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Analysis of liver transcriptome data to identify the genes affecting lipid metabolism during the embryonic and hatching periods in ROSS breeder broilers

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Abstract Upon transfer of incubating eggs from the setter stage to the hatcher, the metabolism of the energy source for the rapid metabolism also changes rapidly when the adipose tissue may play an important part. To comprehend the molecular processes underlying the alterations in fat metabolism, identification of genes, processes, and pathways related to fat metabolism is imperative. This research aimed to identify the important genes in lipid metabolism during the embryonic and hatching periods in Ross breeder broilers. The embryonic transcriptomics data were extracted from the Gene Expression Omnibus (GEO) database with accession number GSE109451 and analyzed using Gene Expression Omnibus 2R (GEO2R). Common genes between the setter and hatcher periods were identified with Venn web tool. The Database for Annotation, Visualization, and Integrated Discovery (DAVID) tool was used to identify the biological processes and pathways. The protein-protein network was drawn using the String software and analyzed with the Cytoscape software. Overall, 580 genes in the setter period and 711 genes in the hatcher period showed differential expressions, and 205 common genes were identified between these periods. The most important pathways and processes related to lipid metabolism with common genes between the setter and hatcher periods were the cell cycle, retinol metabolic process, activation of protein kinase activity, nucleic acid metabolism, and metabolic pathways. The key genes associated with lipid metabolism included *PBK*, *CDK1*, *CCNB2*, *AURKA*. The risks associated with excess fat tissue in chickens present a dual challenge that encompasses animal health and product quality. Targeted research in this area holds the potential to yield effective interventions, ultimately contributing to the sustainability and profitability of poultry production. Enhanced understanding and control of fat metabolism are essential for fostering a healthier and more productive poultry industry.

Keywords: embryonic period, hatching period, key genes, lipid metabolism

Introduction

One of the main problems in raising broilers is the increase in fat deposition (Emmerson, 1997) that reduces the feed efficiency and increases the cost of meat production (Liu et al., 2018; Moreira et al., 2018). In poultry, the liver is the main site of lipogenesis, and liver lipid metabolism disorders lead to excessive fat deposition, fatty liver disease, and great economic loss (Liu et al., 2020). Oxidation of fatty acids increases during the setter period, but it decreases after hatching (Liu et al., 2020). In the first

few days after hatching, the yolk is rapidly absorbed. Therefore, the function of subcutaneous adipose tissue may coincide with fat absorption in the post-hatch period (Zhao et al., 2021). The digestive tract is not complete after hatching as a result, the extra energy supply can be related to the deposition of subcutaneous fat during the embryonic period and after hatching (Halevy, 2020; Lu et al., 2020; Nyuiadzi et al., 2020). During the last 7 days of the embryonic period and the first few days after hatching, 90% of the energy required for chick growth comes from the oxidation of fatty acids (Noble and Cocchi, 1990). Chick subcutaneous adipose tissue increases during embryonic day 12, but chick abdominal fat develops later than subcutaneous adipose tissue and is related to nutrition and management (Mellouk et al., 2018; Hicks and Liu, 2021) Previous studies have shown that fats stored in adipocytes are transferred to muscle fibers for growth during the embryonic life of chicks (Chartrin et al., 2007; Liu et al., 2016). There is no detailed information about the growth of subcutaneous adipose tissue in the setter and hatch period (Mellouk et al., 2018; Xiao et al., 2019). Previous studies showed that genes involved in lipid metabolism, beta fatty acid oxidation, play an important role in early muscle growth (Liu et al., 2020). Development of the adipose tissue during the setter and hatching period determines the growth trend during the whole life of chickens (Ailhaud et al., 1992; Guo et al., 2011). Therefore, the study of hepatic lipid metabolism during the embryonic and hatching periods may lead to better understanding of the molecular processes of hepatic lipid metabolism in chickens. Liver transcription data analysis is a powerful tool that provides crucial insights into the underlying genetic mechanisms of lipid metabolism. Moreover, gene expression and the emergence of a specific phenotype are influenced by various factors and complex mechanisms. Some of these mechanisms, such as long non-coding RNAs, DNA methylation, chromatin microRNAs, histone tail modifications, and remodeling that affect the epigenome interact with other factors, such as climate, nutrition, and pathogens (Shokri et al., 2023; Safaei et al., 2024). It is also possible that these environmental factors, epigenomic mechanisms and the genome itself interact at different levels (Mohamadipoor Saadatabadi et al., 2021). Various evidences have shown that the production, reproduction and health of animals are affected by the diversity in the epigenome (Bordbar et al., 2022; Safaei et al., 2022). Thus, this research aimed to identify the effective genes in the process of lipid metabolism during the embryonic and hatching periods in Ross breeder broilers.

