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Fasting and Refeeding Affect the Goose Liver Transcriptome Mainly Through the PPAR Signaling Pathway

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Nutrition and energy are essential for poultry growth and production performance. Fasting and refeeding have been widely used to study the effects of nutrition, energy, and related mechanisms in chicken. Previous studies have shown that geese have a strong capacity for fat synthesis and storage; thus, changes in the goose liver transcriptome may be different from those in chicken assessed with a model of fasting and refeeding. However, the responses of the goose liver transcriptome to fasting and refeeding have not yet been addressed. In this study, 36 70-day-old Si Ji geese with similar body weight were randomly assigned to three groups: control (*ad libitum* feeding), fasting (fasted for 24 h), and refeeding (fast for 24 h followed by 2-h feeding) groups. After treatment, eight geese per group were sacrificed for sample collection. Liver samples from four geese in each group were subjected to transcriptome analysis, followed by validation of differentially expressed genes (DEGs) using quantitative polymerase chain reaction with the remaining samples. As a result, 155 DEGs (73 up-regulated) were identified between the control and fasting groups, and 651 DEGs (321 up-regulated) were identified between the fasting and refeeding groups. The enrichment analyses of Gene Ontology terms and Kyoto Encyclopedia of Genes and Genomes pathways showed that fasting mainly influenced material metabolism in the liver, especially lipid metabolism; in contrast, refeeding affected not only lipid metabolism but also glucose and amino acid metabolism. In addition, the peroxisome proliferator-activated receptor (PPAR) signaling pathway may play an important role in lipid metabolism. In conclusion, fasting and refeeding have a strong effect on lipid metabolism in the goose liver; specifically, fasting promotes fatty acid oxidation and inhibits fatty acid synthesis, and refeeding has the opposite effect. The model of fasting and refeeding is suitable for goose nutrition studies.

Key words: goose, lipid metabolism, liver, nutrition, transcriptome analysis

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Introduction

The growth and development of animals require a continuous supply of nutrition and energy, and the nutrition and energy needs of animals differ according to different life stages. For example, laying hens need to be fed appropriately during the late stages of laying. A diet that provides more nutrition and energy than needed by hens could lead to the development of fatty liver hemorrhage syndrome. By contrast, geese should be overfed to allow for the rapid development of fatty liver. Evidence indicates that nutritional factors have a strong impact on animal growth, reproduction, and immunity. Many metabolism-related diseases (*e.g.*, diabetes, non-alcoholic fatty liver disease, metabolic syndrome, and other obesity-related metabolic diseases) are also closely related to nutritional and energy problems (Streba *et al*., 2008; American Diabetes Association, 2014; Buzzetti *et al*., 2016). Carbohydrates, proteins, fats, vitamins, minerals, and other nutrients are not only the raw materials for building cells but some of these nutrients and their derivatives can also act as signal molecules (*e.g.*, sphingolipids) to participate in signaling regulation, thus influencing gene expression and cell function.

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Fasting (or starvation) and feeding are two completely different nutritional statuses. Fasting can cause a slump in nutrition and energy in animals, leading to a switch in energy supply from glucose metabolism to fat metabolism. Refeeding has the opposite effect, which can cause a dramatic increase in nutrition and energy, leading to the rapid elevation of blood glucose, and an increase in fat synthesis and storage. Previous studies have indicated that fasting, even for a short period of time, can dramatically reduce the capacity of lipogenesis in the chicken liver, whereas refeeding for 1 h can restore the reduced capacity (Leveille *et al*., 1975). Thus, identifying the drastic changes in nutrition and energy levels caused by fasting and refeeding may facilitate gaining an in-depth understanding of the molecular mechanisms by which nutrition and energy metabolic processes affect animal physiological functions and disease development.

