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Transcriptome analysis reveals key genes and signalling pathways related to residual feed intake in meat-type ducks

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ABSTRACT

The feed utilisation rate is a key factor that affects the economic benefits of meat-type duck breeding. In recent years, residual feed intake (**RFI**) has been routinely used in poultry breeding as an index for evaluating feed utilisation. However, the genetic mechanism underlying RFI in meat-type ducks remains poorly understood. In the present study, 1 000 meat-type ducks with similar BW were randomly selected to measure BW gain and feed intake from 21 to 42 d of age to assess RFI. Six high- and six low-RFI meat-type ducks were randomly selected for a transcriptome survey of livers. Protein–protein interaction (**PPI**) network and gene set enrichment (**GSEA**) analyses were used to elucidate the molecular basis of RFI. We identified 1 297 differentially expressed genes (**DEGs**) in the LRFI group, of which 686 and 611 were markedly up- and downregulated, respectively. Functional annotation showed that DEGs were mainly enriched in gene ontology terms related to the regulation of biosynthetic and metabolic processes. Kyoto encyclopedia of genes and genomes (**KEGG**) pathway analysis showed significant enrichment of insulin signalling and multiple pathways related to energy metabolism. Furthermore, the PPI network analysis revealed that *PRKACB*, *PRKAR2A*, *FYN*, *PTK2*, *ITGB1*, *ESR1*, and *PIK3CB* were primarily related to the biological processes of lipid metabolism, glucose transport, and immunological stress and may affect RFI. Moreover, the GSEA suggested that upregulated genes in the LRFI group were associated with immune, lipid transport, and insulin signalling. *ACLY* and *SLC50A1* were the most markedly upregulated and downregulated DEGs, respectively. In the PPI network, *PRKACB*, *PRKAR2A*, and *FYN* were identified as pivot genes. The aforementioned DEGs were mainly involved in lipid and glucose metabolism and inflammatory response, consistent with the KEGG and GSEA results. Therefore, these results revealed *PRKACB*, *PRKAR2A*, and *FYN* as potentially key genes for improving feed efficiency traits in meat-type ducks. Our results provide insights into the biological basis of RFI in meat-type ducks and will be useful for the selection of meat-type ducks with a greater feed efficiency phenotype in future breeding programmes.

Implications

As a common indicator for evaluating the feed efficiency of poultry, residual feed intake is closely related to economic benefits of poultry breeding. However, molecular mechanisms underlying residual feed intake require further investigation. In this study, RNA-sequencing was used to screen and compare differentially expressed genes between high- and low-residual feed intake groups in meat-type duck livers. Results showed that differentially expressed genes were chiefly enriched in insulin signalling and multiple pathways related to energy metabolism. Six important candidate genes that may affect residual feed intake values were also identified. Further research examining these genes will help to select ducks with higher feed efficiency phenotypes in future meat-type duck breeding programmes.

Introduction

China has the largest duck industry worldwide and accounts for approximately 70% of global duck breeding and consumption. Feed cost is the largest expenditure of breeding programmes (60–70%) and therefore an important factor affecting total income from the meat-type duck industry (Chen et al., 2021). Such concerns have led to research focusing on improving feed efficiency (Zeng et al., 2016).

Residual feed intake (**RFI**) was first proposed 50 years ago and is now widely used in the selection of efficient poultry feed (Mebratie et al., 2019). RFI is inversely proportional to feed efficiency (Koch et al., 1963) and influenced by factors, such as heredity, nutrient

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absorption, and energy utilisation (Herd and Arthur, 2009). RFI is a relatively independent trait with moderate heritability that can be used as a breeding index (Zhang et al., 2019b). The liver is intricately involved in metabolic processes, such as digestion, absorption, excretion, and biological transformation (Zaefarian et al., 2019; Zhang et al., 2019a). Many previous studies have reported on the importance of the relationship between RFI and lipid deposition (Lee et al., 2015; Xiao et al., 2021; Mota et al., 2022). Hepatic nutrient partitioning directly affects energy-utilisation efficiency and potentially plays a vital role in feed efficiency (Horodyska et al., 2019). However, the molecular mechanism by which the liver affects RFI in meat-type ducks remains unclear. RNA-sequencing is an important means to explore the molecular mechanisms of complex traits (Xing et al., 2020; Yuan and Lu, 2021).

To comprehensively explore the molecular mechanism underlying the regulation of RFI in meat-type ducks, we analysed differentially expressed genes and conducted functional annotation and transcriptome analyses in the livers of meat-type ducks in low-RFI and high-RFI groups using high-throughput RNA-Seq data. Our results provide a clearer understanding of differentially expressed genes and signalling pathways involved in RFI and serve as a reference for duck breeding with efficient feed utilisation and a useful guide for duck breeding companies.

Material and methods

Animals and experimental design

All meat-type ducks used in the current experiment had the complete pedigree records and were supplied by Huangshan Qiangying Duck Breeding Co., Ltd. (Huangshan, China). All ducks were derived from genetically unrelated sources and selected as closed populations for 12 generations based on feather colour, growth, feed efficiency and slaughter traits within each generation. All meat-type ducks were wing-banded, weighed on the day of hatching, and reared on the floor for the first 2 weeks. At 21 d of age, 1 000 male ducks with similar BW (1042.1 ± 87.2 g) were selected as the experimental population and transferred to individual cages (55 × 50 × 40 cm). All ducks were fed with the same diet at room temperature, and management protocols were carried out in accordance with the company's guidelines. Within 72 h after hatching, a constant 24 L: 0 D photoperiod was provided for the birds in the house, which were then maintained in a 20 L: 4 D light environment until 42 d of age. The raising process included a mesh bed for feeding and nipple drinkers for water supply. The animals were allowed access to feed and water *ad libitum* during the raising process. Ingredient nutrient levels of the basal diet used in the present study are shown in Supplementary Table S1.