Materials and methods

Transcriptomics data during the setter and hatcher periods in Ross breeder broilers were extracted from the GEO database (National Center for Biotechnology Information) with accession number GSE109451. The GEO database contains a large portion of gene expression data with high functionality and generality (Hunt et al., 2022). The liver transcriptome data included 24 liver samples with four biological replicates and, at six developmental ages (e16, e18, and e20 embryos; and day 1, day 3, and day 9 hatchling chicks). This study used the data of e16, and e20 embryos for setter period and day 1, and day 9 chicks for the hatcher period (Table 1). The GEO2R bioinformatics tool was used to identify the genes that were differentially expressed. This tool allows the users to analyze gene expression on microarray data sets of the GEO database (Barrett et al., 2012). The GEO2R uses the R packages to analyze the GEO data and displays the results as a table of genes that can be visualized with GEO Profile graphics (Barrett et al., 2012). Detection of genes with different expressions in setter and hatcher periods was based on 1<LogFC<-1 and adjp-Value <0.05 (Figure 1). Using the Venn Diagram web tool, the common genes with different expression between setter and hatcher periods were determined (Figure 2). The DAVID's bioinformatics tool was utilized to identify the biological processes and pathways in which the genes with common different expression between the setter and hatchery periods are involved. The DAVID provides a set of tools for functional annotation and enrichment (Huang et al., 2007). The STRING online software was used to create a network of identified genes with common different expression between the setter and hatchery periods. The purpose of the STRING database is to collect information, and synthesize association data of known and predicted proteins for a large number of organisms (Szklarczyk et al., 2017). The STRING network analysis was done with the CytoNCA plugin of the Cytoscape software. This software is used for integration, visualization, and analysis of biological networks (Saito et al., 2012). The genes that have the greatest effect on fat metabolism in the Cytoscape software were identified based on the betweenness centrality, and degree.

Table 1. Accession numbers of the samples used for embryonic and hatching periods

Period	Developmental stage	Accession number of samples
setter	e16	GSM2943545
		GSM2943546
		GSM2943547
		GSM2943548
	e20	GSM2943553
		GSM2943554
		GSM2943555
		GSM2943556
hatcher	day 1	GSM2943557
		GSM2943558
		GSM2943559
	day 9	GSM2943560
		GSM2943565
		GSM2943566
		GSM2943567
		GSM2943568

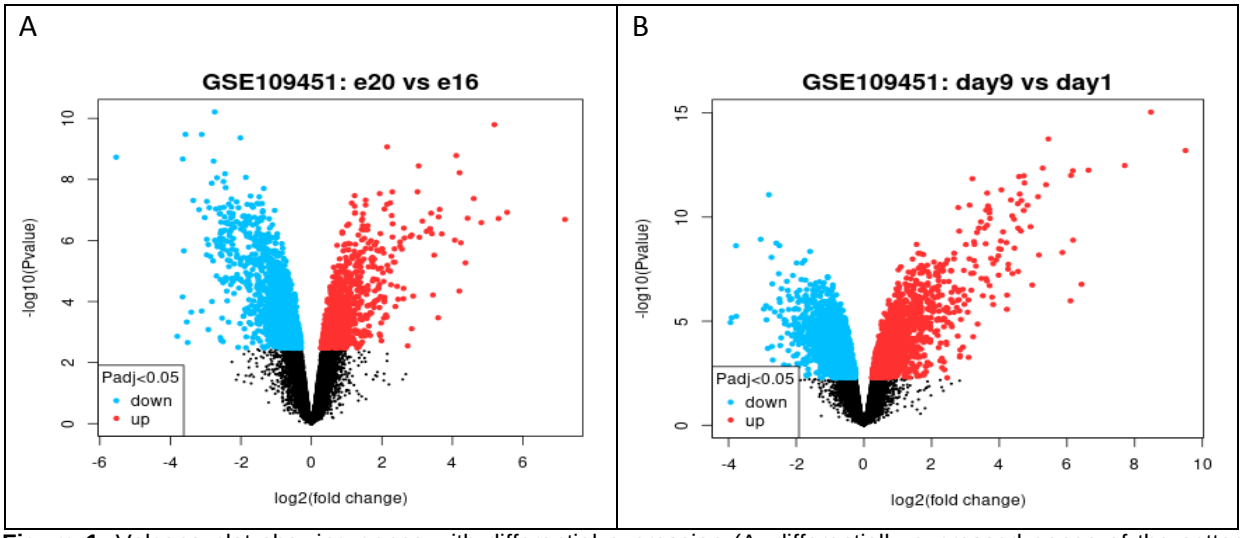

Figure 1. Volcano plot showing genes with differential expression (A: differentially expressed genes of the setter period, B: differentially expressed genes of the hatcher period)

