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Preliminary comparative transcriptomic profiling of leg muscle in Pekin and Hanzhong Ma ducks

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Abstract

Sustainable poultry production depends on the identification of duck breeds capable of maintaining productivity with efficient resource utilization and improved environmental adaptability. The present study investigated the leg muscle transcriptomes of two genetically distinct duck breeds, Pekin and Hanzhong Ma (HZM), using publicly available RNA-Seq datasets retrieved from the National Center for Biotechnology Information Sequence Read Archive under BioProjects PRJNA665337 and PRJNA665334. Four paired-end transcriptome samples representing six-month-old ducks, including two biological replicates from each breed, were analyzed to identify molecular mechanisms associated with muscle growth and metabolic efficiency. Raw sequencing reads were quality-checked, trimmed and aligned against the duck reference genome (GCF_015476345.1) using HISAT2, achieving mapping efficiencies greater than 92% across all samples. Differential gene expression analysis using DESeq2 identified 3,398 significantly differentially expressed genes (adjusted p-value < 0.01 and $|\log_2$ fold change| > 2) between Pekin and Hanzhong Ma ducks. Genes upregulated in Pekin ducks were mainly enriched in oxidative phosphorylation, mitochondrial ATP synthesis, glycolysis and fatty acid β -oxidation pathways, indicating enhanced energy metabolism and rapid muscle growth. Important mitochondrial genes, including *NDUFB9*, *NDUFB5*, *NDUFB4*, *NDUFB8*, *NDUFA6* and *NDUFA7*, were highly expressed and associated with improved ATP production and metabolic efficiency. In contrast, genes downregulated in Pekin ducks relative to Hanzhong Ma ducks were primarily involved in mitotic cell cycle regulation, DNA replication and chromosome segregation. Key genes in this category included *CDC45*, *MCM2*, *MCM5*, *ORC1*, *BUB1* and *CDK1*. Protein-protein interaction analysis identified several hub genes associated with muscle hypertrophy, metabolic regulation and stress adaptation. These findings provide insights into breed-specific molecular mechanisms influencing muscle development and may support future breeding strategies for sustainable and resource-efficient duck farming.

Keywords: Duck transcriptomics, RNA-Seq, sustainable poultry production, muscle growth, differential gene expression, metabolic efficiency

Introduction

Duck farming represents an important component of the poultry sector because of its contribution to meat and egg production, adaptability to diverse agro-climatic conditions and suitability for low-input farming systems. In many Asian countries, particularly China and India, ducks are valued for their ability to efficiently utilize natural feed resources and maintain productivity under semi-intensive and scavenging production systems. Improvement of economically important traits such as muscle growth, feed efficiency and adaptability has therefore become a major objective in modern duck breeding programs. Skeletal muscle is one of the most important tissues influencing growth performance and meat yield in poultry species. Apart from its essential role in locomotion and body support, skeletal muscle also contributes significantly to metabolic regulation and energy homeostasis within the body (Mitchell *et al.*, 2002) [14]. Muscle development is regulated through myogenesis, a highly coordinated biological process involving the proliferation, differentiation and fusion of myogenic precursor cells. These processes are controlled by complex gene regulatory networks and signaling pathways that influence muscle fiber formation, hypertrophy and metabolic activity (Buckingham, 2017) [4]. Variations in the expression of genes associated with these pathways can lead to substantial differences in muscle growth and production efficiency among breeds.

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Advances in transcriptomic technologies have considerably improved the understanding of molecular mechanisms underlying growth and development in livestock species. The concept of the transcriptome emerged following the development of gene expression profiling approaches such as Serial Analysis of Gene Expression (SAGE) and Expressed Sequence Tags (ESTs), which enabled large-scale identification and characterization of expressed genes (Adams *et al.*, 1992; Velculescu *et al.*, 1995) ^[1, 15]. The term “transcriptome” was later introduced by Velculescu *et al.* (1997) ^[16] during studies on gene expression profiling in yeast. More recently, RNA sequencing (RNA-Seq) has become a powerful and widely adopted technique for studying gene expression patterns, identifying differentially expressed genes and understanding biological pathways associated with economically important traits. Comparative transcriptomic studies have been extensively used to investigate muscle development and growth-related traits in poultry species. Davis *et al.* (2015) ^[7] compared the breast muscle transcriptomes of modern and legacy broiler chickens and identified several genes associated with enhanced muscle growth, including *IGF1*, *IGF1R* and *WFIKK2*, along with negative regulators such as *MSTN* and *ACE*. Their findings demonstrated that altered expression of myogenic regulators contributes significantly to growth performance in commercial poultry lines. Similarly, transcriptome studies in ducks have provided valuable insights into the molecular basis of muscle development, lipid metabolism and breed-specific production traits.

The Pekin duck is one of the most commercially important meat-type duck breeds worldwide and has been intensively selected for rapid growth, superior feed conversion efficiency and increased muscle deposition. In contrast, indigenous duck breeds generally exhibit slower growth but possess greater adaptability and desirable reproductive characteristics. Hanzhong Ma duck is a native duck breed distributed mainly in Hanzhong City of Shaanxi Province, China. This breed is relatively small in body size and light in weight, with average adult body weights of approximately 1.172 kg in males and 1.238 kg in females, which limits its meat production potential. However, Hanzhong Ma ducks are recognized for their adaptability and dual-purpose utility for egg and meat production. Previous studies have shown that phenotypic differences between duck breeds are strongly influenced by artificial selection and genetic regulation of muscle growth and lipid deposition. Wang *et al.* (2017) ^[17] reported that modern meat-type Pekin ducks and native Pekin ducks exhibit distinct differences in muscle growth and lipid metabolism despite sharing similar genetic backgrounds, suggesting that intensive selection has significantly shaped their transcriptomic profiles. Furthermore, Hu *et al.* (2021) ^[8] analysed skeletal muscle transcriptomes of Hanzhong Ma ducks during embryonic and postnatal developmental stages and identified several candidate genes associated with muscle growth and development. These findings highlight the importance of transcriptomic analysis for understanding breed-specific molecular mechanisms regulating skeletal muscle formation and metabolism.

