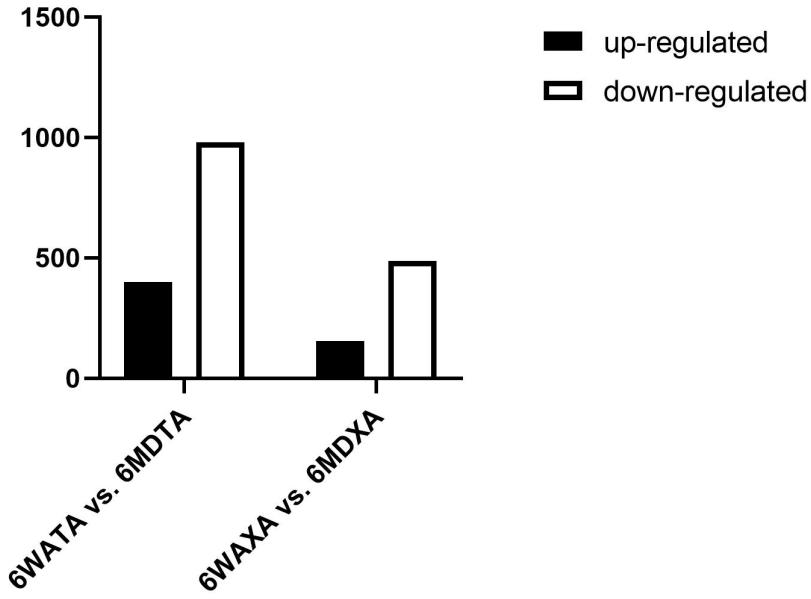
215 A

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217 B

218 Figure 1. Number of differentially expressed genes (DEGs) between two

219 samples. **(A)** Numbers of unique or shared DEGs between two samples. **(B)** Numbers of

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

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

Additionally, in the 6WATA *vs.* 6MDTA and 6WAXA *vs.* 6MDXA comparisons, 222 DEGs were shared. These DEGs might correlate with the origin of the meat flavor between the breeds. The genes with large differences in expression are shown in table 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\|118094871\| ref\|XM_422690.2\| |
| 422618 | 4 | 45 | 1.14 | 13.24 | 2.10E-10 | 2.30E-09 | TECRL | gi\|118090172\| ref\|XM_420576.2\| |
| 416571 | 243 | 967 | 68.99 | 284.43 | 2.13318E-108 | 1.70918508E-106 | NDUFAB1 | gi\|118097960\| ref\|XM_414872.2\| |
| 768706 | 40 | 83 | 11.36 | 24.41 | 4.22E-05 | 0.000232 | PCCB | gi\|118094795\| 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\|118111165\| ref\|XM_001231725.1\| |
| 396435 | 4065 | 624 | 1154.04 | 183.54 | 0 | 0 | TPI1 | gi\|45382060\| ref\|NM_205451.1\| |