BW was recorded at 21 d and 42 d. Additionally, BW gain, feed intake, average daily gain (**ADG**), average daily feed intake (**ADFI**), feed conversion ratio, RFI, and metabolic BW (**MBW$^{0.75}$**) from 21 to 42 d of age were calculated. The RFI was calculated using the following formula: $RFI = ADFI - (a_0 + a_1 \times MBW^{0.75} + a_2 \times ADG)$.

where a_0 = intercept, a_1, a_2 = partial regression coefficients. The RFI was calculated using the linear model fit function in SAS version 9.4 (SAS Institute Inc, Cary, NC, USA). The base model formula is described in Supplementary Material S1.

RNA extraction and sequencing

After deleting outlier data, the RFI value was calculated for each duck. Moreover, the 30 ducks with the highest RFI (inefficient, HRFI) and 30 ducks with the lowest RFI (efficient, LRFI) were selected to prioritise the samples because the average RFI rank was subjected to outlier or extreme values. Finally, six HRFI and six LRFI ducks were randomly selected from each group to represent the two distinct RFI groups. Liver tissues were collected from each individual and stored at −80 °C until analysis. Total RNA was extracted from the liver tissues using TRIzol (Invitrogen Life Technologies, Thermo Fisher Scientific, Carlsbad, CA, USA). The integrity and purity of the RNA were analysed through agarose gel electrophoresis (1%) and spectrophotometry using the Nanodrop 2000 (Thermo Fisher Scientific, Wilmington, DE, USA), respectively. The A260/A280 ratio was checked to ensure a range of between 1.8 and 2.0. Twelve cDNA libraries were constructed (Genedenovo Biotechnology Co., Ltd., Guangzhou, China) using qualified samples and sequenced on the Illumina HiSeq 4000 platform (Illumina, San Diego, CA, USA). The raw sequence data generated in this study are publicly available in the NCBI Short Reads Archive (BioProject ID: PRJNA396466).

Analysis of differentially expressed genes

FastQC v0.11.2 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) was used to evaluate the GC content, quality score, and other statistics for the quality control of data. Cutadapt v1.14 was used to remove sequencing adaptors and low-quality reads. Filtered reads were aligned to the duck genome [BGI_duck_1.0 (GCA_000355885.1)] using TopHat2 v2.0.3.12 and mapped with Bowtie2 v2.2.3. Segmentation and location of the reads were inferred. Cuffinks v2.2.1 was used to calculate the expression level of each gene (fragments per kilobase million). Subsequently, the fold change of gene expression between the two groups was calculated. The edgeR package (https://www.r-project.org/) was employed to filter differentially expressed genes (**DEGs**), using the selection criteria of $|\log_2 FC| > 1$ and false discovery rate < 0.05. Positive and negative $\log_2 FC$ values indicated upregulated and downregulated genes, respectively.

Functional annotation

The gene ontology (https://www.geneontology.org/) and Kyoto encyclopedia of genes and genomes (**KEGG**) (https://www.genome.jp/kegg/) databases were used to screen the biological processes and related pathways considerably enriched by DEGs. Statistical significance was considered at false discovery rate ≤ 0.05.

Construction of the protein–protein interaction network

Protein–protein interaction (**PPI**) information for DEGs was first obtained from the online STRING database. Subsequently, it was imported into the Cytoscape software for processing to generate the PPI network. According to the scores of the three centrality algorithms, screening was performed to identify the Top 10 DEGs in the PPI network, and the intersection was taken as the core genes of the network. The modules and seed genes of the PPI network were filtered with the plug-in molecular complex detection in Cytoscape (degree cut-off ≥ 2, node score cut-off ≥ 0.2, K-core ≥ 2, and max depth = 100).

Gene set enrichment analysis

Gene set enrichment analysis (**GSEA**) is a routinely employed method used to identify potential pathways. To explore the key genes and important gene ontology terms and KEGG pathways related to RFI traits in ducks, we used GSEA to rank genes based on the relationship between gene expression and category. An enrichment score for each gene set was calculated to obtain the distribution of each gene set in the list. Subsequently, a normalised enrichment score was confirmed for each gene set assay. Positive and negative normalised enrichment scores reflected low and high expression levels of the LRFI group, respectively. The gene set was

considered significantly enriched at normalised enrichment score > 1 and a nominal *P*-value ≤ 0.05.

Validation using quantitative PCR

The qualified RNA samples were used as templates and reverse transcribed into cDNA according to the EasyScript®One-Step gDNA Removal and cDNA Synthesis SuperMix kit instructions (TransGen Biotech, Beijing, China). Twelve DEGs were selected for quantitative PCR analysis. Corresponding primers were designed using Primer Premier 5.0 software (Supplementary Table S2). An ABI 7500 Real-Time PCR System (Applied Biosystems Inc., Foster City, CA, USA) was used to perform quantitative PCR using NovoStart® SYBR qPCR SuperMix Plus (Novoprotein Scientific, Shanghai, China). Livers from six high- and six low-RFI meat-type ducks were randomly selected for validation using quantitative PCR. All experiments were performed in triplicate.

Statistical analysis

Duck RFI values were calculated based on the formula model described in Supplementary Material S1. Differences in phenotypic traits between the HRFI and LRFI groups were analysed using a Students′ *t*-test in IBM SPSS Statistics 25 (IBM Corp., Armonk, NY, USA). Gene relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). The expression values of each gene obtained using qPCR and RNA-Seq were subjected to linear regression analysis using GraphPad Prism v8.0 (GraphPad Software, San Diego, CA, USA). Differences were considered significant at $P < 0.05$.

Results

Phenotypic traits

Table 1 shows the comparative analysis results of phenotypic traits related to feed efficiency in meat-type ducks of the HRFI and LRFI groups. The two groups of meat-type ducks were selected from the two ends of the RFI value ranking. Phenotypic determination showed that the RFI value of the LRFI group was significantly lower than that of the HRFI group ($P < 0.01$). In addition, the ADFI and feed conversion ratio (**FCR**) values of the LRFI group were markedly lower than those of the HRFI group ($P < 0.01$).