Despite the availability of transcriptomic information in ducks, comparative studies focusing on molecular differences between commercial meat-type breeds and indigenous dual-purpose breeds remain limited.

Understanding these differences is important for developing sustainable breeding strategies aimed at improving productivity while maintaining adaptability and efficient resource utilisation. Therefore, the present study was undertaken to compare the leg muscle transcriptomes of Pekin and Hanzhong Ma ducks using publicly available RNA-Seq datasets retrieved from the National Center for Biotechnology Information database. Differential gene expression analysis and functional enrichment analysis were performed to identify genes and biological pathways associated with muscle growth, energy metabolism and adaptive regulation. The findings of this study may contribute to the identification of potential molecular markers for sustainable and efficient duck production systems.

Materials and Methods

Retrieval of RNA-Seq datasets

Publicly available RNA sequencing datasets of duck skeletal muscle tissues were retrieved from the National Center for Biotechnology Information Sequence Read Archive (SRA) database under the BioProject accessions PRJNA665337 and PRJNA665334. The datasets represented two genetically distinct duck breeds, namely Pekin duck and Hanzhong Ma (HZM) duck. Transcriptome data generated from leg muscle tissues of six-month-old ducks were selected for comparative analysis. A total of four paired-end RNA-Seq samples were included in the study, comprising two biological replicates from each breed. The Pekin duck samples corresponded to SRR12702265 and SRR12702256, whereas the Hanzhong Ma duck samples corresponded to SRR12701866 and SRR12701875. All libraries were sequenced using the Illumina HiSeq X Ten platform following cDNA library preparation protocols.

Quality assessment of raw reads

The quality of raw sequencing reads was evaluated using FastQC (Andrews, 2010) ^[3]. Several quality parameters, including per-base sequence quality, GC content distribution, sequence duplication levels, adapter contamination and overrepresented sequences, were examined to assess the suitability of the sequencing data for downstream transcriptomic analysis.

Read preprocessing and trimming

Raw reads were subjected to preprocessing using fastp (v0.20.1) (Chen *et al.*, 2018) ^[5] to remove low-quality reads, adapter sequences and ambiguous bases. Trimming procedures were carried out to improve the overall quality of the sequencing data before genome alignment. Only high-quality filtered reads obtained after preprocessing were retained for subsequent analyses.

Reference genome preparation

The duck reference genome assembly GCF_015476345.1 and its corresponding annotation files were downloaded from the National Center for Biotechnology Information database. The reference genome index required for transcriptome alignment was constructed using HISAT2.

Alignment of sequencing reads

High-quality filtered reads from each sample were aligned against the duck reference genome using HISAT2. HISAT2 is a splice-aware aligner designed for efficient mapping of

RNA-Seq reads to reference genomes and was used to generate alignment files in Sequence Alignment Map (SAM) format. The resulting SAM files were converted into Binary Alignment Map (BAM) format and sorted using SAMtools for efficient downstream analysis and transcript quantification.

Assessment of alignment quality

Alignment quality and mapping statistics were evaluated using SAMtools flagstat analysis. Parameters assessed included total reads, mapped reads, properly paired reads, singleton reads and reads aligned to different chromosomes. These analyses were performed to verify the reliability and overall quality of the transcriptome alignment.

Quantification of gene expression

Gene-level read counts were generated from the aligned BAM files using transcript annotation information corresponding to the duck reference genome. The generated count matrix was used for downstream differential gene expression analysis.

Differential gene expression analysis

Differential gene expression analysis between Pekin and Hanzhong Ma ducks was performed using the DESeq2 package (Love *et al.*, 2014) [12] implemented in R statistical software. Read counts were normalized to account for differences in sequencing depth and library size among samples. For statistical comparison, Hanzhong Ma ducks were considered the reference group, while Pekin ducks were used as the comparison group. Differentially expressed genes (DEGs) were identified based on an adjusted p-value threshold of less than 0.01 and an absolute log₂ fold change greater than 2.

Functional enrichment analysis

Functional annotation and enrichment analyses of differentially expressed genes were carried out in DAVID (Huang *et al.*, 2009) [9] to identify significantly enriched biological processes and metabolic pathways associated with skeletal muscle growth and metabolism. Gene Ontology (GO) analysis was performed to classify genes into biological process, molecular function and cellular component categories. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis was conducted to identify metabolic and signalling pathways enriched among

the differentially expressed genes.

Protein-protein interaction network analysis

Protein-protein interaction (PPI) network analysis was performed to investigate functional interactions among differentially expressed genes and to identify central regulatory genes within the interaction network. Networks were visualised in Cytoscape (v3.10.3) and hub genes were identified using the cytoHubba plugin based on the Maximal Clique Centrality (MCC) algorithm (Chin *et al.*, 2014) [6]. Hub genes were identified based on their interaction connectivity and biological significance in muscle growth, metabolism and cellular regulation.

Data visualization

Visualization analyses including volcano plots, heatmaps and principal component analysis (PCA) plots were generated using R packages to evaluate transcriptomic variation and expression patterns between the two duck breeds. Volcano plots were used to visualize significantly differentially expressed genes, whereas PCA analysis was performed to assess clustering patterns among samples based on gene expression profiles.