## Validation using qRT- PCR

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

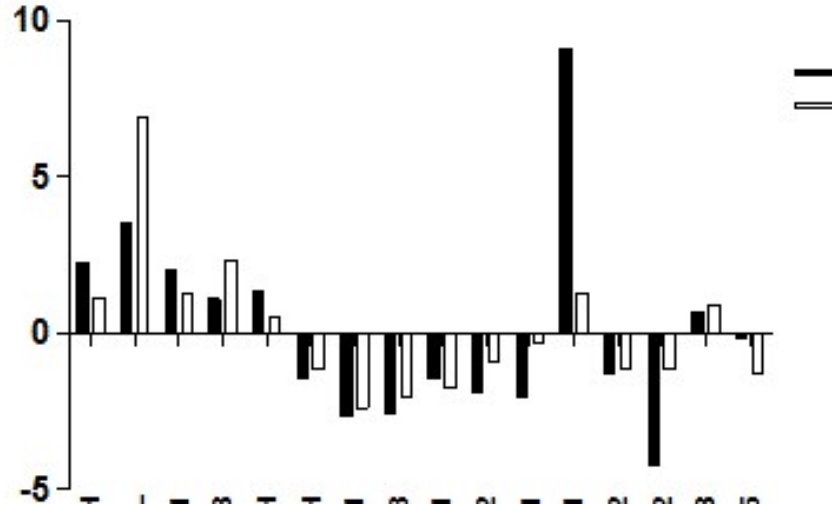
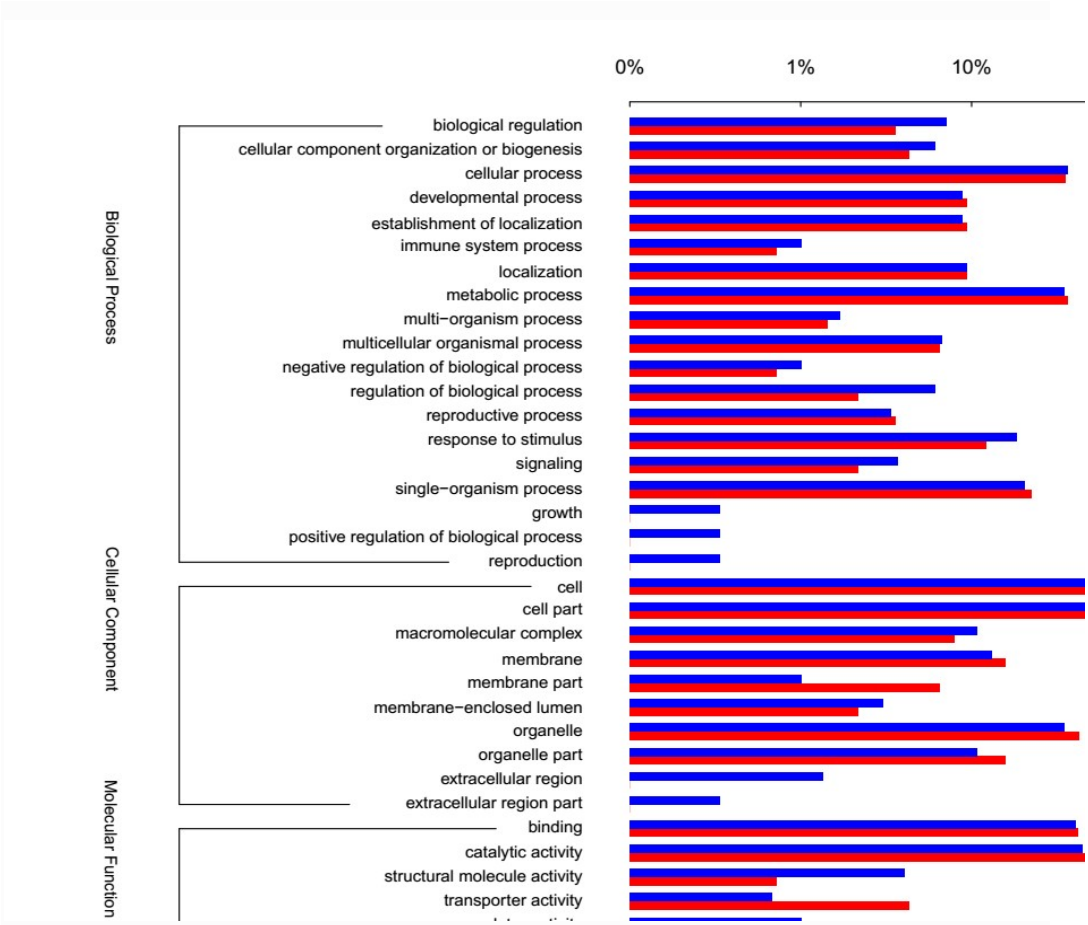