Existing evidence shows that fasting and feeding can modulate gene expression and signal transduction in chickens. For example, Wang *et al*. (2020) showed that fasting activated AMP-activated protein kinase (AMPK), leading to inhibition of glycogen synthase, fatty acid synthase (*FASN*), and sterol regulatory element binding protein 1 (*SREBP1*) expression, and induction of the downstream gene glucose-6-phosphatase catalytic subunit 2 in the liver of 20-day-old chickens. Fujita *et al*. (2018) reported that 6 h of fasting significantly increased the mRNA expression levels of insulin-like growth factor binding protein-1 and 2 in the livers of 8-day-old chickens, which could be reversed by 6 h of refeeding. Li *et al*. (2011) found that fasting for 24 or 48 h induced the mRNA expression of F-box protein 32 and tripartite motif-containing 63 in the skeletal muscle of 7-day-old chicks, and refeeding suppressed this induction. Based on transcriptome analysis, Ji *et al*. (2012) identified 2016 differentially expressed genes (DEGs), including 917 up-regulated and 863 down-regulated genes, in the fasted vs. fed adipose tissue of 17-day-old commercial broiler chickens from three groups (*i.e*., chickens fed *ad libitum*, fasted for 5 h, and those fed but deprived of insulin by injections of anti-insulin serum). These DEGs were enriched in a broad series of metabolism, signaling, and adipogenesis pathways such as those related to sphingolipid metabolism, peroxisome proliferator-activated receptor (PPAR) signaling, fatty acid metabolism, and the peroxisome. Desert *et al*. (2018) performed transcriptome analysis on the livers of 4-week-old broiler chickens that were fasted for 16 or 48 h and those fed *ad libitum*, and identified 1162 DEGs; up-regulated genes in the fasted group were associated with fatty acid oxidation, ketogenesis, and gluconeogenesis, whereas the down-regulated genes were associated with fatty acid and cholesterol synthesis. In contrast to other transcriptomic studies that mainly focused on juvenile chickens (1, 2, or 4 weeks old), Cogburn *et al*. (2020) carried out transcriptome analysis on the livers of newly hatched chicks that were fasted for 4, 24, or 48 h in comparison to those of fully fed chicks. They identified several highly expressed upstream regulators, including those related to the fasting-lipolytic state (*PPARA, NR3C1, NFE2L2, SERTAD2, FOX01, NR0B1,* and *RXR*) and those related to the fully fed lipogenic/thermogenic state (*THRSPA, SREBF2, PPARG, PPARD, JUN, ATF3,* and *CTNNB1*). Accordingly, the downstream genes, including the lipogenic genes (e.g., *FASN, ME1, SCD*) and lipolytic genes (*e.g*., *ALDOB, LDHB, LPIN2*) were down-regulated and up-regulated by fasting, respectively, and these changes could be reversed by refeeding (Cogburn *et al*., 2020). In summary, these studies provided deep insight into the mechanisms by which energy/material metabolism is affected by fasting and feeding in chickens.

Compared to chickens, the effects of fasting and refeeding have barely been investigated in geese. Previous studies have indicated that the goose, as a descendant of a migrant bird, has an excellent capacity to deposit fat in the liver. For example, the goose liver can grow to be 8−10-times heavier (about 800−1200 g) than the normal liver after a short period (3−4 weeks) of artificial overfeeding, and the fat content in this overfed liver can reach up to ~60% (Fournier *et al*., 1997). This unique feature suggests that goose lipid metabolism may differ from that of chicken in some respects. Therefore, this study was designed to investigate the effects of fasting and refeeding on the liver transcriptome of geese, and to screen for DEGs and associated enriched signaling pathways. These findings may provide a foundation for studying the mechanisms by which nutritional/energy levels affect production performance and some metabolism-related diseases (such as fatty liver) in geese.

Materials and Methods

Experimental Animals and Sample Collection

All animal protocols were approved by the Animal Care and Use Committee of Yangzhou University (IACUC certificate number NSFC2020-DKXY-22). Thirty-six healthy 1-day-old Si Ji geese were purchased from Jiangsu Si Ji Goose Breeding Company (Jurong, Jiangsu, China) and raised in Mali Agricultural Ecological Park (Jurong, Jiangsu, China). During the brooding period, the goslings were raised on a net with 24 h lighting per day and the temperature maintained at 28−30℃ with infrared lamps. The goslings had free access to feed and water. The formula for the brooding diet is presented in Supplementary Table 1. At 28 days of age, the geese were raised on a net under natural temperature and lighting conditions until the age of 70 days. The geese also had free access to feed and water. The formula for the growing diet is shown in Supplementary Table 2. At 71 days old, 24 healthy geese with similar body weight were randomly assigned into three groups (eight geese per group): the control group (average body weight 4.18±0.13 kg, *ad libitum* feeding for 24 h from 12 PM to 12 PM), fasting group (average body weight 4.14±0.11 kg, fasted for 24 h from 12 PM to 12 PM), and refeeding group (average body weight 4.13±0.12 kg, fasted for 24 h followed by refeeding for 2 h from 10 AM to 12 PM the next day). All geese were sacrificed within a short period (<15 min) before the liver samples were harvested. Four liver samples from each group were used for the transcriptome sequencing analysis. Eight