Summary of mapping statistics

After preprocessing sequencing data for the samples, 82 035 712–160 489 464 clean reads were generated for each library. The sequencing data quality evaluation is shown in Supplementary Table S3. Sequencing quality was high; therefore, the data

Table 1
Descriptive statistics of feed efficiency traits in meat-type ducks.

Traits	HRFI[1]	LRFI[2]	SEM	*P*-value
ADFI (g/d)	299.37	252.48	7.61	<0.001
ADG (g/d)	133.61	135.43	3.71	0.830
$MBW^{0.75}$ (g/d)	360.66	350.46	8.60	0.263
FCR (g/g)	2.24	1.88	0.01	<0.001
RFI (g/d)	21.65	−20.02	0.56	<0.001

Abbreviations: ADFI = average daily feed intake; ADG = average daily gain; $MBW^{0.75}$ = metabolic BW; FCR = feed conversion ratio; RFI = residual feed intake; HRFI = high residual feed intake; LRFI = low residual feed intake.
$P < 0.05$ was considered significant; $P < 0.001$ was considered highly significant.
[1] n = 6 (HRFI);
[2] n = 6 (LRFI).

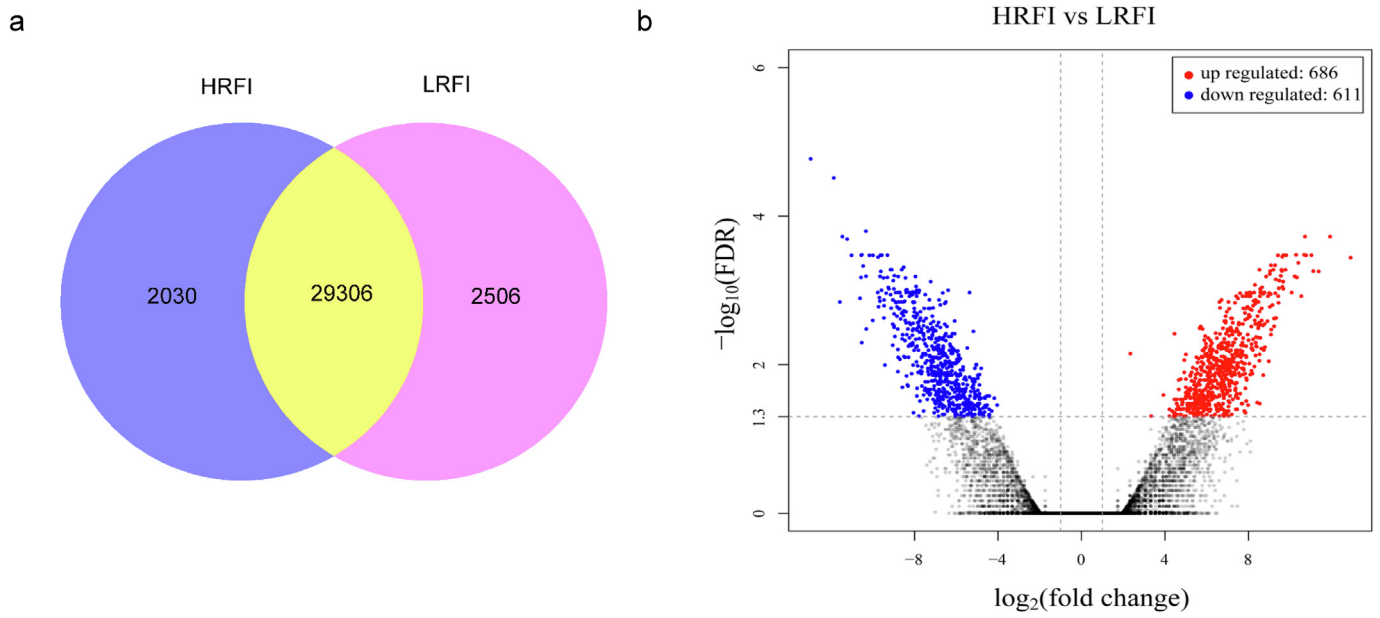

Fig. 1. Differentially expressed genes (DEGs) detected using transcriptome analysis in meat-type ducks. (a) Venn diagram showing genes expressed in only the HRFI group (light red circle), only the LRFI group (blue circle), or common to both groups (intersection). (b) Volcano plot. Red dots (up) denote markedly upregulated genes, blue dots (down) denote markedly downregulated genes ($|\log_2 FC| \geq 1$ and false discovery rate (FDR) < 0.05), and black dots (no) indicate non-significant DEGs. Abbreviations: HRFI = high residual feed intake; LRFI = low residual feed intake. n = 6 (HRFI); n = 6 (LRFI).

could be used for subsequent analysis. Among all mapped reads, the vast majority fell into annotated exons, followed by those in the intron regions, with the lowest number of reads mapped to the intergenic regions (Supplementary Fig. S1). The number of reads distributed in the exon region in the HRFI was marginally higher, and the number of reads in the intron and intergenic regions was marginally lower than those in the LRFI group.

Differentially expressed genes related to feed efficiency

The Venn diagram presents the number of detected genes expressed in both groups (Fig. 1a). A total of 1 297 differentially expressed genes were screened in the duck liver (Fig. 1b); 686 genes were upregulated and 611 genes were downregulated in the LRFI group, thus indicating that both up- and downregulated genes played an important role in RFI. Supplementary Table S4