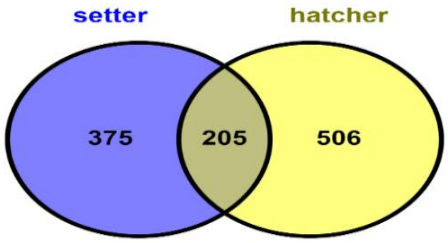

Figure 2. Using the Venn diagram web tool, number of common genes with differential expression between setter and hatcher periods

Results

In the setter period, in general, 580 genes were expressed differently, of which 373 genes showed an increase in expression and 207 genes showed a decrease in expression, and in the hatcher period, a total of 711 genes were expressed differently, of which 384 genes showed an increase in expression and 327 genes showed a decrease in expression. Using the Venn Diagram web tool, 250 common genes with differential expressions between the setter and hatcher periods were identified (Table 2). The process of gene expression is an essential process that communicates between the information encoded in a gene and the final functional gene product (Volgin, 2014).

Using the DAVID database to identify the biological terms (GO terms and KEGG pathways) at $p<0.05$ the most important pathways and processes related to lipid metabolism with common genes between the setter and hatcher periods were the cell cycle, retinol metabolic process, activation of protein kinase activity, nucleic acid metabolism, and metabolic pathways (Table 3).

With String software, a network of protein-protein interactions of common genes with differential expression between the setter and hatcher stages was drawn and for further analysis of this network, it was placed in the Cytoscape software (Figure 3). Genes with greater effect on lipid metabolism were identified based on grade index and vision indices (Table 4).

Table 2. The symbol of the common genes between setter and hatcher periods

Symbol of the common genes	E2F8,RTKN2,PTPRR,RCBTB1,TPX2,SLC2A5,DCK, CYP2W2,FABP7,AVDL,SERPINF1,EPT1,BRCA1,H NMT,KPNA2,DGUOK,SEL1L3,ANLN,BIRC5,PLK1, AvBD8,KNTC1,ANGPTL3,TM4SF4,HBAD,GSTA4, MGST3,BORA,CDK1,OSMR,C14ORF1,FABP5,SLC 16A5,PPM1K,KIF11,FZD2,LOC423347,SLC6A6,RA CGAP1,CENPH,KNSTRN,LOC418424,CCNG2,TEN M2,CCNB3,TM6SF1,IRF6,NEBL,TNNC1,RNF103,A URKA,HDAC9,MAD2L1,FGF19,KIAA1524,CKS1B, MAP3K7CL,UPP2,KIAA1161,SGOL1,NCAPD3,FST, RIMBP2,KIF4A,RAB20,ARHGEF39,BARD1,CA9,KI F2C,LOC419851,GNG4,ADH1C,IGFBP2,IMPA2,CT H,SPTBN5,FKBP5,TYMS,DNA2,ABCB1LA,MELK,C DC20,NDC80,PLK4,SOCS3,CCNA2,GTSE1,BUB1, ANKRD9,PBK,NUF2,CHODL,PKP2,HBAA,PTTG1, HMGB2,AvBD10,STMN1,CREM,NSDHL,WEE1,NU P210,ECT2,DEPDC1,GNLY,SKA3,PLCXD1,DIAPH 3,ETNPPL,DMBT1L,GGACT,IP6K2,SMC2,ABHD5, GSTA,LEAP2,MTFR2,CENPE,ASPM,ZYG11B,NCK AP5,BRCA2,TTR,PTGDS,HAUS1,CDCA3,CA4,LOC 396380,TACC3,ITGBL1,C8B,SLC25A4,UBE2C,FBX O9,CCNB2,MASP2,CENPW,CKAP2,AKR1D1,EML4 ,TOP2A,TSPO2,HBE1,ALDH1A1,SFTPA,CDC45,DI O2,P22,FAM72A,KIF18A,KIF15,CETP,ADSL,BUB1 B,CTD2510F5.6,RRM1,DLGAP5,MSMO1,HJURP,C APSL,MOGAT1,MKI67,BACH1,LHFPL5,FAM83D,L OC418836,ACMSD,NT5C2,PGPEP1L,CENPP,SER PINB10,GSTZ1,LOC418892,CENPI,KIF20A,LOC42 4919,APOA4,CPT1A,TERF1,DCTD,CEPT1,LPIN1, KCNK5,BLB1,CYP2C45,APITD1,JUN,ADAMTS5,TT K,CDKN3,NCAPG,ALDH9A1,CES1,INSIG1,NEK2,L OC428141,CENPF,C6,NUSAP1,INCENP,LOC1008 59853,BCKDHB,EAF2,HBG1,S100A9