Table 1. **Primer sequences for qPCR analysis**

Gene	Forward (5′ −3′)	Reverse (5′ −3′)
ACSBG2	TACCTCCTCCCTCCAGTGTG	GCTTTATCCACTGGCCACCT
ACOX1	AGCCGCTGGATCTTCATCTG	GTGGGGCTGTTGAGGATGAA
CPT1A	GCATTGACCGCCATCTGTTC	GCCAGCATCTCAGGGTTCTT
ACSL5	TTCCTCCCGCTGACTTGAAC	TCCAGAAGCATGCAGTCCTC
ACAA1	AAGCCAGGCTGTGTACTGTG	CCATGCCAGTTCCAATGCAC
CPT1B	CTTCACCCTGCCCACTGTAG	CCCGTGGGTTGATGTTCTGA
EHHADH	ACTCAGCGACTACCCAGACT	TTTGCCAAGCGAATTCCTGC
SCD	CTTCACCCTGCCCACTGTAG	CCCGTGGGTTGATGTTCTGA
PCK1	TTACCCAGGGGGATCTGGAG	AGAGCCAACCAGCAGTTCTC
GAPDH	CTGATGCTCCCATGTTCGTG	CCACGATGCCAAAGTTGTCA
β-actin	GCACCCAGCACGATGAAAAT	GACAATGGAGGGTCCGGATT

liver samples from each group were used for quantitative polymerase chain reaction (qPCR) verification of the expression levels of the DEGs identified by transcriptome analysis.

Extraction and Purification of Total RNA, Reverse Transcription, and qPCR Analysis

Total RNA was extracted from the liver samples using TRIzol (Cat. No. DP424, Tiangen Biotech Co., Ltd., Beijing, China), HiScript TMQ RT Super Mix Reverse Transcription kit (Cat# R123-01, Vazyme Biotech Co., Ltd., Nanjing, China) was used to synthesize the first-strand cDNA, and the Vazyme AceQTM qPCR SYBR Green Master Mix kit (Cat# Q111-02/03, Vazyme Biotech Co., Ltd., Nanjing, China) was used for fluorescence qPCR analysis. All procedures were performed according to the manufacturers' instructions. Using the online software Primer 3.0 (http://bioinfo.ut.ee/primer3-0.4.0/), primers were designed according to the mRNA sequences of genes of interest retrieved from GenBank or from our local database established based on previous sequencing analysis of the goose liver transcriptome. The binding specificity of the primers was checked using the National Center for Biotechnology Information Primer-Blast online program. The glyceraldehyde phosphate dehydrogenase (*GAPDH*) gene and *β*-actin gene were used as internal reference genes. The primer sequences are listed in Table 1.

Transcriptome Sequencing Analysis

The quantity and quality of total RNA samples were analyzed using a Qubit 2.0 fluorometer (ThermoFisher Scientific Inc.) and Agilent 2100 Bioanalyzer (Agilent Technologies), respectively. The cDNA library was subsequently constructed by reverse transcription using qualified total RNA samples. In brief, eukaryotic mRNA samples were enriched with Oligo (dT) bound to magnetic beads, followed by random interruption of mRNA in fragmentation buffer. The first strand of cDNA was then synthesized by reverse transcription with six-base random primers (random hexamers), and the second strand of cDNA was synthesized by adding reaction buffer, dNTPs, RNase H, and DNA polymerase I to the previous solution. cDNA samples were purified using AMPure XP beads. After repairing the ends of cDNA, connecting with sequencing adapters, and selecting the appropriate size of fragments with AMPure XP beads, a cDNA library was constructed by PCR enrichment. The quality of the cDNA library was then checked and sequenced on an Illumina high-throughput sequencing platform (HiSeq X-ten) using sequencing-by-synthesis technology.