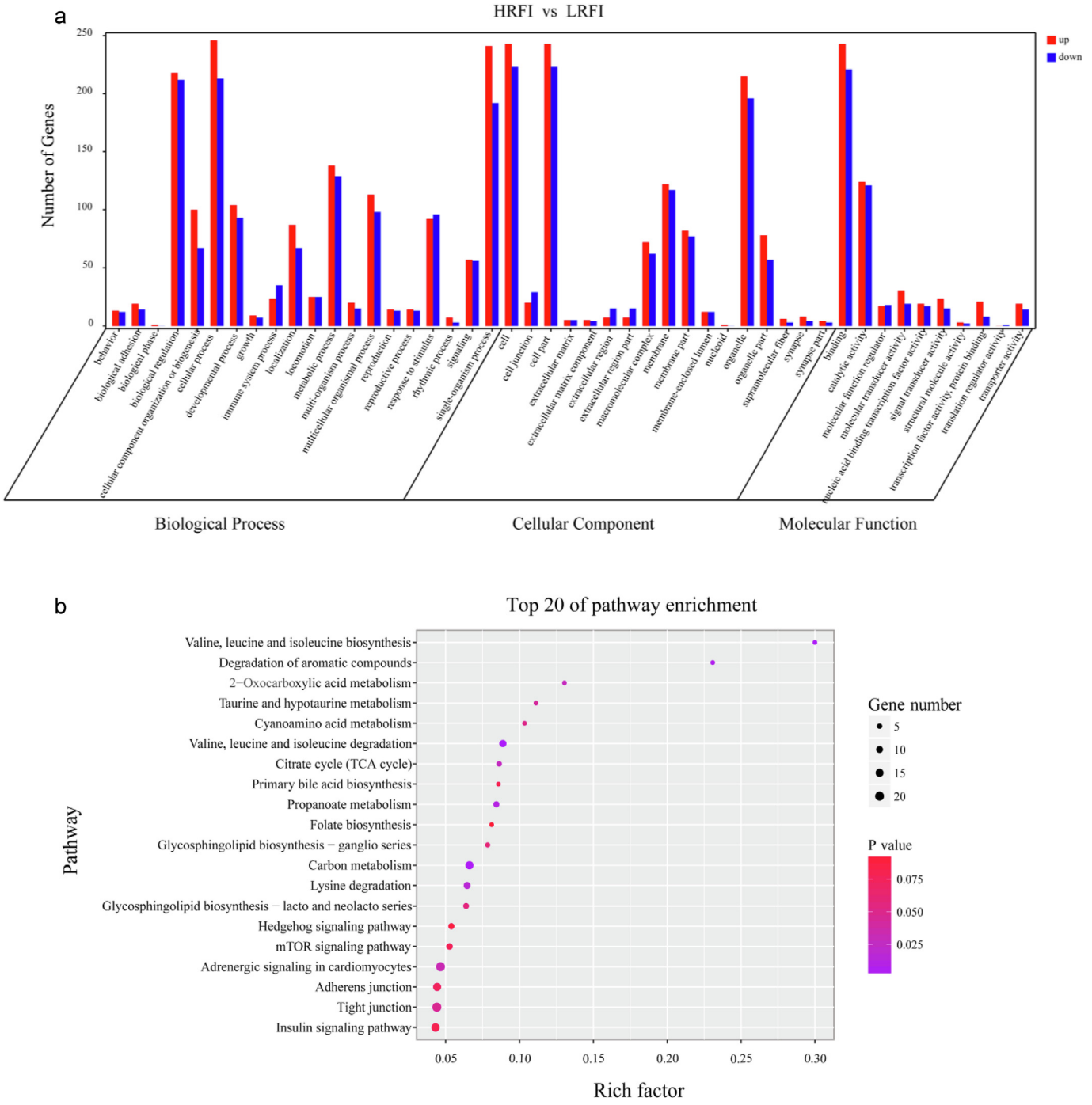

Fig. 2. Functional analysis of gene ontology terms (a) and Kyoto encyclopedia of genes and genomes pathways (b) for differentially expressed genes in meat-type ducks. Abbreviations: HRFI = high residual feed intake; LRFI = low residual feed intake.

shows the top 10 genes with markedly upregulated and downregulated expression.

Gene ontology functional analysis of differentially expressed genes

The identified DEGs were mainly involved in biosynthesis gene regulation, regulating cell biosynthesis and metabolism regulation, regulation of polymer biosynthesis, ATP metabolism regulation, regulation of cell metabolism, primary metabolic process of regulation and histone acetylation, and protein acetylation (Supplementary Table S5, Fig. 2a). Over 100 DEGs were enriched in GO terms related to biosynthesis regulation. Moreover, a higher number of genes were upregulated than downregulated.

Kyoto encyclopedia of genes and genomes pathway analysis of differentially expressed genes

Kyoto encyclopedia of genes and genomes pathway analysis of 1 297 DEGs showed the enrichment of significant pathways, namely, the mTOR signalling pathway, insulin signalling pathway, FOXO signal pathway, and MAPK signal pathways associated with energy metabolism, amino acid metabolism, carbon metabolism,