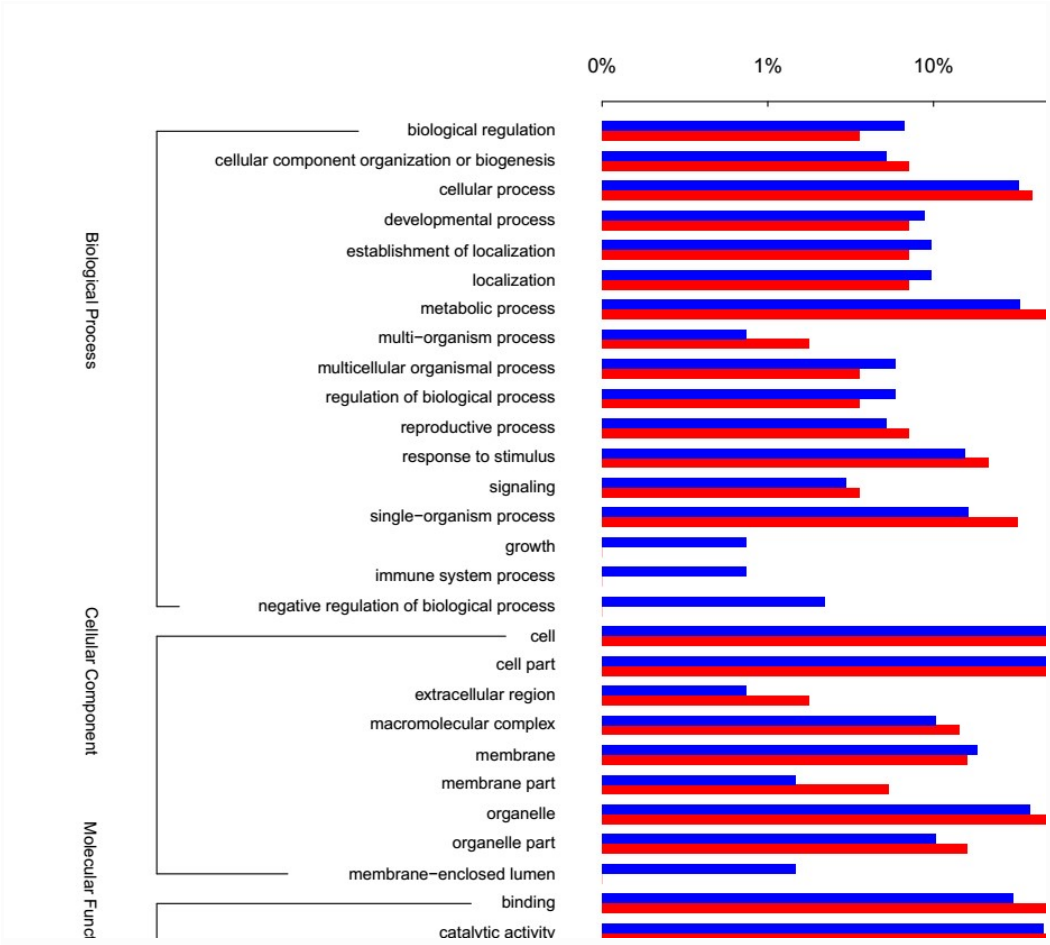

Figure 2. QRT-PCR validation of the gene expression profiles.

## GO classification analysis of the DEGs

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


0%
1%
10%
Biological Process
Cellular Component
Molecular Function
biological regulation
cellular component organization or biogenesis
cellular process
developmental process
establishment of localization
immune system process
localization
metabolic process
multi−organism process
multicellular organismal process
negative regulation of biological process
regulation of biological process
reproductive process
response to stimulus
signaling
single−organism process
growth
positive regulation of biological process
reproduction
cell
cell part
macromolecular complex
membrane
membrane part
membrane−enclosed lumen
organelle
organelle part
extracellular region
extracellular region part
binding
catalytic activity
structural molecule activity
transporter activity

A




B

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.