Clean data with a quality score of Q30 or more were selected for further analysis according to the calculated Phred score on a large amount of raw data. By aligning against the sequence of the designated reference genome, clean data were annotated using the HISAT2 system. Single genes (*i.e*., unigenes) were assembled by comparing the reads with the StringTie program. By standardizing the reads to units of fragments per kilobase of transcript per million fragments mapped (FPKM), the expression level of a single gene was calculated. On this basis, DEGs were identified by comparing the expression level of each gene between the different groups. The criteria for a DEG were fold change ≥ 2 or ≤ 0.5 and false discovery rate (FDR) <0.05. With the identified DEGs, Gene Ontology (GO) function and enrichment analyses were performed using the Kolmogorov-Smirnov analysis method in the top GO R data package (the standard for significant enrichment was set to a KS-value <0.05). Enrichment analysis of Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways was performed using the KOBAS program (version 2.0). The standard of significant enrichment for KEGG pathway analysis was set as a q-value <0.05.

Statistical Analysis

The results from the qPCR analysis were calculated using the $2^{-\Delta\Delta Ct}$ method. All data are expressed as the mean$\pm$ SEM. One-way analysis of variance in SPSS software (version 16.0) was used to evaluate the statistical significance of the differences among groups, followed by pairwise comparisons using Tukey's post-hoc test. $P<0.05$ and $P<0.01$ were judged as significant and extremely significant differences, respectively.

Results

Identification of DEGs in the Goose Liver Transcriptome

By sequencing the transcriptomes of four liver samples from each group, a total of 98.19 Gb clean data was obtained, and the clean data across the samples reached an average of 6.71 Gb. The Q30 base percentage was above 93.93%, the