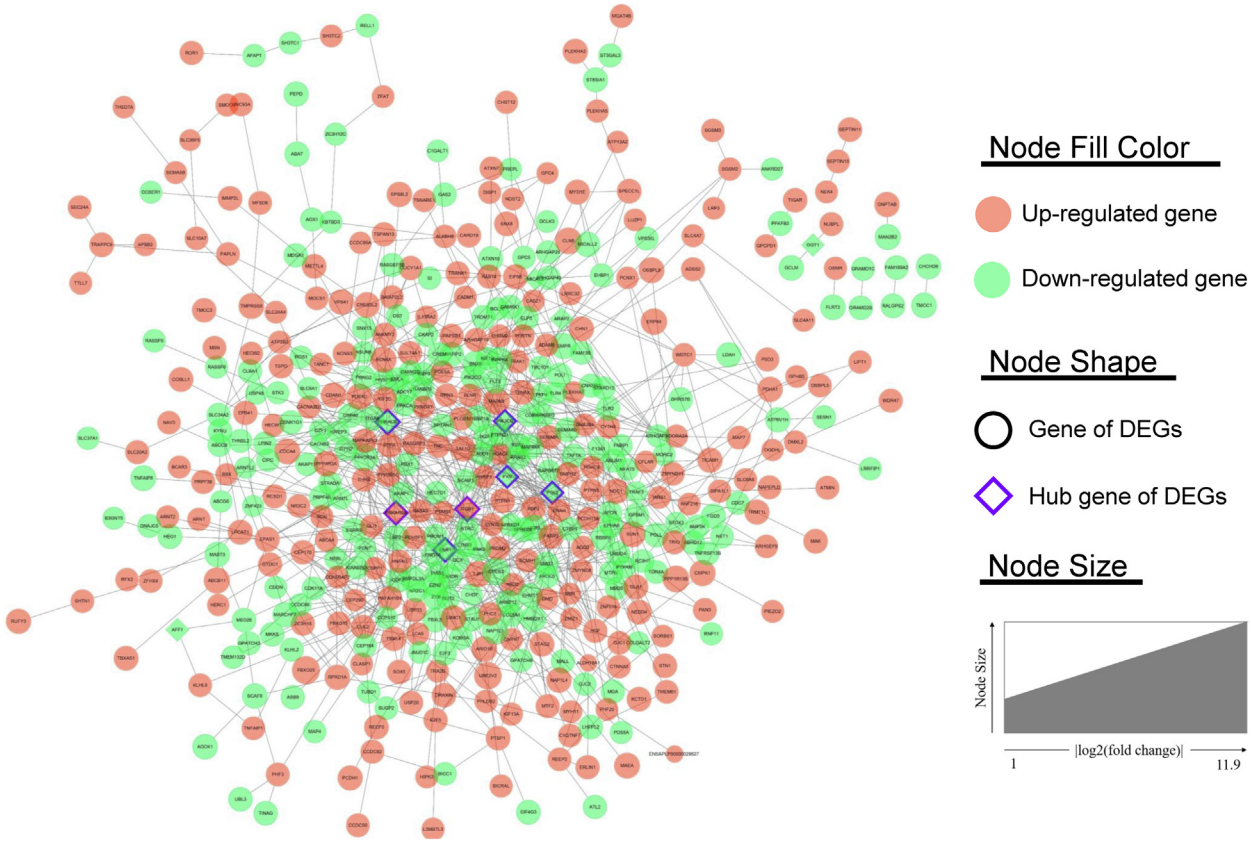

Fig. 3. Protein–protein interaction network analysis in meat-type ducks. Red and green nodes represent up- and downregulated genes, respectively. Diamonds with a purple border indicate nodes with the seven hub genes screened by three central algorithms. Node size corresponds to the fold change of each gene. Abbreviation: DEG = differentially expressed gene.

Table 2
Top 10 genes evaluated in the protein–protein interaction network in meat-type ducks.

Gene	Degree	Gene	EPC	Gene	MNC
PRKACB	31	*FYN*	114.039	*PRKACB*	26
FYN	27	*PRKACB*	113.542	*FYN*	23
ESR1	23	*PTK2*	113.244	*PTK2*	22
ITGB1	23	*ITGB1*	110.389	*ITGB1*	19
PTK2	22	*PRKAR2A*	109.63	*PRKAR2A*	18
PRKAR2A	19	*PIK3CB*	107.333	*ESR1*	15
PAFAH1B1	18	*ESR1*	106.046	*PIK3CB*	14
CTBP1	18	*PPP2R5C*	104.758	*PPP2R5C*	12
PIK3CB	17	*CD4*	103.215	*CD4*	12
PSMB4	17	*PAFAH1B1*	101.129	*TLR4*	12

Abbreviations: *PRKACB* = protein kinase cAMP-dependent catalytic subunit β; *FYN* = FYN proto-oncogene; *ESR1* = estrogen receptor 1; *ITGB1* = integrin subunit β1; *PTK2* = protein tyrosine kinase 2; *PRKAR2A* = protein kinase cAMP-dependent type II regulatory subunit α; *PAFAH1B1* = platelet–activating factor acetylhydrolase 1b regulatory subunit 1; *CTBP1* = C-terminal binding protein 1; *PIK3CB* = phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit β; *PSMB4* = proteasome 20S subunit beta 4; *PPP2R5C* = protein phosphatase 2 regulatory subunit B'gamma; *CD4* = CD4 molecule; *PAFAH1B1* = platelet–activating factor acetylhydrolase 1b regulatory subunit 1; *TLR4* = toll-like receptor 4. EPC = edge percolated component; MNC = maximum neighbourhood component.