Table 3. Pathways and processes related to lipid metabolism with common genes of setter and hatcher periods

Term	p-Value	Count
GO:0007049~cell cycle	1.45E-05	9
GO:0042572~retinol metabolic process	0.011903	3
GO:0032147~activation of protein kinase activity	0.014141	3
gga01232:Nucleotide metabolism	2.72E-04	8
gga01100:Metabolic pathways	0.020356	31

Discussion

Some of the genes found in this research are consistent with some previous studies and are involved in the pathways and biological processes related to lipid metabolism. The cell cycle is the first biological process identified, which plays an important role in fat metabolism. Considering the role of some lipids during cell division, some studies showed that the metabolism of specific lipids and phospholipids may be regulated in specific cell cycle models (Jackowski, 1994; Saitoh et al., 1996; Witkin et al., 2012; Fagone and Jackowski, 2013). The regulation of lipid metabolism during the periods of increased growth, such as the G1 phase of the cell cycle, is very weak compared to other phases (Sanchez-Alvarez et al., 2015). Coordinated processes of cell cycle progression and lipid metabolism are essential for proper cell proliferation (Ow et al., 2020). The cell cycle is fundamental in regulating metabolic processes, including lipid metabolism that in Ross broilers, the coordination between cell growth and metabolism is critical. The second significant biological process is the retinol metabolic process. Vitamin A (retinol) plays an important role in the regulation of cell homeostasis (Chen and Chen, 2014; Blaner, 2019). Vitamin A is directly implicated in the metabolism of lipids. Adequate retinol levels promote efficient fat utilization and deposition, which is vital for energy production and overall productivity in broiler chickens (Yuan et al., 2014). The last significant process identified is the activation of protein kinase activity. AMP-activated protein kinase is a protein kinase that is activated by lipid metabolites and regulates several key enzymes of lipid metabolism (Hardie et al., 1989). Lipid metabolism regulation through AMPK pathways can affect overall growth performance, feed efficiency, and body composition in Ross broilers (Huang et al., 2017). The significant pathways related to lipid metabolism in this study were nucleic acid metabolism and metabolic pathways. Adenine nucleotides, and their metabolites, play a role in regulating glucose and fat metabolism (Ge et al., 2021). Uridine is related to glucose homeostasis, lipid metabolism and amino acid metabolism (Zhang and Knapp, 2016). Nucleic acid metabolism pathways in Ross breeder broilers significantly influence lipid metabolism through genetic expression, environmental adaptation, and various signaling pathways. The regulation of genes involved in lipid biosynthesis, fatty acid transport, and energy utilization is critical for optimizing the growth and health of broilers in production settings (Huang et al., 2017; Lim et al., 2022). The metabolic pathways involved in lipid metabolism have a substantial impact on the lipid profiles and overall health of Ross breeder broilers. These pathways include a range of biochemical processes that regulate lipid synthesis, transport, and storage. In chickens, lipids are represented by triglyceride (TG) and are first made in hepatocytes and then stored in fat cells (Nematbakhsh et al., 2021). The difference in lipid metabolism in chickens compared to mammals is the transfer of dietary lipids to the liver, hepatic lipogenesis, and the presence of certain lipoproteins in the blood. With the synthesis of fatty acids, lipids are secreted by the liver in the form of very low-density lipoprotein (VLDL) and transported through the circulation to target tissues such as adipose tissue, where lipoproteins are hydrolyzed by lipoprotein lipase for immediate use or deposition (Cui et al., 2018).