Ethical statement

The present investigation was based entirely on publicly available RNA-Seq datasets obtained from the National Center for Biotechnology Information database. No live animals or biological samples were directly used in this study; therefore, separate institutional ethical approval was not required.

Results

RNA-Seq data quality assessment and alignment statistics

High-throughput RNA sequencing generated transcriptomic datasets from the leg muscle tissues of Pekin and Hanzhong Ma (HZM) ducks. Following quality assessment using FastQC and preprocessing with fastp, clean paired-end reads were aligned to the duck reference genome (GCF_015476345.1) using HISAT2. All four samples showed mapping percentages greater than 92%. The HZM sample SRR12701866 showed the highest mapping rate (97.06%), while Pekin sample SRR12702256 showed the lowest mapping rate (92.61%). Properly paired reads ranged from 83.03% to 85.43%. Alignment statistics for all samples are summarized in Table 1.

Table 1: Summary of alignment statistics

Sample ID	Breed	Total Reads	Mapped Reads (%)	Properly Paired Reads (%)
SRR12702265	Pekin	60,700,283	95.55	84.94
SRR12702256	Pekin	53,207,800	92.61	83.03
SRR12701866	Hanzhong Ma	66,443,455	97.06	85.43
SRR12701875	Hanzhong Ma	58,391,600	96.72	85.21

Identification of differentially expressed genes

Differential expression analysis was performed using DESeq2 with Hanzhong Ma ducks considered as the reference group and Pekin ducks as the comparison group. Genes with adjusted p-value <0.01 and |log₂FoldChange| >2 were considered significantly differentially expressed. A total of 3,398 differentially expressed genes (DEGs) were

identified between Pekin and Hanzhong Ma ducks. Among these, 1,823 genes were significantly upregulated in Pekin ducks, whereas 1,523 genes were downregulated relative to Hanzhong Ma ducks. The volcano plot displayed the distribution of significantly upregulated and downregulated genes between the two breeds (Figure 1).

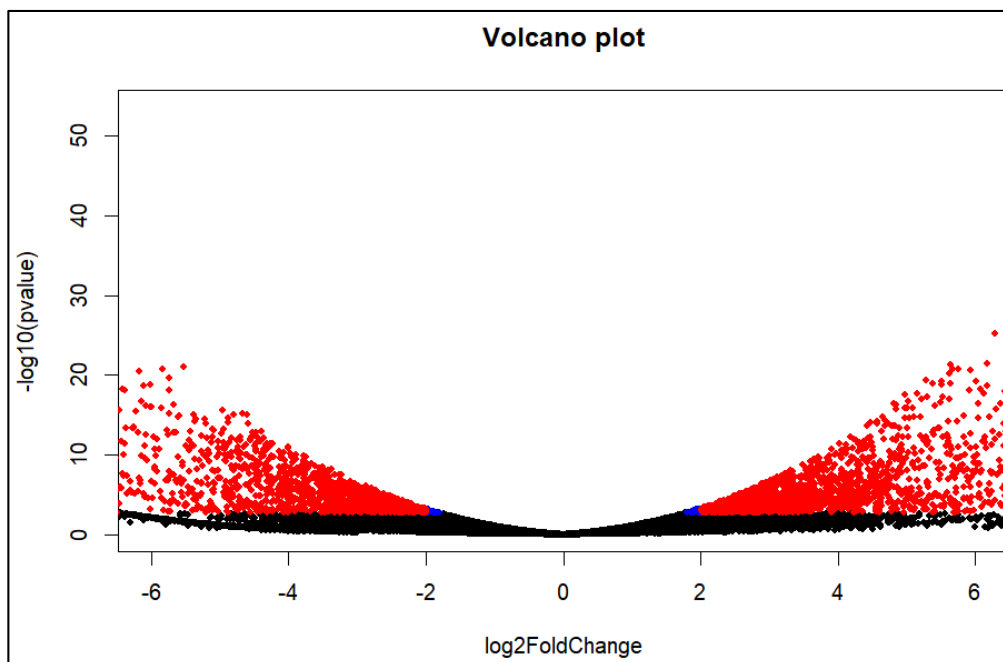


Fig 1: Volcano plot showing differentially expressed genes between Pekin and Hanzhong Ma ducks. Red dots represent significantly differentially expressed genes with adjusted p-value < 0.01 and $|\log_2\text{FoldChange}| > 2$.

Protein-protein interaction network analysis of upregulated DEGs

A protein-protein interaction (PPI) network was constructed for 919 significantly upregulated DEGs using STRING with a high-confidence interaction score (>0.9). The network contained 919 nodes and 756 edges, with an average node

degree of 1.65 and an average local clustering coefficient of 0.186. The observed number of edges (756) was higher than the expected number of edges (209), with a PPI enrichment p-value $< 1.0 \times 10^{-16}$. The PPI interaction network of upregulated genes is presented in Figure 2.