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

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

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

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

**Analysis of DEGs using KEGG pathway enrichment**

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

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

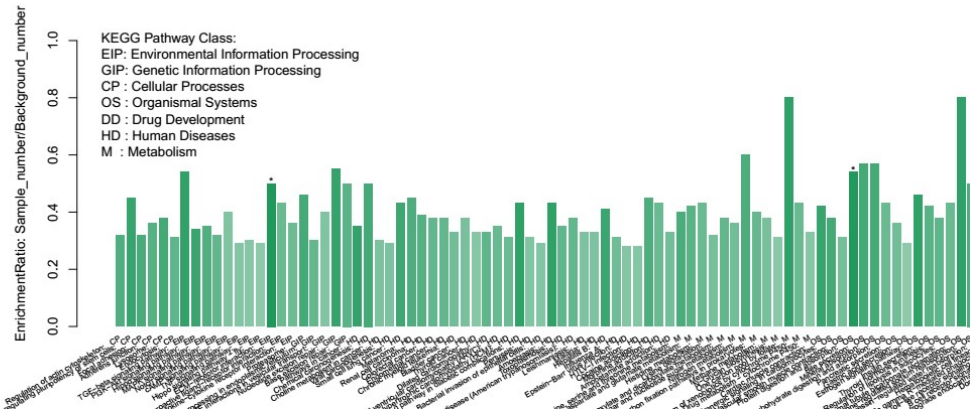
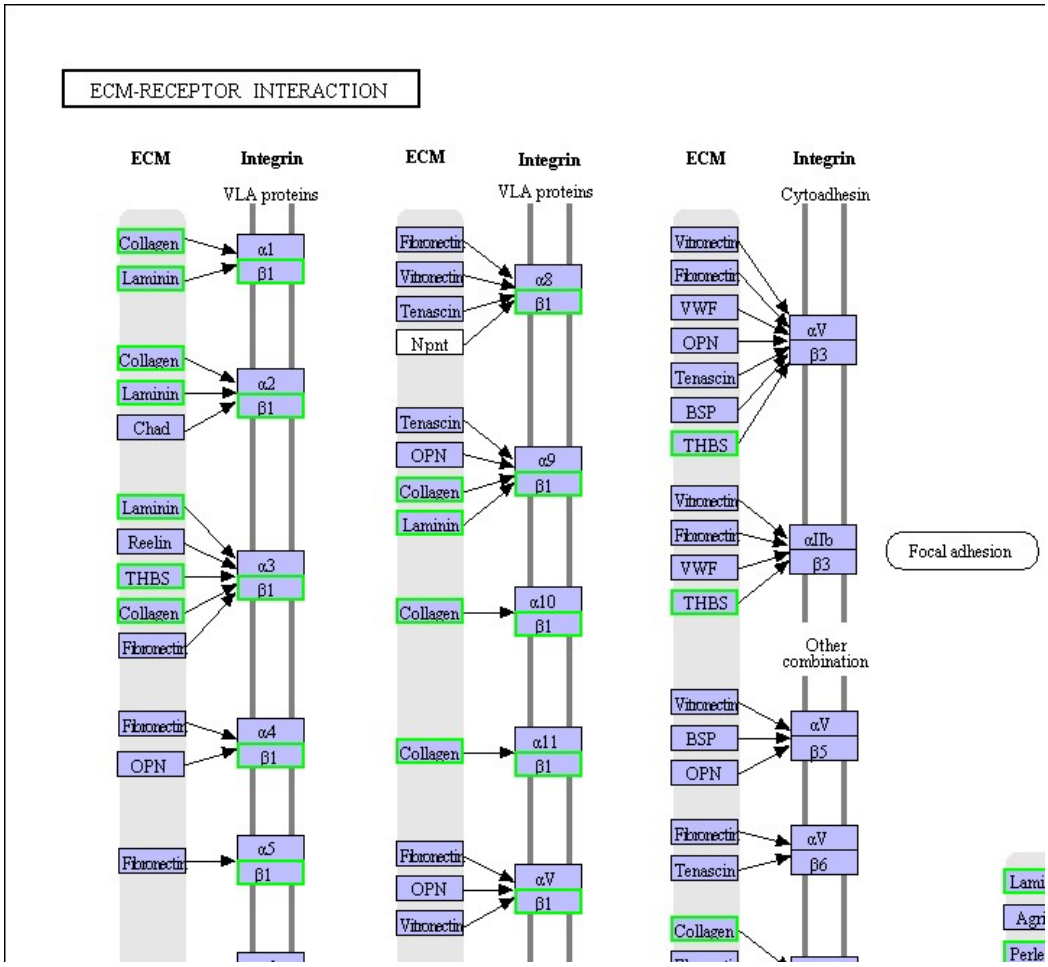



Figure 4. Histogram of the KEGG enrichment analysis of the up- and downregulated genes between 6WATA and 6MDTA. The x-axis shows the name and classification of the path; the y-axis represents the enrichment rate; * indicates $p < 0.05$. The side-color gradient represents the increasing P value (faint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

**Visualization of KEGG pathways associated with the DEGs**

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

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

## Discussion

The Zhuanghe dagu chicken originated from Zhuanghe city, Dalian, China, and is a unique local breed that was bred during a particular historical period and under special geographical and climatic conditions. The ZD chicken has characteristics of rough feeding, cold resistance, and strong adaptability, and is famous for its large body, deep and broad chest, wide and long back, tall and stout legs, and plump belly [25]. The Zhuanghe dagu chicken has strong disease resistance, a high egg yield, and delicious meat taste. It is a famous local fine breed for meat and eggs in China, and was listed as a national livestock and poultry genetic resources protected breed in 2006 [26]. With the industrialized production of the Zhuanghe dagu chicken, the product coverage area has gradually expanded and the market share is increasing year by year. The processed products are sold to 12 countries and regions in five continents, including Europe, Asia, America, and Africa [27]. In recent years, researchers have studied the characteristics of big-bone chickens using cytological polymorphisms, protein polymorphisms, DNA polymorphisms, and candidate genes [28, 29]. These studies showed that the meat quality of big-bone chickens is significantly better than that of commercial broiler chickens. Chicken meat contains less fat and a higher proportion of polyunsaturated fatty acids (PUFA) compared with meat from other species, making it a better choice for healthy diets [30]. In western countries, chicken

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

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

Low lysine incorporation or reduced protein diets (RPD) [36] in pigs represent a successful nutritional strategy to increase muscle fat accumulation, which does not affect other fat depots like the abdominal or subcutaneous depots (i.e., improved fat partitioning). Furthermore, enhanced pork sensory acceptability might result from the increased IMF induced by RPD [37, 38]. In beef, the analysis muscle growth-associated DEGs [39] suggested that the biological mechanisms controlling fat deposition and muscle growth are different. In ducks, tenderness and flavor were promoted by increasing lipid levels in the breast muscle [13]. Although the IMF is useful in improving meat quality, the mechanism underlying in increased IMF content 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*, *TPI1*, *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 β1 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 β1 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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# Figures