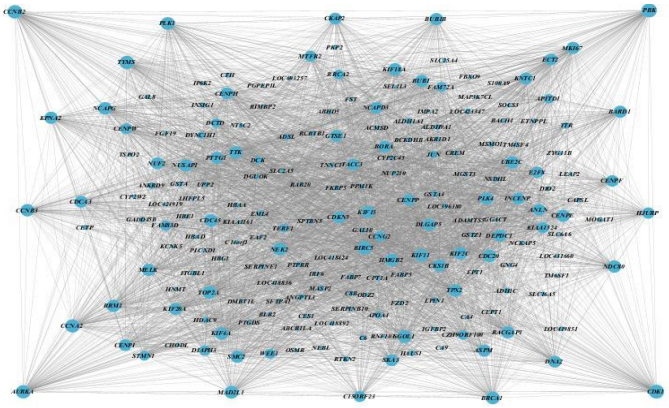

Figure 3. The network of protein-protein interactions with common genes with different expression between the two stages of setter and hatcher is the size of the nodes based on the degree index.

Table 4. Key genes based on degree and betweenness centrality among shared genes with differential expression

Gene	Degree	Betweenness
PBK	98	1504.616
CDK1	97	1149.7775
CCNB2	94	596.7219
AURKA	92	643.72906

With String software, a network of protein-protein interactions of common genes with differential expression between the setter and hatcher stages was drawn and for further analysis was placed in the Cytoscape software (Figure 3). During the incubation of Ross breeder broilers, two critical periods are identified: the setter period (first 18 days) and the hatcher period (last 3 days before hatching). During the embryonic period, broiler chickens heavily rely on the yolk for energy, while in the hatching period, there is a marked shift to lipid metabolism as they prepare for birth and subsequent growth (Su et al., 2020). The up regulation of lipid metabolism-related genes during the hatcher period underscores the critical transition into utilizing fat as a primary energy source, which is vital for optimal growth and development post-hatching. Understanding these mechanisms may lead to better nutritional

strategies and breeding practices for enhancing growth efficiency and meat quality in poultry production (Liu et al., 2019; Su et al., 2020; Wang et al., 2022). Here, the genes that had a greater effect on fat metabolism were identified based on grade index and betweenness indexes (Table 4). PBK gene was identified (with degree 98) as a key gene in fat metabolism. This gene encodes a serine/threonine protein kinase belonging to the dual mitogen-activated protein kinase family. Mitogen-activated protein kinases are important and highly active proteins that regulate lipid metabolism (Guo et al., 2020) by activating receptor tyrosine kinases (RTK) that plays an important role in regulating glucose and lipid metabolism (Zhao et al., 2020).

The second key gene with degree 94 is *CDK1*, which is involved in cell cycle regulation. Previous studies showed that by removing CDK1 in hepatocytes, hepatocyte division is prevented *in vivo* (Diril et al., 2012). Hepatocyte proliferation contributes to liver maintenance and regeneration. Based on previous results, loss of hepatic *CDK1* leads to changes in lipid metabolism (Ow et al., 2020). Our results showed that this gene is directly and indirectly involved in lipid metabolism.

The next key gene with degree 94 is *CCNB2*. Cyclin B2, an important regulator of mitosis in the cell cycle (Brandeis et al., 1998), is involved in the development of chicken breast muscle (Li et al., 2019). There is a positive correlation between the lipid metabolism pathway and the processes related to cell division (Yano, 2012).

The last key gene identified in this research is the AURKA gene with degree 92. *AURKA* is involved in metabolism and cell cycle (Ruiz-Pérez et al., 2017). Previous findings showed that this gene can directly control hepatocytes and macrophages in the liver (Shi et al., 2023). According to the standard poultry diet containing less than 10% lipids, the liver (the main place of lipid metabolism) plays the main role in supplying the desired fats to the body. Our data confirmed the previous findings on the effective role of this gene in lipid metabolism.

Conclusions

The primary objective of this study was to identify the genes effective in the process of lipid metabolism in the setter and hatcher periods in Ross breeder broilers. The results of this research provide a new insight by understanding the changes in the gene expression of lipid metabolism. Glucose and lipid metabolisms are influenced by genetic polymorphisms and environmental factors. Excessive absorption and accumulation of lipids cause obesity and problems in glucose metabolic homeostasis. Liver diseases in which the proliferation of liver cells decreases are due to irregular lipid metabolism. A better understanding of the molecular regulation of lipid metabolism can offer new insights into the use of key genes in selecting against excessive fat deposition to improve chicken production efficiency and meat quality.

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