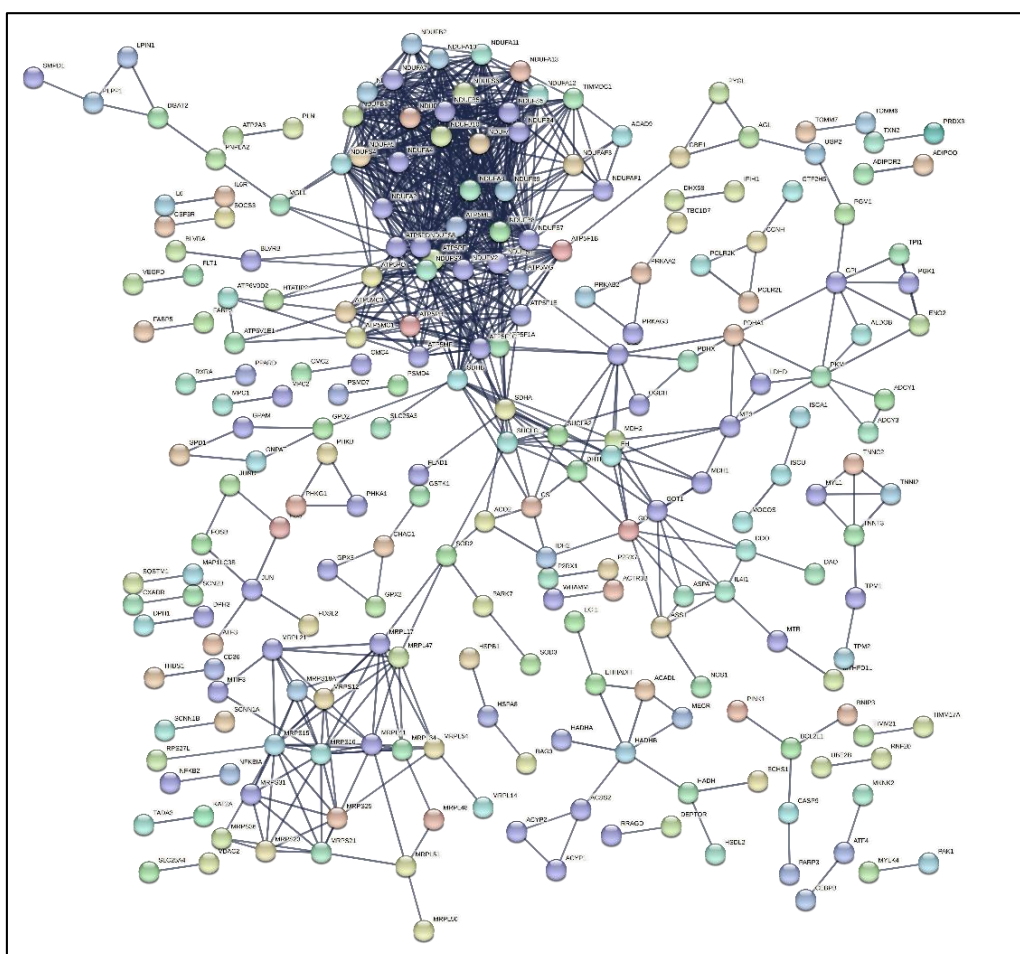


Fig 2: Protein-protein interaction network of significantly upregulated DEGs in Pekin ducks generated using STRING.

Topological analysis identified 20 major hub genes within the upregulated network, including *NDUFB9*, *NDUFB5*, *NDUFB4*, *NDUFB8*, *NDUFA6*, *NDUFA5*, *NDUFA7*, *NDUFB3*, *NDUFA2*, *NDUFB6*, *NDUFS7*, *NDUFV2*,

NDUFS3, *NDUFS4*, *NDUFB10*, *NDUFA8*, *NDUFA11*, *NDUFS8*, *NDUFS5* and *NDUFA13*. The top 20 hub genes identified from the upregulated network are shown in Figure 3.

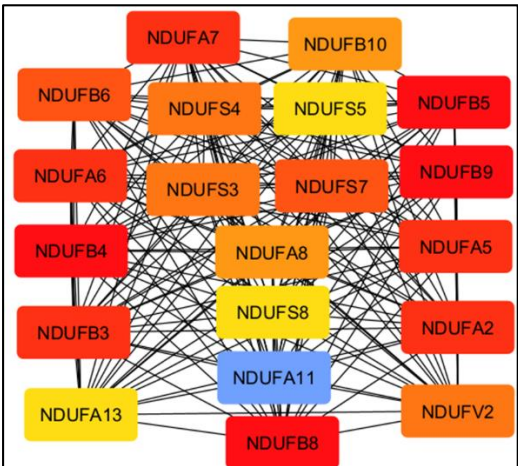


Fig 3: Top 20 hub genes identified from the upregulated DEGs PPI network.

GO and KEGG enrichment analysis of upregulated DEGs

Gene Ontology (GO) enrichment analysis of upregulated DEGs identified significant enrichment of biological process, cellular component and molecular function categories. The significantly enriched biological process terms included mitochondrial respiratory chain complex I assembly, mitochondrial electron transport from NADH to ubiquinone, proton motive force-driven ATP synthesis, tricarboxylic acid cycle, glycolytic process, reactive oxygen species metabolic process and fatty acid beta-oxidation. Cellular component enrichment included mitochondrion, mitochondrial inner membrane, mitochondrial matrix,

mitochondrial large ribosomal subunit and proton-transporting ATP synthase complex. The major molecular function categories included NADH dehydrogenase activity, proton transmembrane transporter activity and ATP synthase activity. KEGG pathway analysis identified oxidative phosphorylation, metabolic pathways, carbon metabolism, citrate cycle (TCA cycle), glycolysis/gluconeogenesis, pyruvate metabolism, PPAR signalling pathway, fatty acid degradation and muscle contraction pathways as significantly enriched pathways. GO and KEGG enrichment results for upregulated DEGs are presented in Figure 4a and 4b.

