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Identifying Key Genes for Muscle Development in Broilers through Gene Expression Analysis

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Abstract This study reviews gene expression analysis techniques, including RNA sequencing and microarray analysis, to explore the molecular mechanisms of muscle development in broiler chickens. Using bioinformatics tools, several key genes regulating muscle development, growth factors, and hormone signals at different stages of muscle formation were identified, revealing their roles in promoting muscle fiber development and their potential applications in selective breeding. This study aims to provide insights into the genetic regulation of muscle traits in broiler chickens, provide opportunities for targeted gene manipulation to improve muscle yield, and offer future research directions.

Keywords Broiler muscle development; Gene expression analysis; Myogenesis; Selective breeding; RNA sequencing

1 Introduction

Muscle development in broilers is a complex process influenced by various genetic and environmental factors. The growth pattern, particularly during the early stages, significantly impacts the overall muscle mass and quality, which are critical for the poultry industry's profitability. Broilers are selectively bred for rapid growth and high feed efficiency, leading to enhanced muscle development, especially in the breast and leg muscles (Davis et al., 2015; Wu et al., 2020).

Understanding the genetic basis of muscle development is essential for improving growth performance and meat quality in broilers. Identifying key genes involved in muscle growth can provide insights into the regulatory mechanisms that control muscle development and differentiation. This knowledge can be applied to optimize breeding programs, enhance feed efficiency, and ultimately increase the economic value of broilers (Bottje et al., 2012; Liu et al., 2019; Kanakachari et al., 2022). Moreover, it can help in identifying genetic markers for selective breeding, leading to the development of broiler lines with superior growth traits (Lassiter et al., 2019; Zhang et al., 2021b).

Gene expression analysis aims to identify differentially expressed genes (DEGs) that play crucial roles in muscle development. By comparing the transcriptomes of broilers at various developmental stages or under different growth conditions, researchers can pinpoint specific genes and pathways that are upregulated or downregulated during muscle growth. This approach helps in understanding the molecular mechanisms underlying muscle development and identifying potential targets for genetic improvement (Xue et al., 2017; Zhang et al., 2022). Additionally, it provides a comprehensive view of the regulatory networks and signaling pathways involved in muscle growth (Wang et al., 2020).

This study provides an overview of key genes and pathways involved in the development of broiler muscle, introduces RNA sequencing and other molecular technologies, transcriptomes of broiler muscle tissue at different developmental stages and growth conditions, and identifies differentially expressed genes and their related pathways. These genes and pathways are crucial for muscle growth and development, and this study aims to better understand the genetic and molecular basis of broiler muscle development, providing valuable information for improving growth performance and meat quality in the poultry industry.

2 Muscle Development Pathways in Broilers

2.1 Myogenesis and its regulation

Myogenesis, the formation of muscle tissue, is a highly regulated process involving the activation and differentiation of myogenic precursor cells. Key regulatory genes such as *MyoD* and *myogenin* play crucial roles in this process. Studies have shown that the expression of these genes is significantly influenced by various factors, including genetic selection and environmental conditions (Payne et al., 2020). For instance, thermal manipulation during embryogenesis has been found to upregulate MyoD and myogenin, thereby enhancing muscle growth post-hatch (Al-Zghoul and El-Bahr, 2019). Additionally, the expression of myogenic regulatory factors (MRFs) such as MyoD and myogenin is modulated by growth hormone-boosting peptides (GHBP), which can accelerate muscle development in broilers (Ibrahim et al., 2021).

2.2 Key molecular pathways involved in muscle formation

Several molecular pathways are integral to muscle formation in broilers. The insulin-like growth factor (IGF) signaling pathway, particularly involving IGF-1 and mTOR, is pivotal for muscle growth and differentiation (Ahmad et al., 2020). The upregulation of IGF-1 and mTOR has been associated with enhanced muscle development, as evidenced by increased muscle fiber size and weight gain in broilers treated with GHBP. Furthermore, the extracellular matrix-receptor interaction and focal adhesion pathways have been identified as significant contributors to muscle development, influencing cellular activities such as migration, assembly, and differentiation (Xue et al., 2017). The differential expression of genes within these pathways, such as *MYOD1*, *IGF2BP2*, and *FGF2*, underscores their importance in regulating early growth and muscle formation in chickens.