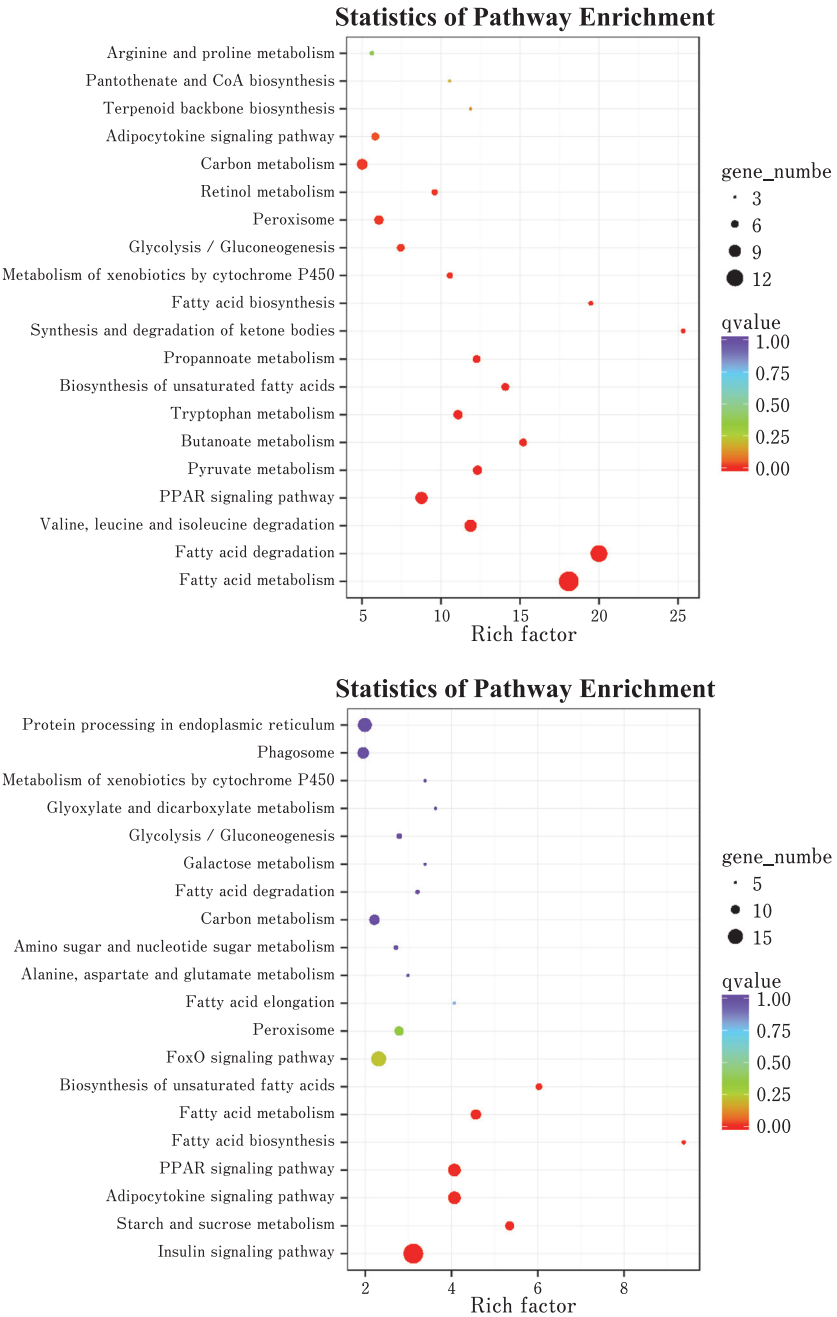

Fig. 1. **The top 20 KEGG pathways enriched with differentially expressed genes identified in the fasting vs. control groups (A) or in the refeeding vs. fasting groups (B) of geese.** The x-axis shows the enrichment factor and the y-axis indicates the associated KEGG pathways. The sizes of the dots reflect the numbers of differentially expressed genes associated with each pathway, and the colors of the dots indicate the q-values; the smaller the q-value, the more significant the enrichment.

mapping efficiency of clean reads to the reference genome was 76.48–81.41% (Supplementary Table 3), and a total of 15,517 genes were annotated (Supplementary Table 4). DEGs were identified by comparing the expression level of each gene between groups. The heat map of all DEGs indicated that the samples for sequencing had good repeatability (Supplementary Fig. 1). Compared with the control group, the fasting group had 155 DEGs (73 up-regulated and 82 down-regulated; Supplementary Table 5). Compared with the fasting group, the refeeding group had 651 DEGs

Table 2. **Top 10 up/down-regulated differentially expressed genes between the fasting vs. control groups**

	Gene	Log_2 fold change	False discovery rate
Up-regulated			
	NRAP	3.73	4.00E-32
	AZIN2	2.47	1.60E-14
	ABHD3	2.75	6.15E-10
	BSN	4.61	5.41E-08
	PCK1	7.24	7.27E-08
	LDHB	1.98	4.12E-06
	HMGCS1	1.88	0.000117
	HMGCS2	1.76	0.000146
	EHHADH	1.58	0.000146
	LOC106032502	1.42	0.000147
Down-regulated			
	LOC106045899	−3.16	1.84E-10
	LOC106044188	−1.77	1.87E-07
	AHSG	−1.83	1.02E-05
	LOC106033760	−1.91	1.28E-05
	LOC106044189	−2.03	7.61E-05
	PCOLCE2	−2.04	0.000106
	SLC51A	−1.52	0.0001246
	GATM	−4.11	0.000146
	HGD	−1.48	0.000150
	ANKH	−1.38	0.000152

Table 3. **Top 10 up/down-regulated differentially expressed genes between the refeeding vs. fasting groups**

	Gene	Log_2 fold change	False discovery rate
Up-regulated			
	GATM	4.79	4.80E-54
	ACSBG2	5.64	1.91E-43
	ANGPTL3	6.94	2.62E-41
	FASN	4.51	9.02E-28
	TCAIM	3.20	6.88E-27
	UGP2	4.78	5.07E-23
	GLDC	2.92	7.48E-23
	ME1	2.77	9.01E-21
	RRBP1	2.81	9.01E-21
	DOK5	2.99	1.18E-20
Down-regulated			
	HMGCL	−6.10	2.34E-80
	CPT1A	−4.86	1.95E-55
	BACH2	−4.02	2.81E-35
	ACSL1	−2.97	5.07E-24
	NDRG1	−3.40	5.70E-23
	ABHD3	−4.33	1.06E-19
	LRR1	−2.63	1.06E-19
	PSME4	−2.56	4.46E-17
	HMGCS1	−3.27	8.98E-17
	PANK1	−2.41	8.98E-17

(321 up-regulated and 330 down-regulated; Supplementary Table 6). Among the DEGs, 94 were common to the fasting and refeeding groups. The 10 most significant (or Top 10) up-regulated and down-regulated DEGs according to the FDR are listed in Tables 2 and 3, respectively.