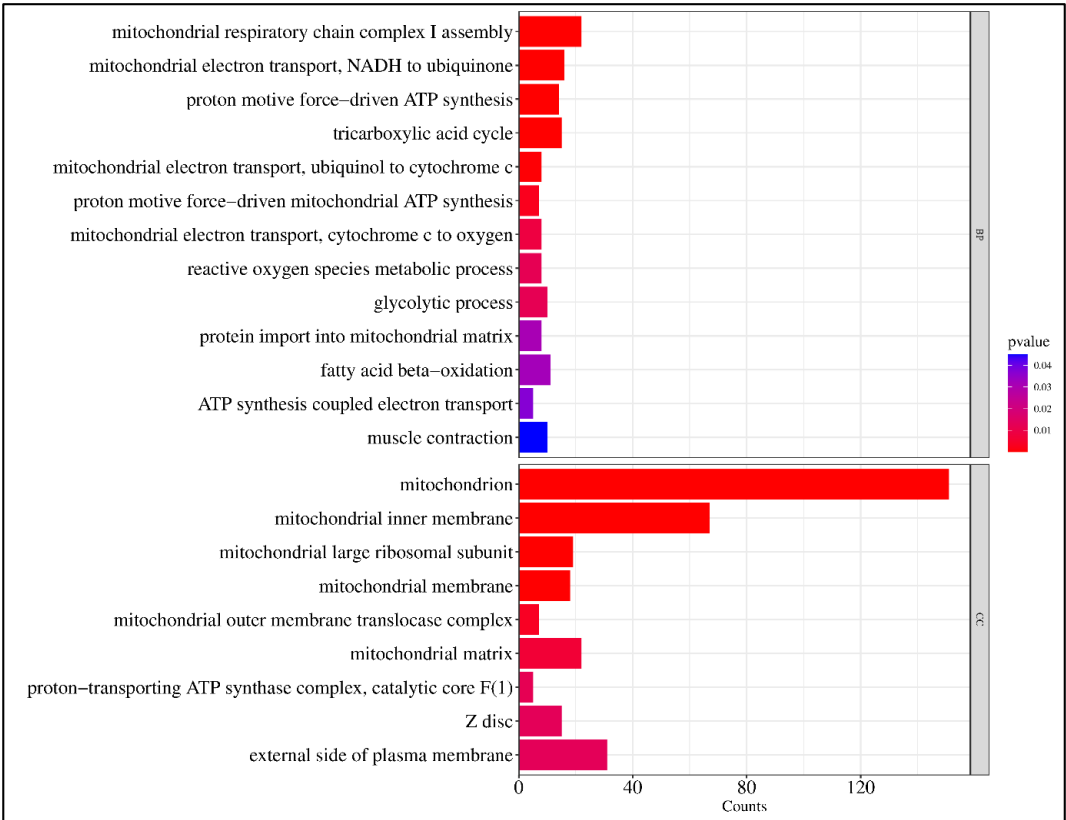


Fig 4a: Bar plot showing significantly enriched BP and CC terms among upregulated DEGs in Pekin ducks.

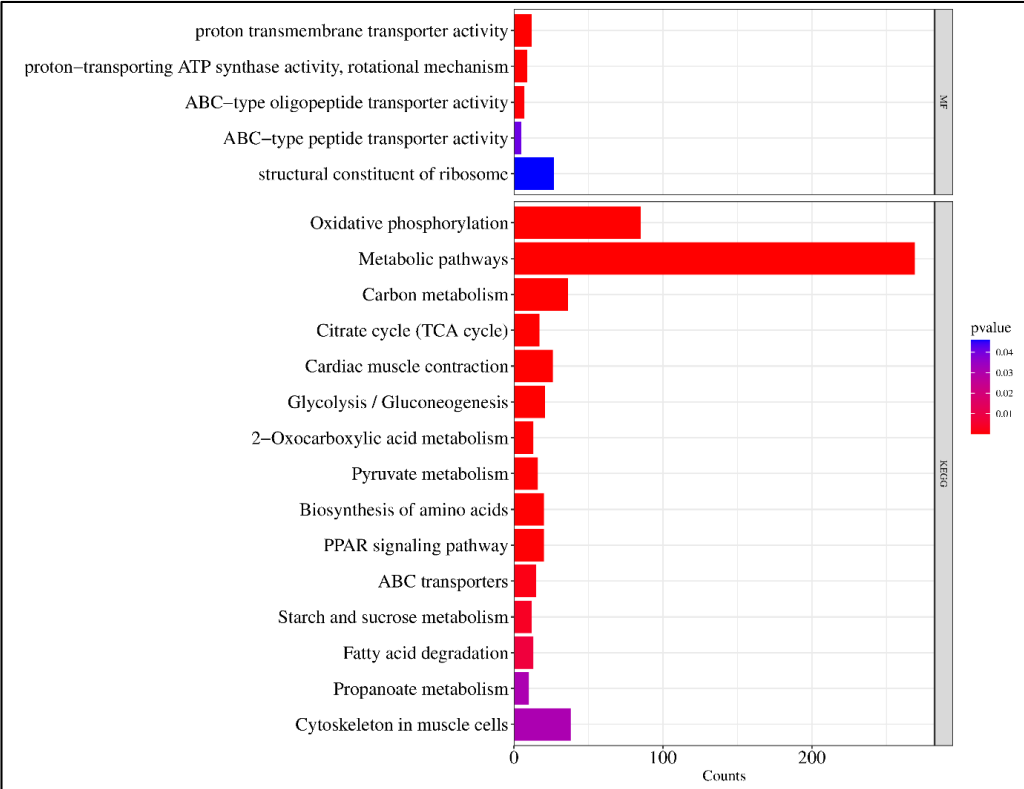


Fig 4b: Bar plot showing significantly enriched MF and KEGG terms among upregulated DEGs in Pekin ducks.