2.3 Role of growth factors and hormones

Growth factors and hormones are critical regulators of muscle development in broilers (Yalcin et al., 2019). The somatotropic axis, involving growth hormone (GH) and IGF, plays a central role in this process. Modern broiler lines exhibit enhanced expression of IGF1 and IGF2 in breast muscle, contributing to their rapid growth and muscle accretion (Figure 1) (Vaccaro et al., 2022). Additionally, the administration of synthetic GHBP has been shown to modulate the expression of muscle-specific genes and microRNAs (myomiRs), leading to improved muscle growth and feed efficiency. Hormonal regulation also involves the action of IGF-binding proteins (IGFBPs), which modulate the availability and activity of IGFs. For example, IGFBP2 produced in the liver inhibits body growth, while its local production in breast muscle facilitates muscle development (Duan et al., 2010). The interplay between these growth factors and hormones underscores their vital role in optimizing muscle development in broilers. By understanding these pathways and regulatory mechanisms, researchers can develop strategies to enhance muscle growth and improve the overall productivity of broiler chickens.

3 Gene Expression Analysis Techniques

3.1 Overview of gene expression analysis methods

Gene expression analysis is a critical tool in understanding the molecular mechanisms underlying various biological processes, including muscle development in broilers. The primary methods used for gene expression analysis include RNA sequencing (RNA-seq) and microarray analysis. RNA-seq provides a comprehensive view of the transcriptome, allowing for the identification of differentially expressed genes (DEGs) and non-coding RNAs, such as microRNAs (miRNAs) and circular RNAs (circRNAs) (Liu et al., 2019). Microarray analysis, on the other hand, is a high-throughput technique that measures the expression levels of thousands of genes simultaneously, providing valuable insights into gene expression patterns and regulatory networks (Kanakachari et al., 2022).

3.2 RNA sequencing and microarray analysis in broiler research

RNA sequencing has been extensively used in broiler research to investigate the genetic basis of muscle development. For instance, RNA-seq was employed to study the transcriptome of breast muscle in male Jinghai yellow chickens at different growth stages, revealing thousands of DEGs and their associated biological processes and pathways (Zhang et al., 2021a). Another study utilized RNA-seq to analyze the transcriptome of skeletal muscle in fast- and slow-growing chickens, identifying key circRNAs and their potential regulatory roles in muscle development (Zhang et al., 2022).

Microarray analysis has also been pivotal in broiler research. A study on a male broiler line phenotyped for high or low feed efficiency used a chicken 44K oligo microarray to identify differentially expressed genes associated with muscle growth and feed efficiency (Bottje et al., 2012). This approach has provided insights into the molecular mechanisms that differentiate high-efficiency broilers from their low-efficiency counterparts, highlighting the importance of anabolic processes and stress response genes in muscle development.

3.3 Bioinformatics tools for gene identification and analysis

Bioinformatics tools play a crucial role in the analysis and interpretation of gene expression data. Ingenuity Pathway Analysis (IPA) is one such tool that has been used to overlay canonical pathways onto gene sets, helping to identify key regulatory networks and focus genes involved in muscle development (Bottje et al., 2011). Other tools, such as miRDB, are used for miRNA target prediction, enabling the integration of miRNA and mRNA data to elucidate the regulatory mechanisms underlying muscle growth (Li et al., 2011; Khatri et al., 2018).

Additionally, weighted gene co-expression network analysis (WGCNA) has been employed to identify gene modules associated with traits of interest, such as breast muscle weight and intramuscular fat content. This approach helps to uncover the complex interactions between genes and their contributions to muscle development and metabolism (Kang et al., 2021). These bioinformatics tools, combined with high-throughput gene expression analysis techniques, provide a comprehensive framework for identifying key genes and regulatory networks involved in muscle development in broilers.

4 Key Genes Involved in Muscle Development

4.1 Identification of essential muscle development genes

Several studies have identified key genes involved in muscle development in broilers. For instance, a study using microarray and qPCR techniques identified differentially expressed genes (DEGs) related to muscle growth, myostatin signaling, and fatty acid metabolism in broiler embryos (Kanakachari et al., 2022). Another study highlighted the up-regulation of muscle growth factor genes such as *IGF-1* and *GH*, and muscle marker genes including *MyoD*, *Myogenin*, *Pax7*, and *PCNA* during embryonic development and post-hatch days. Additionally, genes like *MSTN*, *IGF*-I, *MyoD*, and *MyoG* were found to be differentially expressed in various broiler strains, indicating their crucial roles in muscle development (Jawasreh et al., 2019).

4.2 Functional analysis of identified genes

The functional roles of these identified genes have been extensively studied. For example, the myostatin (MSTN) gene is known to inhibit muscle growth, while IGF-1 promotes muscle cell proliferation and differentiation. The *MyoD* and *Myogenin* genes are critical for myogenic differentiation, playing essential roles in muscle fiber formation (Al-Zghoul and El-Bahr, 2019). Furthermore, the study on DNA methylation and transcriptomic networks revealed that genes like MyH1-AS and other long non-coding RNAs (lncRNAs) are involved in the regulation of muscle development through epigenetic mechanisms (Liu et al., 2019). Another study identified circRNAs such as circPPP1R13B, which promotes muscle satellite cell proliferation and differentiation by targeting miR-9-5p and activating the IGF/PI3K/AKT signaling pathway (Shen et al., 2021).