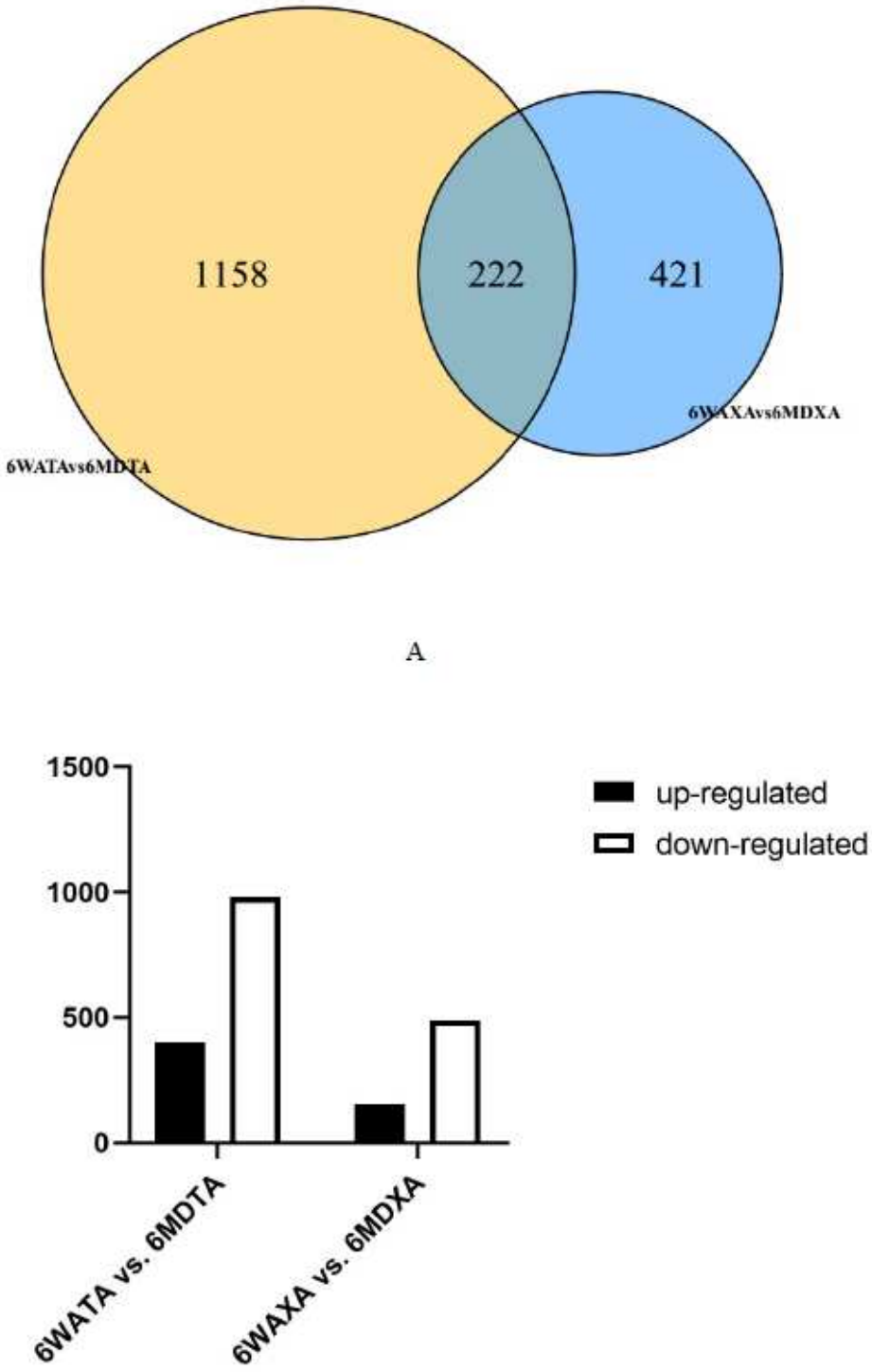