GO Functional Annotation and Enrichment Analysis

A total of 72 DEGs in the fasting group vs. the control group and 271 DEGs in the refeeding group vs. the fasting group were annotated for GO functional terms. The DEGs enriched in the secondary tier of functional GO categories are

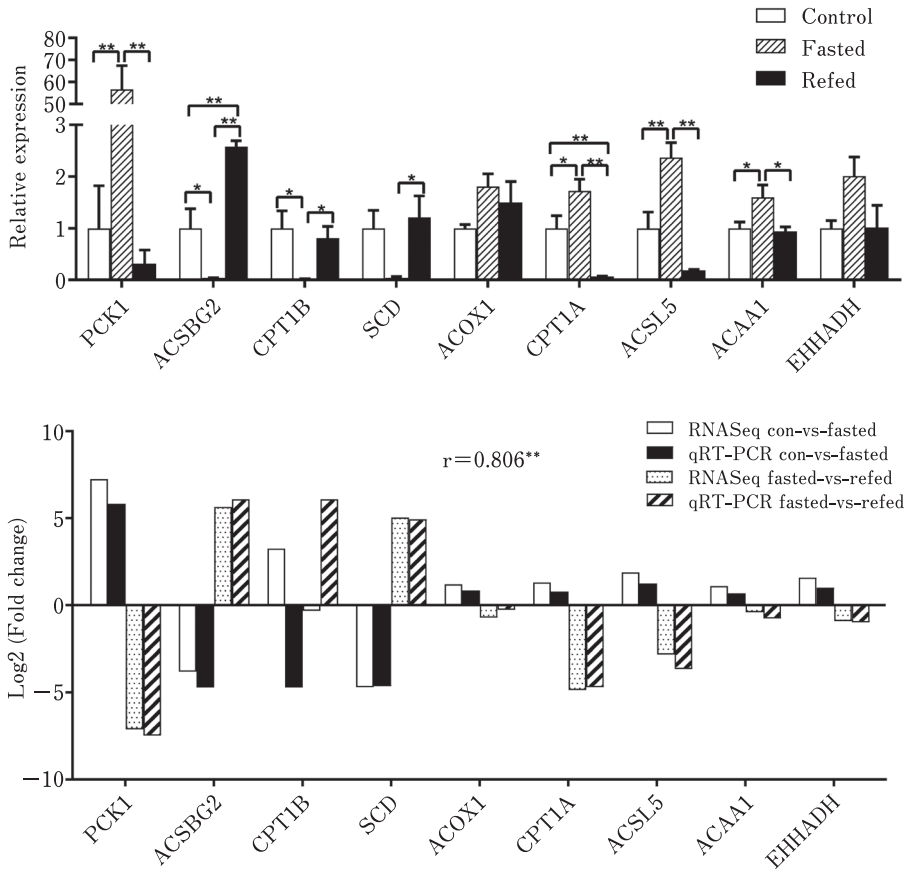

Fig. 2. **Validation of selected differentially expressed genes in the livers of control, fasted, and refed geese determined by quantitative reverse transcription-PCR (qRT-PCR) with *GAPDH* as the reference gene.** (A) The expression level is presented as the fold change relative to the control group ($n=8$); * $p<0.05$ and ** $p<0.01$. The data are expressed as means±SE. (B) Comparisons of gene expression levels between transcriptome analysis and qRT-PCR analysis based on the fold change of gene expression. "r" denotes the Pearson correlation coefficient and ** denotes $p<0.01$.