Protein-protein interaction network analysis of downregulated DEGs

A PPI network was constructed for 1,067 significantly downregulated DEGs using STRING with a high-confidence interaction score (>0.9). The downregulated network consisted of 1,067 nodes and 328 edges, with an

average node degree of 0.615 and an average local clustering coefficient of 0.166. The observed number of edges (328) exceeded the expected number of edges (78), with a PPI enrichment p-value <1.0e⁻¹⁶. The PPI interaction network of downregulated genes is shown in Figure 5.

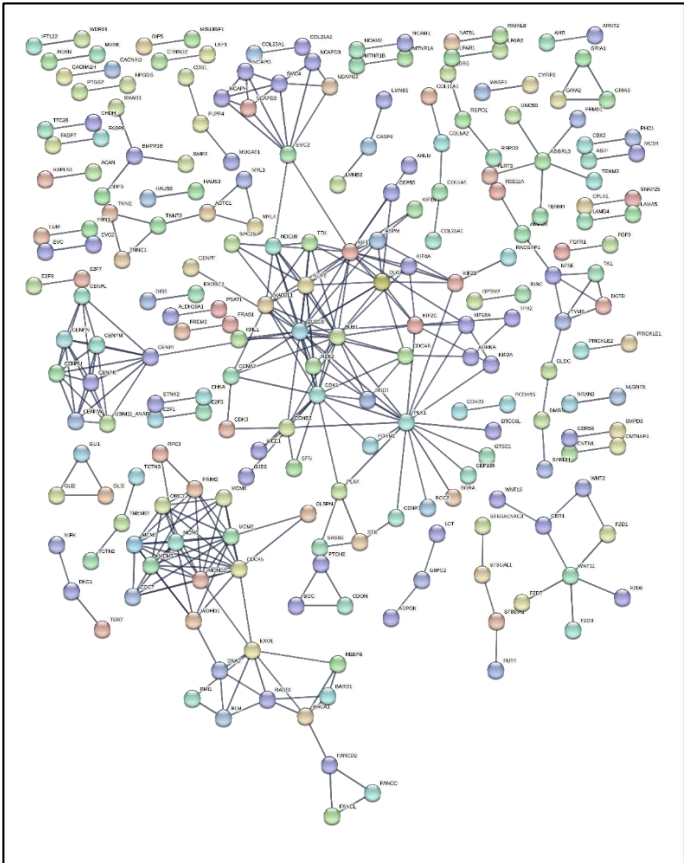


Fig 5: Protein-protein interaction network of significantly downregulated DEGs in Pekin ducks generated using STRING.

The major hub genes identified among the downregulated DEGs included *CDC45*, *MCM2*, *MCM5*, *MCM3*, *MCM10*, *ORC1*, *BUB1B*, *BUB1*, *KIF11*, *NUF2*, *MCM6*, *MAD2L1*,

NDC80, *CENPI*, *TTK*, *CENPM*, *CENPK*, *CENPN*, *CENPU* and *CDK1*. The top 20 hub genes identified from the downregulated network are presented in Figure 6.

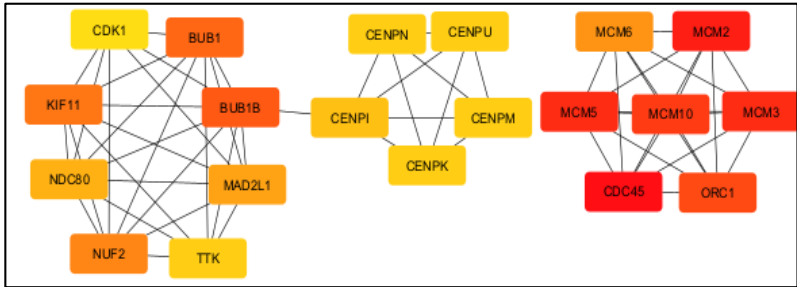


Fig 6: Top 20 hub genes identified from the downregulated DEG PPI network.

GO and KEGG enrichment analysis of downregulated DEGs

GO enrichment analysis of downregulated DEGs identified significant enrichment in biological process, cellular component and molecular function categories. The major enriched biological process categories included extracellular matrix organization, cell division, mitotic cell cycle, chromosome segregation, microtubule-based movement, collagen fibril organization, spindle assembly checkpoint signalling, DNA replication initiation and nervous system development. Cellular component enrichment included extracellular matrix, neuron projection, glutamatergic synapse, spindle microtubules, condensin complex,

kinetochore, chromosome centromeric regions and collagen-containing extracellular matrix. The significantly enriched molecular function categories included microtubule binding, microtubule motor activity, calcium ion binding, single-stranded DNA helicase activity, extracellular matrix structural constituent activity and metalloendopeptidase activity. KEGG pathway enrichment analysis identified motor proteins, cytoskeleton in muscle cells, cell cycle regulation, ECM-receptor interaction and neuroactive ligand-receptor interaction as significantly enriched pathways. GO and KEGG enrichment results for downregulated DEGs are shown in Figure 7a and 7b.