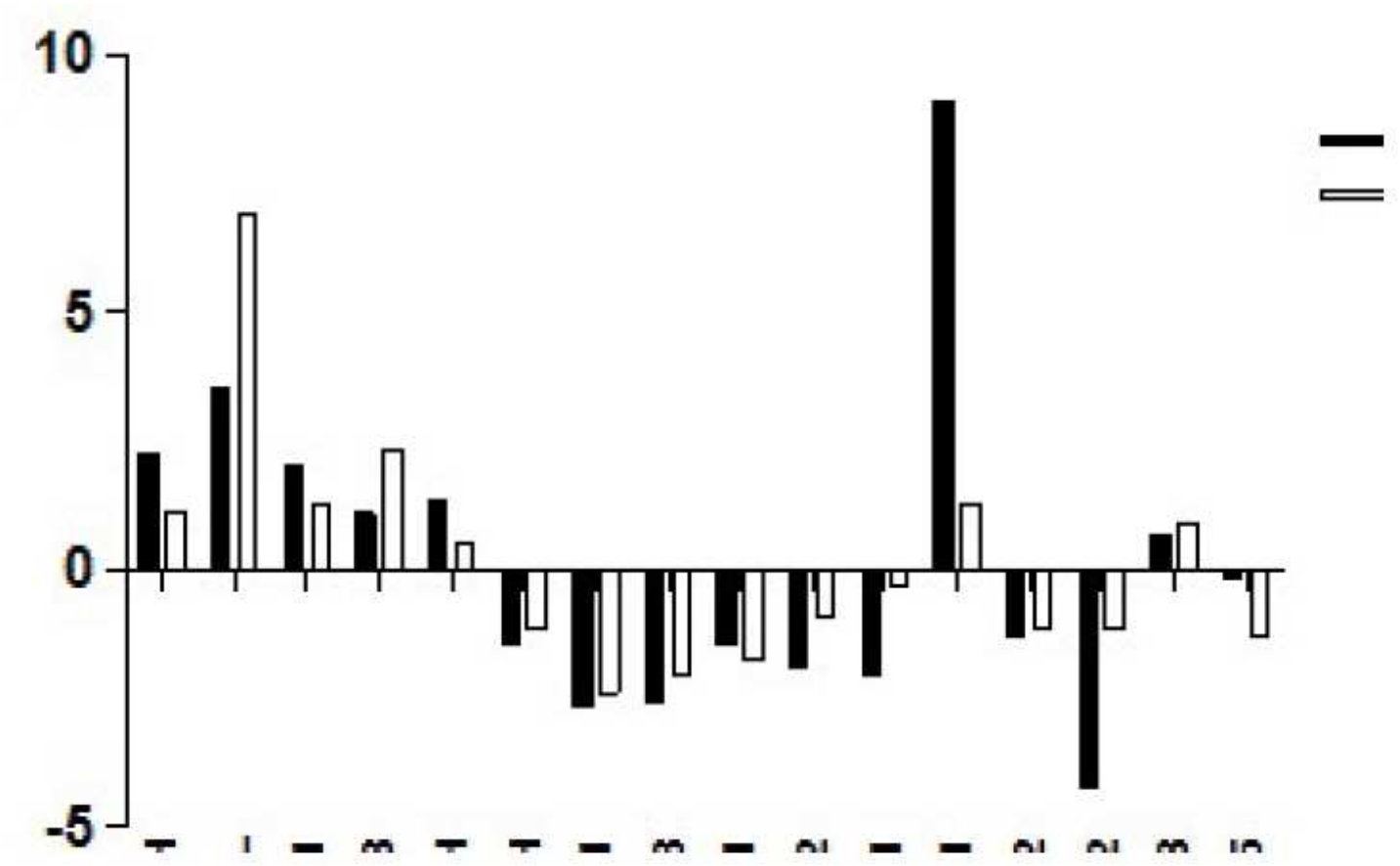