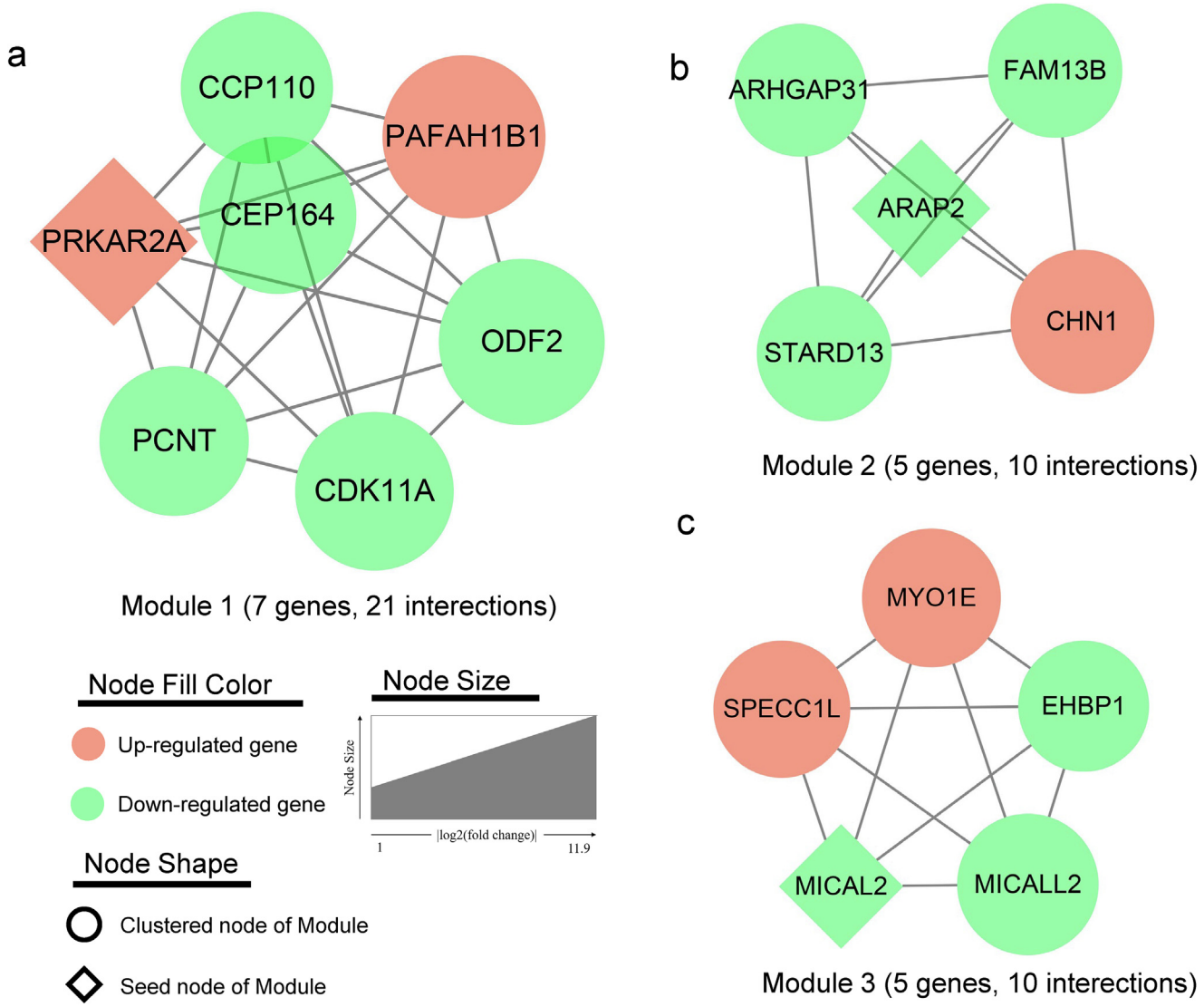

Fig. 4. Top three protein–protein interaction hub network modules in the differentially expressed genes in meat-type ducks. Red and green nodes depict up- and downregulated genes, respectively. Diamond symbols represent the seed node of each module. Node size corresponds to the fold change of each gene.

tricarboxylic acid (**TCA**) cycle, and 2-oxidation metabolic pathways, such as those of carboxylic acid (Supplementary Table S6, Fig. 2b).

Protein–protein interaction analysis

To comprehensively explore connections between DEGs, we created a PPI network based on DEG interactions (Fig. 3). The resulting PPI network contained 473 genes and 976 interaction relationships. Seven core genes were analysed and predicted using the centrality methods of degree, maximum neighbourhood component, and edge percolated component (Table 2, Supplementary Fig. S2). Three modules with the highest scores were established (Fig. 4).

Gene set enrichment analysis

The distinction between the gene expression in the HRFI and LRFI groups was examined in depth using GSEA (Supplementary Table S7). Regarding the GO- and KEGG-based lists, higher gene expression in the HRFI group showed the strongest correlation with immunity, such as the positive regulation of interferon γ production (Fig. 5a) and regulation of the toll–like receptor 4 signalling pathway (Fig. 5b). In the LRFI group, the gene sets with higher expression were mainly related to the lipid transport function and insulin signalling pathway, such as the import into cells (Fig. 5c) and the regulation of the insulin receptor signalling pathway (Fig. 5d).

Validation using quantitative PCR

Trends for the 12 selected genes were largely consistent with those determined using RNA-Seq, as shown in Fig. 6a. Regression analysis of the quantitative PCR and RNA-Seq results showed a significant correlation with $R^2 = 0.9634$ ($P < 0.001$), indicating that the regression equation was meaningful (y = 0.4020x–0.0209; Fig. 6b). Therefore, the RNA-Seq results were considered reliable and accurate.

Discussion

In recent decades, RFI has been widely used as an index of feed efficiency in livestock (Zhang et al., 2022; Zhao et al., 2023) and poultry (Miyumo et al., 2022; Zou et al., 2023) production. In the