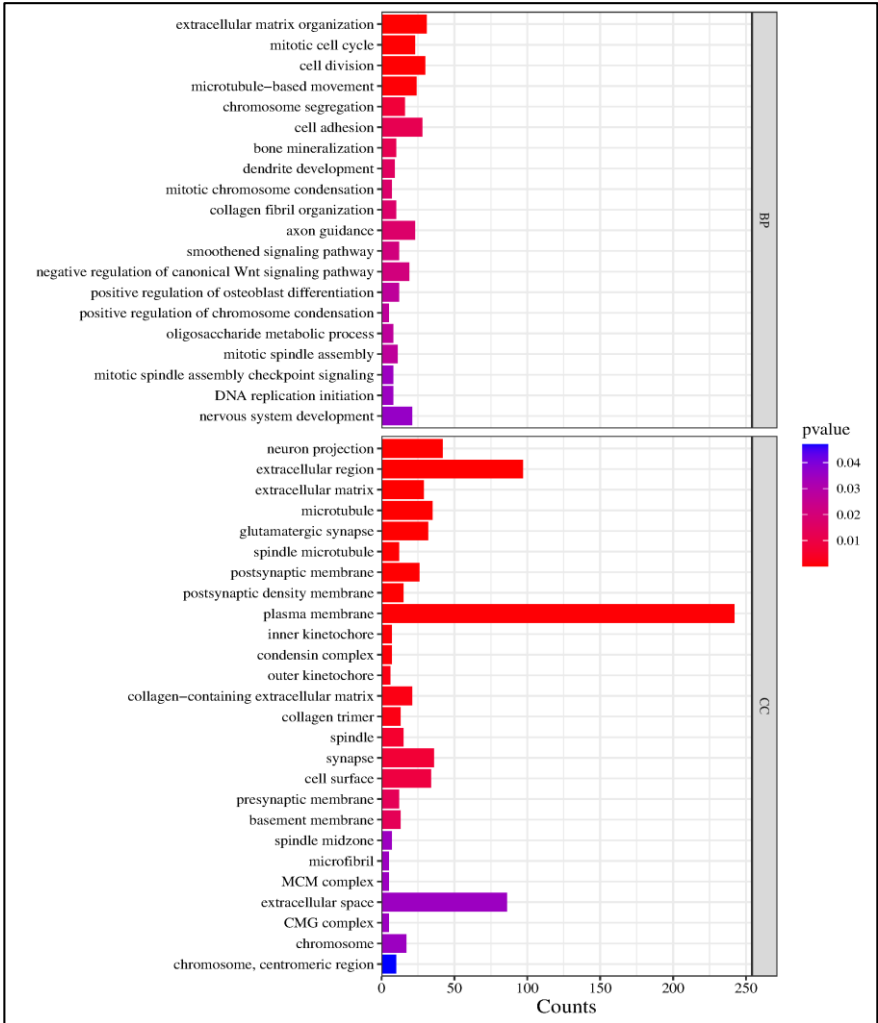


Fig 7a: Bar plot showing significantly enriched BP and CC terms among downregulated DEGs in Pekin ducks.

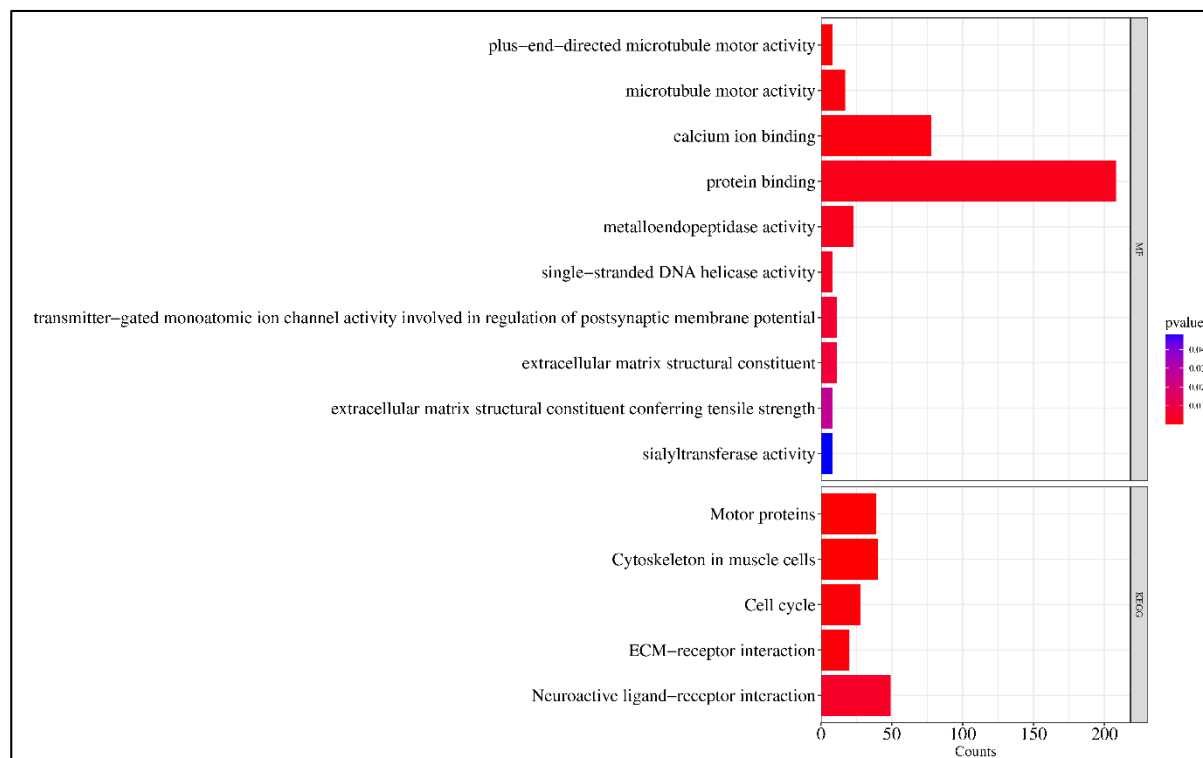


Fig 7b: Bar plot showing significantly enriched MF and KEGG terms among downregulated DEGs in Pekin ducks.