**Figure 1**

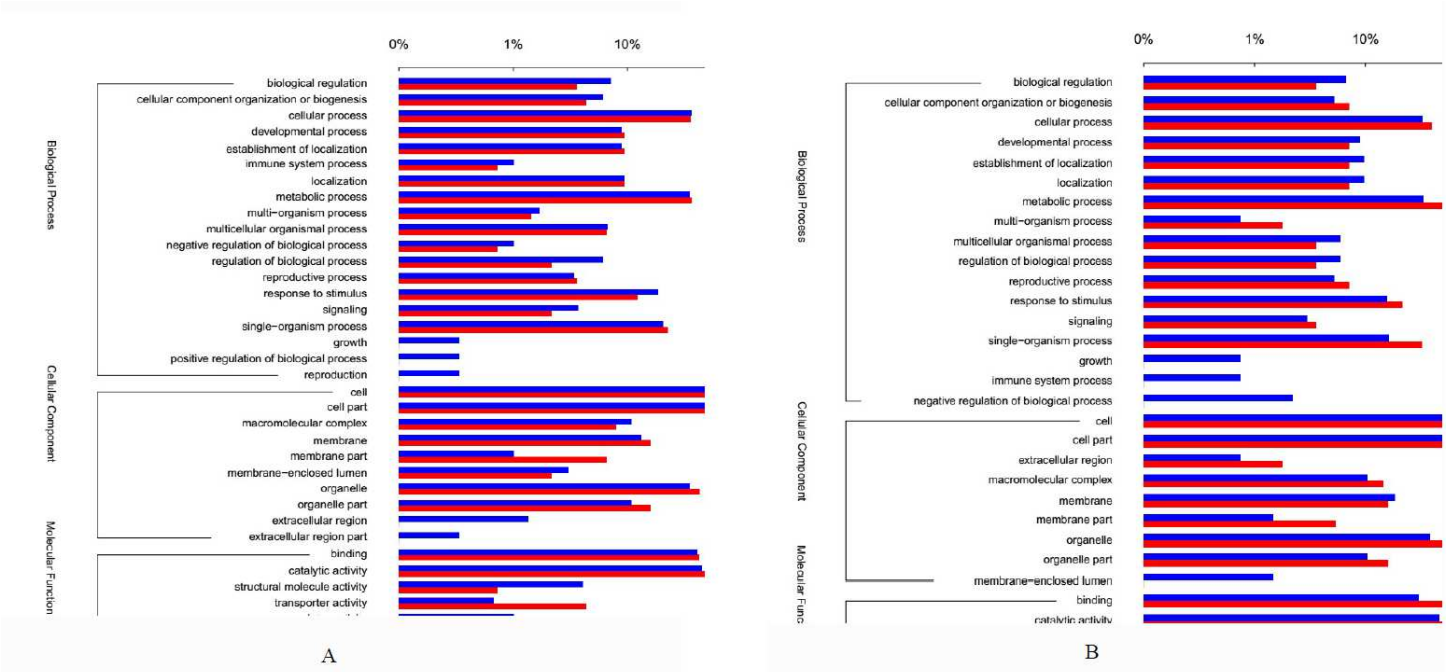
Number of differentially expressed genes (DEGs) between two samples. (A) Numbers of unique or shared DEGs between two samples. (B) Numbers of DEGs showing upregulated or downregulated expression in

in each comparison. W, week; M, month; A, AA broiler; D, Zhuanghe Dagu chicken; TA, leg muscle; BA, breast muscle.



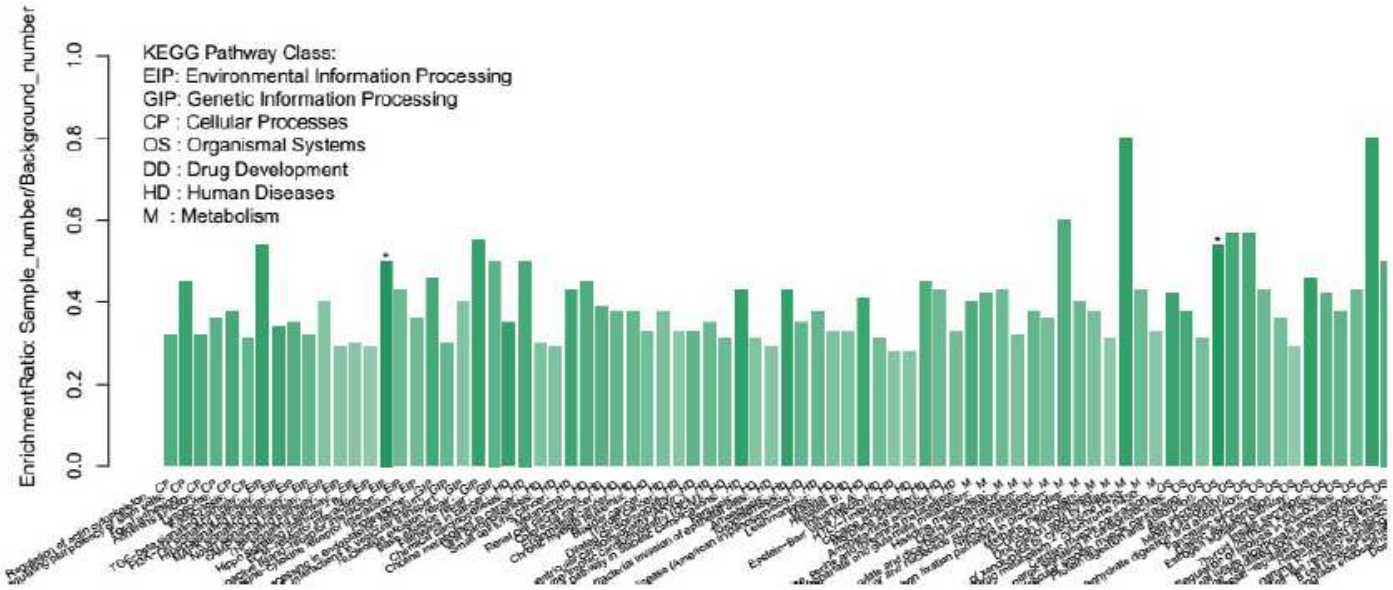

## Figure 2

QRT-PCR validation of the gene expression profiles.