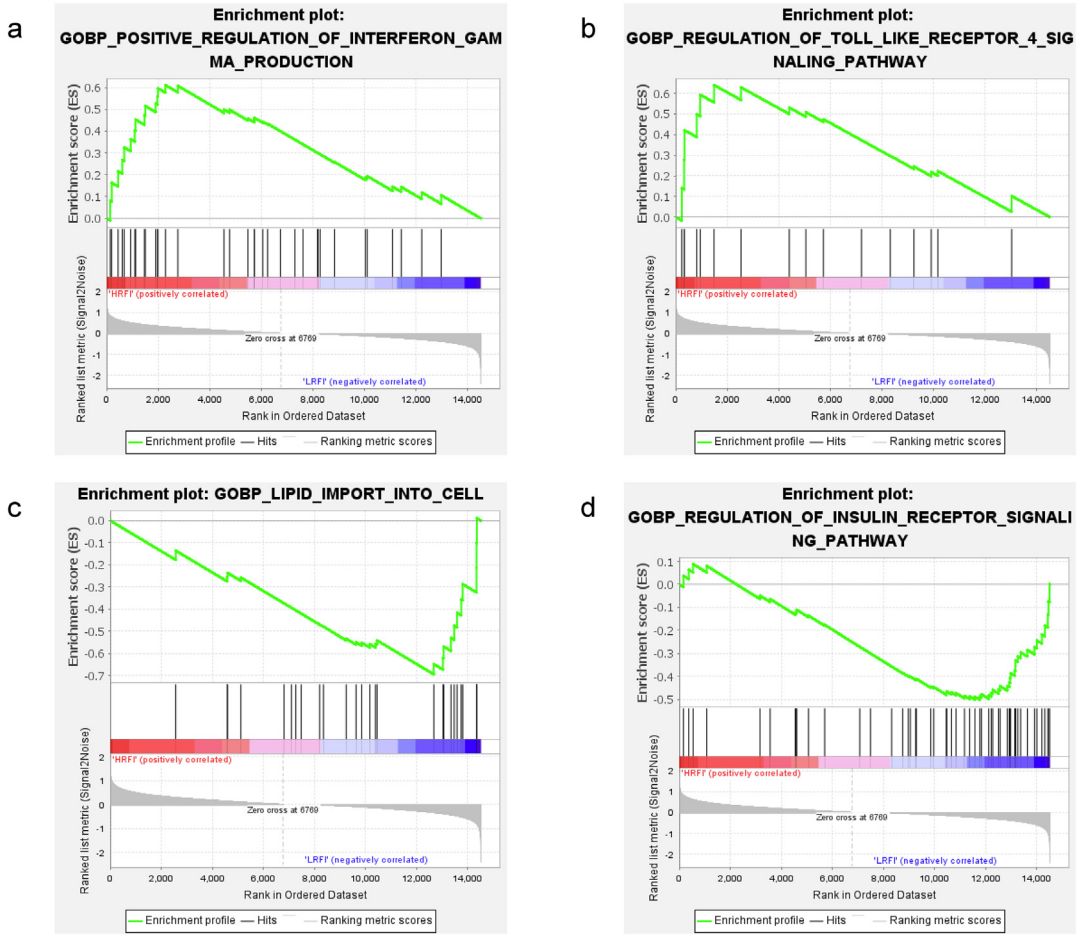

Fig. 5. Gene set enrichment analysis of the HRFI and LRFI groups in meat-type ducks. The gene set enrichment analysis algorithm scored the enrichment of genes in the pathway, which were in the ranked gene list. Enrichment score (ES) > 0 represents a gene set distribution that is biased upstream of the ranking list, and ES < 0 represents a gene set distribution that is biased downstream of the ranking list. The analysis demonstrated that (a) positive regulation of interferon gamma production and (b) regulation of toll-like receptor 4 signalling pathway were enriched in HRFI groups, whereas (c) lipid import into cell and (d) regulation of insulin receptor signalling pathway were enriched in LRFI groups. Abbreviations: HRFI = high residual feed intake; LRFI = low residual feed intake.

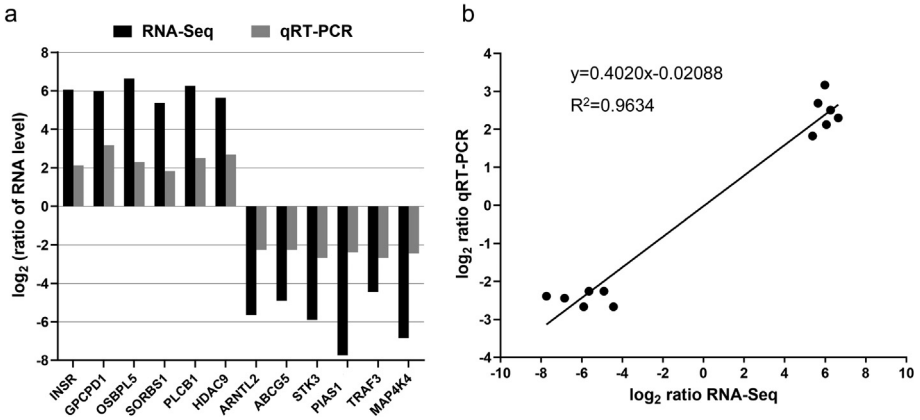

Fig. 6. Verification of RNA sequencing data via quantitative PCR in meat-type ducks. (a) Comparison between qPCR and RNA-Seq measurements of expression levels of 12 differentially expressed genes. (b) Regression analysis of $\log_2$(LRFI/HRFI) ratios between quantitative PCR and RNA-sequencing. Abbreviations: HRFI = high residual feed intake; LRFI = low residual feed intake. *INSR* = insulin receptor; *GPCPD1* = glycerophosphocholine phosphodiesterase 1; *OSBPL5* = oxysterol binding protein–like 5; *SORBS1* = sorbin and SH3 domain containing 1; *PLCB1* = phospholipase C beta 1; *HDAC9* = histone deacetylase 9; *ARNTL2* = aryl hydrocarbon receptor nuclear translocator like 2; *ABCG5* = ATP binding cassette subfamily G member 5; *STK3* = serine/threonine kinase 3; *PIAS1* = protein inhibitor of activated STAT 1; *TRAF3* = TNF receptor–associated factor 3; *MAP4K4* = mitogen-activated protein kinase kinase kinase kinase 4.

present study, meat-type duck phenotypic data showed that the ADFI, FCR, and RFI of LRFI values in the LRFI group were markedly lower than those in the HRFI group, consistent with the results of Bai et al. (Bai et al., 2022). These findings indicate that feed costs can be reduced and income increased by reducing RFI.

Previous studies have found that lipid metabolism (Xu et al., 2016; Liu et al., 2018) and immune stress (Liu et al., 2019; Yang et al., 2020) are important factors affecting RFI in chickens. Consistent with these findings, in the present study, we found identified that the two factors affected RFI in meat-type ducks, especially lipid metabolism. Our functional annotation and GSEA revealed that most of the DEGs closely related with lipid metabolism, glycolysis, and other physiological processes were upregulated in LRFI ducks, thus indicating that lipid transport and insulin metabolism were strengthened in LRFI ducks. Lipid metabolism and glucose metabolism were speculated to be key factors affecting RFI traits in LRFI ducks, similar to the functional annotation results for Beijing-You chicken based on whole-genome sequencing (Liu et al., 2018). Increased expression of lipid transport genes can imply a reduction in lipid deposition and corresponding energy consumption in LRFI ducks, resulting in decreases in RFI and FCR (Jin et al., 2020). A previous study showed that reducing the efficiency of body fat deposition may have a beneficial effect by improving the performance of animal production (Vatish et al., 2009). Therefore, it can be speculated that RFI traits may be regulated by genes pertinent to lipid metabolism.