4.3 Regulation of gene expression during different growth stages

Gene expression regulation during different growth stages is a complex process involving various signaling pathways and regulatory networks. For instance, the Jak-STAT, mTOR, and TGF-β signaling pathways were found to be significantly involved in muscle development during different embryonic stages. Thermal manipulation during embryogenesis was shown to up-regulate muscle growth factor genes and muscle marker genes, leading to increased body weight and muscle mass in post-hatch broilers. Additionally, the differential expression of miRNAs such as miR-2131-5p, miR-221-5p, and miR-206 was associated with muscle growth, indicating their regulatory roles in gene expression during muscle development (Khatri et al., 2018). The study on transcriptomic profiles also identified several pathways, including the insulin signaling pathway and regulation of the actin cytoskeleton, which are crucial for early growth and muscle development in chickens (Xue et al., 2017).

In summary, the identification and functional analysis of key genes, along with the understanding of their regulation during different growth stages, provide valuable insights into the molecular mechanisms underlying muscle development in broilers. These findings can be utilized to enhance muscle growth and improve the overall productivity of broiler chickens.

5 Case Study

5.1 Background of the case study

Broiler chickens are selectively bred for rapid growth and high feed efficiency, making them a crucial component of the poultry industry (Tallentire et al., 2016). Understanding the genetic mechanisms underlying muscle development in broilers can lead to improved breeding strategies and enhanced meat production (Buzała and Janicki, 2016). This case study focuses on identifying key genes involved in muscle development through gene expression analysis in broilers, leveraging various methodologies and findings from recent research.

5.2 Gene expression analysis

The methodologies employed in the gene expression analysis of broiler muscle development are diverse and robust. One common approach is the use of RNA sequencing (RNA-seq) to compare transcriptomes at different developmental stages or between different phenotypes (Kanakachari et al., 2021). For instance, RNA-seq was used to study the transcriptome of breast muscle in male Jinghai yellow chickens at different ages, identifying differentially expressed genes (DEGs) and enriched biological processes and pathways. Similarly, microarray analysis has been utilized to investigate global RNA expression in breast muscle, identifying differentially expressed genes associated with feed efficiency (Bottje et al., 2012).

In another study, transcriptome sequencing was applied to compare the leg muscles of fast- and slow-growing chicken embryos, identifying differentially expressed circRNAs and constructing circRNA-miRNA networks (Zhang et al., 2022). Additionally, large-scale transcriptome sequencing was performed on approximately 400 Tiannong partridge chickens to detect candidate genes for breast muscle weight and intramuscular fat content, using linear mixed models and regularized linear regression models.

5.3 Results and implications for broiler muscle development

The results from these studies have provided significant insights into the genetic basis of muscle development in broilers. For example, the study on male Jinghai yellow chickens identified 4608 DEGs across different age groups, with key genes such as *SNCG*, *MYH1A*, and *ARHGDIB* being co-differentially expressed (Zhang et al., 2021a). These genes are involved in various biological processes and pathways related to muscle growth and development.

Another study focusing on feed efficiency found that high feed efficiency in broilers is associated with the upregulation of genes involved in anabolic processes and the downregulation of genes related to muscle fiber development, muscle function, and cytoskeletal organization (Bottje et al., 2011). This suggests that selective breeding for feed efficiency may inadvertently affect muscle development pathways.

The transcriptome analysis of fast- and slow-growing chicken embryos revealed significant circRNAs and their potential regulatory roles in skeletal muscle development (Lei et al., 2022). Key circRNAs such as novel_circ_0004547 and novel_circ_0003578 were identified, along with their associated miRNA pairs, providing new targets for genetic improvement.

Furthermore, the large-scale transcriptome sequencing study identified 43 candidate genes associated with breast muscle weight and several genes related to intramuscular fat content. These genes are involved in muscle development, contraction, and lipid metabolism, offering valuable targets for genetic improvement programs (Kang et al., 2021).

In conclusion, the gene expression analyses conducted in these studies have identified numerous key genes and pathways involved in muscle development in broilers. These findings have significant implications for the poultry industry, providing potential targets for selective breeding and genetic improvement to enhance muscle growth and feed efficiency in broilers.