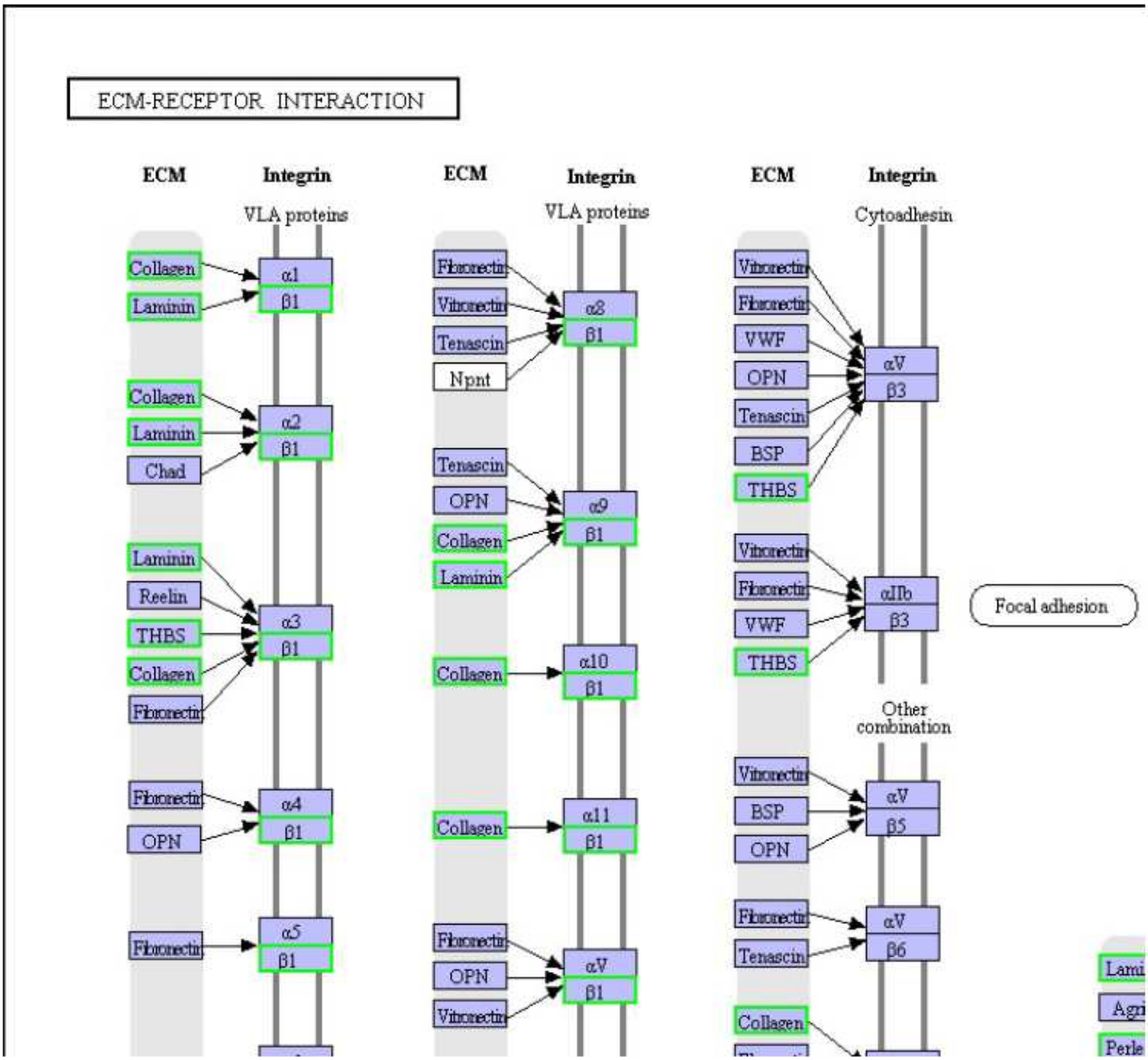
## Figure 3

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 gen




## Figure 4

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




## Figure 5

KEGG annotation pathway map of the downregulated ECM–receptorinteraction 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 with green borders represent the protein products of downregulated differentially expressed genes.