Discussion

Several studies have investigated meat quality in many domesticated animals, including pig, cattle and chicken. The present study compared the leg muscle transcriptomic profiles of Pekin and Hanzhong Ma (HZM) ducks to identify molecular mechanisms associated with muscle growth and production-related traits. Differential expression analysis was performed using a stringent threshold of $|\log_2 \text{fold change}| > 2$ and false discovery rate (FDR) < 0.05 , following criteria adopted in previous transcriptomic studies (Luo *et al.*, 2021) [13]. Using these parameters, a large number of significantly differentially expressed genes (DEGs) were identified between the two duck breeds, indicating substantial transcriptional divergence associated with their contrasting phenotypes. One of the major findings of this study was the strong enrichment of mitochondrial and oxidative metabolism-related genes in Pekin ducks. Upregulated genes were predominantly associated with oxidative phosphorylation, mitochondrial electron transport, ATP synthesis, glycolysis, tricarboxylic acid (TCA) cycle and fatty acid β -oxidation pathways. In particular, the top hub genes identified from the protein-protein interaction (PPI) network, including *NDUFB9*, *NDUFB5*, *NDUFB4*, *NDUFB8*, *NDUFA6* and *NDUFS7*, were mainly components of mitochondrial respiratory chain complex I. These genes encode subunits of mitochondrial respiratory chain Complex I and are essential for electron transport and ATP synthesis during oxidative phosphorylation (Alston *et al.*, 2018) [12]. The enrichment of these pathways indicates that Pekin ducks prioritize mitochondrial energy metabolism and oxidative ATP generation, which likely supports rapid muscle hypertrophy, higher feed conversion efficiency and superior meat yield. Previous transcriptomic studies in ducks have also identified genes associated with muscle growth, ATP generation, lipid metabolism and muscle organization, supporting the role of metabolic regulation in breed-specific growth performance (Wang *et al.*, 2017) [17].

In contrast, genes relatively more expressed in Hanzhong Ma ducks were enriched in pathways related to cell cycle regulation, DNA replication, extracellular matrix (ECM) organization, chromosome segregation, spindle assembly and neural development. Hub genes such as *CDC45*, *MCM2*, *MCM5*, *ORC1*, *BUB1* and *CDK1* are known regulators of mitotic progression and cellular proliferation. The enrichment of ECM organization, collagen fibril organization and cytoskeletal pathways suggests active tissue structuring and developmental regulation in Hanzhong Ma ducks. Neural-associated pathways, including axon guidance and nervous system development, were also significantly enriched. These transcriptomic characteristics may align with the indigenous adaptive features of Hanzhong Ma ducks, including structural resilience, slower but sustained growth and egg-laying performance. The comparative transcriptomic patterns observed between the two breeds reflect differences arising from long-term breeding objectives. Pekin ducks have undergone intensive artificial selection for rapid growth, muscle mass accumulation and commercial meat production, whereas Hanzhong Ma ducks have traditionally been maintained as indigenous dual-purpose ducks with emphasis on adaptability and reproductive performance. The present findings indicate that Pekin ducks rely predominantly on metabolic efficiency and mitochondrial energy production, while Hanzhong Ma ducks exhibit greater activity in pathways related to cellular organization and proliferative development.

Protein-protein interaction analysis further highlighted the biological significance of the identified DEGs. The upregulated PPI network in Pekin ducks showed greater interaction density compared to the downregulated network, indicating coordinated regulation of metabolic pathways associated with oxidative phosphorylation and energy production. The identification of the top 20 hub genes provides important molecular targets that may be useful for

future breeding and genomic selection programs. These genes could potentially be leveraged in molecular breeding strategies aimed at improving muscle growth efficiency, feed utilization, and adaptability in duck populations. Although the study identified biologically meaningful transcriptomic differences, several limitations should be acknowledged. First, the analysis was based on a relatively small sample size with only two biological replicates per breed. Consequently, the present findings should be considered exploratory rather than definitive. Several similar comparative transcriptome studies with sample size ($n = 2$) per breed was done (Karimi *et al.*, 2024; King *et al.*, 2026) ^[10, 11]. A limited sample size reduces statistical power and may not fully represent within-breed variation. Second, the study relied on publicly available RNA-Seq datasets and factors such as environmental conditions, nutrition, management practices and genetic diversity could not be independently standardized. In addition, only leg muscle tissue from a single age group was examined, whereas gene expression patterns may vary across developmental stages and tissues.

Despite these limitations, the study provides valuable transcriptomic insights into the molecular basis of breed-specific growth and muscle characteristics in ducks. The identification of mitochondrial metabolism-related genes in Pekin ducks and proliferation-and ECM-related genes in Hanzhong Ma ducks contributes to understanding the biological mechanisms underlying growth performance and adaptation. From a broader perspective, these findings may support sustainable poultry production strategies by identifying genetic markers associated with efficient resource utilization and breed adaptability.

Conclusions

The present study also highlights the potential relevance of integrating molecular breeding approaches with sustainable and eco-friendly farming practices. The identified hub genes and enriched pathways may serve as candidate markers for resource-efficient duck breeding programs, particularly for improving indigenous and regionally adapted duck populations in India. By combining transcriptomic insights with traditional farming systems and AYUSH-based agricultural values emphasizing sustainability and environmental harmony, future breeding programs may contribute toward improved poultry productivity, climate resilience and enhanced rural livelihoods.

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