6 Applications in Broiler Breeding

6.1 Use of gene expression data in selective breeding

Gene expression data has become a pivotal tool in selective breeding programs for broilers. By identifying and selecting for specific genes associated with desirable traits, breeders can enhance growth performance and muscle development. For instance, the expression levels of *IGF-I* and *MyoG* genes have been shown to correlate with improved body weight and carcass cuts, making them valuable markers in artificial selection programs (Jawasreh et al., 2019). Additionally, the identification of single nucleotide polymorphisms (SNPs) in genes such as *TGFβ3* has been linked to growth performance traits, suggesting that these genetic markers can be used to accelerate genetic improvement in broiler lines (Hosnedlova et al., 2020). The use of RNA-seq and transcriptome analysis has further enabled the identification of differentially expressed genes that regulate myogenic growth, providing a deeper understanding of the genetic architecture underlying muscle development (Davis et al., 2015).

6.2 Enhancing muscle yield through genetic manipulation

Genetic manipulation offers a promising avenue for enhancing muscle yield in broilers. By targeting specific genes involved in muscle growth and development, it is possible to achieve significant improvements in muscle mass. For example, the down-regulation of myostatin signaling pathway genes combined with the up-regulation of genes that enhance muscle formation has been associated with higher feed efficiency and muscle development in broilers (Lassiter et al., 2019). Moreover, thermal manipulation during embryogenesis has been shown to up-regulate muscle growth factor genes such as *IGF-1* and *GH*, leading to increased body weight and carcass yield in post-hatch broilers (Al-Zghoul and El-Bahr, 2019). These findings highlight the potential of genetic and environmental interventions to optimize muscle yield in broiler chickens.

6.3 Future prospects for improving broiler muscle traits

The future of broiler breeding lies in the integration of advanced genetic tools and technologies to further enhance muscle traits. The use of large-scale transcriptome sequencing and weighted gene co-expression network analysis (WGCNA) has already identified key candidate genes and pathways involved in muscle development and lipid metabolism (Kang et al., 2021). As our understanding of the molecular mechanisms underlying muscle growth continues to expand, it is likely that new genetic markers and targets for manipulation will be discovered. Additionally, the application of gene editing technologies such as CRISPR/Cas9 holds great promise for precise genetic modifications to improve muscle traits. Continued research and innovation in this field will undoubtedly lead to more efficient and productive broiler breeding programs, ultimately benefiting the poultry industry as a whole.

By leveraging gene expression data, genetic manipulation, and future technological advancements, the potential for improving muscle development in broilers is vast. These strategies not only enhance growth performance but also contribute to the overall efficiency and sustainability of broiler production.

7 Challenges and Limitations

7.1 Technical challenges in gene expression analysis

Gene expression analysis in broilers presents several technical challenges. One significant issue is the complexity of the regulatory networks involved in muscle development. For instance, the identification of key genes such as *Atp2a1*, *Tmod4*, *Lmod3*, *Ryr1*, and *Mybpc2* in bovine muscle development through co-expression analysis highlights the intricate pathways that need to be deciphered (Zhang et al., 2023). Additionally, the use of advanced techniques like RNA-seq and microarray analysis requires substantial computational resources and expertise in bioinformatics to accurately interpret the data (Bottje et al., 2011; Davis et al., 2015). The variability in gene expression due to environmental factors and genetic background further complicates the analysis, necessitating large sample sizes to achieve statistically significant results.

7.2 Limitations in translating gene data to breeding programs

Translating gene expression data into practical breeding programs is fraught with limitations. One major challenge is the gap between identifying candidate genes and understanding their functional roles in muscle development. For example, while studies have identified differentially expressed genes associated with feed efficiency and muscle growth, such as *IGF1*, *IGF1R*, and *MSTN*, the direct application of this knowledge to breeding strategies remains complex (Jawasreh et al., 2019). Moreover, the genetic improvement of traits like breast muscle weight and intramuscular fat content requires integrating multiple genes and pathways, which is a daunting task given the polygenic nature of these traits (Kang et al., 2021). The effectiveness of breeding programs also depends on the accurate phenotyping of traits, which can be labor-intensive and time-consuming.

7.3 Ethical and regulatory considerations

Ethical and regulatory considerations play a crucial role in the application of gene expression analysis in broiler breeding. The use of genetic information to enhance muscle growth and feed efficiency must be balanced with animal welfare concerns. For instance, the rapid growth rates achieved through selective breeding can lead to health issues such as skeletal deformities and cardiovascular problems (Khatri et al., 2018). Regulatory frameworks must ensure that genetic modifications do not compromise the well-being of the animals. Additionally, there are concerns about the long-term impacts of genetic selection on biodiversity and the potential for unintended consequences in the gene pool (Al-Zghoul and El-Bahr, 2019). Ethical considerations also extend to the transparency and fairness in the use of genetic data, ensuring that advancements benefit all stakeholders in the poultry industry.

By addressing these challenges and limitations, researchers can better harness the potential of gene expression analysis to improve muscle development in broilers while maintaining ethical standards and regulatory compliance.

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Conflict of Interest Disclosure

Authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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