Based on the findings of the present and abovementioned studies, we highlight genes pertinent to lipid metabolism, including *ACLY*, *SLC50A1*, *PRKACB*, *PRKAR2A*, and *FYN*. ATP citrate lyase (*ACLY*) was the most markedly downregulated gene in the LRFI group and was enriched in the TCA cycle pathway. In a previous study of RFI in beef cattle, it was found that TCA cycle-related genes may affect RFI (Kong et al., 2016). Furthermore, *ACLY* has been identified as a core transcription factor in the mediation of *de novo* lipogenesis (Desert et al., 2018), which lyses citric acid to acetyl coenzyme A, provides raw materials for the synthesis of cholesterol and fatty acids, participates in the regulation of the synthesis of fatty acids and triglycerides, and plays a pivotal role in lipid metabolism (Nematbakhsh et al., 2021). Previous studies have shown that fat synthesis is a factor that affects RFI (Lee et al., 2015; Zhuo et al., 2015). In the present study, the *ACLY* gene was significantly downregulated in the liver of LRFI ducks, which was similar to that of the bovine subcutaneous adipose tissue reported in a previous study (Zhou et al., 2022).

Energy metabolism is another factor that affects animal RFI (Herd and Arthur, 2009). Solute carrier family 50 member 1 (*SLC50A1*), a member of the novel sugar transporter SLC50 family of uniporters (Wright, 2013), was the most markedly upregulated gene in the liver of the LRFI group. It was also found that the expression of genes related to glycogen utilisation was specifically up-regulated in the muscle of LRFI pigs (Gondret et al., 2017). *SLC50A1* plays a pivotal role in glucose transport and can affect glucose metabolism via alterations in glucose uptake (Wang et al., 2019). Previous studies that performed genome-wide association analysis of glucose metabolism showed that *SLC50A1* was associated with a biomarker of glucose metabolism, suggesting its significance in this process (Li et al., 2017).

The protein kinase cAMP-dependent catalytic subunit β (*PRKACB*), protein kinase cAMP-dependent type II regulatory subunit α (*PRKAR2A*), and FYN proto-oncogene (*FYN*) that were identified as core genes in the PPI network are associated with PKA. *PRKACB* encodes one of the catalytic subunits of PKA (Taylor et al., 2021). PKA is one of the most widely studied serine/threonine kinases that performs several fundamental functions in cells, including the modulation of sugar and lipid metabolism (Schmoker et al., 2018). PKA can phosphorylate and activate hormone-sensitive lipase to regulate lipolysis (Lafontan, 2008). In the liver, PKA is involved in glucose production through the activation of glycogenolysis and gluconeogenesis (Han et al., 2020). Furthermore, PKA regulates intracellular cAMP levels (London et al., 2019). cAMP can indirectly enhance the degradation rate of intracellular triglycerides by reducing intracellular concentrations of fatty acids or fatty acyl-CoA (Mooney and Lane, 1981), thus indicating the mechanism through which they affect the RFI of meat-type ducks. cAMP is produced through glycolysis and the TCA cycle, which was also consistent with the significant enrichment of the TCA cycle observed in the KEGG analysis. In addition, *PRKAR2A* plays an important role in inflammatory regulation (Wei et al., 2021). In the present study, *PRKAR2A* and *PRKACB*, as the hub genes interacting with multiple genes in the PPI network, affected the body's lipid metabolism, glucose metabolism, and inflammatory response. Therefore, *PRKAR2A* and *PRKACB* are considered candidate genes that affect RFI.

FYN is a member of the Src family of kinases and is a non-receptor tyrosine kinase, and *FYN* has been found to affect the feed efficiency trait of pigs (Messad et al., 2019). Another study on the pig liver also demonstrated that *FYN* acts as a putative hub molecule to regulate feed efficiency (Reyer et al., 2017). *FYN* plays an essential role in cell growth and proliferation, cell-cycle entry, skeleton remodelling, and cell migration through the Ras–Mek–ERK, FAK, and PI3K signalling pathways (Yin et al., 2018). Transient stimulation of high glucose levels can promote *FYN* phosphorylation (Ma et al., 2017). In addition, *FYN* can enhance PKA kinase activity (Schmoker et al., 2018). *FYN* is considered one of the most important factors for regulating fatty acid utilisation and glucose homeostasis by affecting the activity of AMP-activated protein kinase (AMPK) (Vatish et al., 2009). Furthermore, *FYN* affects carbohydrate metabolism by regulating insulin sensitivity and glucose metabolism (Bastie et al., 2007), which is consistent with our results of significant gene enrichment in pathways related to insulin metabolism in the GSEA analysis.

Paradis et al. reported that LRFI cattle spend less energy with respect to system validation responses and directed nutrients to growth and protein accumulation (Paradis et al., 2015). It was reported that genes related to immune and stimulus responses may play important roles in differentiating the feed efficiency of chickens (Yang et al., 2020). GSEA showed that the DEGs in the HRFI group were mainly enriched in immune- and inflammation-related biological mechanisms. This may be because *PRKAR2A* plays some part in regulating the inflammatory response after being activated (Wei et al., 2021). *FYN* is mechanistically involved in anaphylactic shock, mast cell activation, and TNF-α release (Saminathan et al., 2020).

Conclusion

In the present study, gene ontology and KEGG pathway analyses showed that the differentially expressed genes were mainly involved in signalling pathways related to amino acid metabolism, carbon metabolism, and insulin signalling associated with energy metabolism. Furthermore, *PRKACB*, *PRKAR2A*, and *FYN* can be used as key genes for the augment of feed efficiency traits in meat-type ducks founded on biological function analysis. More research is needed to characterise the molecular basis underlying the effects of these differentially expressed genes on feed efficiency phenotype formation in poultry and possible alterations during their translation to proteins.

Supplementary material

Supplementary Material for this article (https://doi.org/10.1016/j.animal.2025.101521) can be found at the foot of the online page, in the Appendix section.

Ethics approval

All experimental procedures and sample collection were conducted according to the Regulations for the Administration of Affairs Concerning Experimental Animals (Ministry of Science and Technology, China) and were approved by the Institutional Animal Care and Use Committee of the College of Animal Science and Technology of Anhui Agricultural University, China (approval number: SYXK 2016-007).

Data and model availability statement

The raw sequence data generated in this study are publicly available in the NCBI Short Reads Archive (BioProject ID: PRJNA396466). None of the models was deposited in an official repository. This can be made available upon request from the corresponding author.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

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Declaration of interest

None.

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