shown in Supplementary Fig. 2A and Supplementary Fig. 2B. Notably, in the secondary functional tier "metabolic process" of the first-tier biological process category, the enrichment ratio of DEGs (62.5%) in the fasting group vs. the control group was 24.2% higher than that of all annotated genes (38.3%), whereas the enrichment ratio of DEGs (43.9%) in the refeeding group vs. the fasting group was 5.6% higher than that of all annotated genes (38.3%). In the secondary functional tiers "extracellular region" and "extracellular region part" of the first-tier cellular component category, the enrichment ratios of DEGs in the fasting group vs. the control group (12.5% and 9.7%) were 5.4% and 5.1% higher than those of all annotated genes (7.1% and 4.6%), respectively. In contrast, the enrichment ratio of DEGs in the overfeeding group vs. the fasting group had no secondary functional tiers that were at least 5.0% higher than the enrichment ratio of all annotated genes. In the secondary functional tier "catalytic activity" of the first-tier molecular function category, the enrichment ratio of DEGs in the fasting group vs. the control group (59.7%) was 24.9% higher than that of all annotated genes (34.9%). Similarly, the enrichment ratio (45.0%) of DEGs in the overfeeding group vs. the fasting group was 10.2% higher than that of all annotated genes (34.9%).

There were 356 and 361 GO terms between the fasting group and the control group, and between the refeeding group and the fasting group, respectively, with a significant difference detected between the actual and estimated numbers of DEGs (KS <0.05). Among these GO terms, 81 and 131 terms were significantly enriched (*i.e.*, the actual number of DEGs was significantly higher than the estimated number of DEGs; Supplementary Tables 7 and 8). There were 21 DEGs involved in the GO terms that were significantly enriched between the fasting group and the control group, including *HMGCS2*, *PCK1*, *SOD1*, *CPT1A*, *HADHB*, *HADHA*, *LPIN1*, *ACSL5*, *CTGF*, *LIPC*, *ACOX1*, *ABHD2*,

Table 4. **Gene Ontology (GO) terms enriched with differentially expressed genes identified in the fasting vs. control groups**

GO ID	Term	Annotated	Significant	Expected	KS
GO:0006695	cholesterol biosynthetic process	9	1	0.06	0.00105
GO:0004613	phosphoenolpyruvate carboxykinase (GTP) activity	3	1	0.02	0.004
GO:0006641	triglyceride metabolic process	22	3	0.15	0.00798
GO:0019866	organelle inner membrane	83	5	0.4	0.00838
GO:0031966	mitochondrial membrane	100	6	0.48	0.0084
GO:0004784	superoxide dismutase activity	3	1	0.02	0.00908
GO:0034370	triglyceride-rich lipoprotein particle remodeling	3	1	0.02	0.01093
GO:0034372	very-low-density lipoprotein particle remodeling	3	1	0.02	0.01093
GO:0019752	carboxylic acid metabolic process	224	10	1.5	0.01205
GO:0005743	mitochondrial inner membrane	78	5	0.37	0.01445
GO:0042554	superoxide anion generation	7	1	0.05	0.01497
GO:0016509	long-chain-3-hydroxyacyl-CoA dehydrogenase activity	2	2	0.01	0.01893
GO:0003857	3-hydroxyacyl-CoA dehydrogenase activity	2	2	0.01	0.01893
GO:0001676	long-chain fatty acid metabolic process	11	1	0.07	0.01909
GO:0004030	aldehyde dehydrogenase [NAD(P)+] activity	4	1	0.02	0.01937
GO:0043436	oxoacid metabolic process	244	10	1.63	0.02509
GO:2001234	negative regulation of apoptotic signaling pathway	7	1	0.05	0.02703
GO:1903409	reactive oxygen species biosynthetic process	3	1	0.02	0.03007
GO:0050665	hydrogen peroxide biosynthetic process	3	1	0.02	0.03007
GO:0006082	organic acid metabolic process	247	10	1.65	0.03072
GO:0071450	cellular response to oxygen radical	9	1	0.06	0.03454
GO:0071451	cellular response to superoxide	9	1	0.06	0.03454
GO:0019430	removal of superoxide radicals	9	1	0.06	0.03454
GO:0006631	fatty acid metabolic process	76	6	0.51	0.03477
GO:0006848	pyruvate transport	2	1	0.01	0.03929
GO:0006850	mitochondrial pyruvate transport	2	1	0.01	0.03929
GO:0005741	mitochondrial outer membrane	19	2	0.09	0.04417
GO:0000303	response to superoxide	11	1	0.07	0.0442
GO:0000305	response to oxygen radical	11	1	0.07	0.0442
GO:0016406	carnitine O-acyltransferase activity	2	1	0.01	0.04471
GO:0032787	monocarboxylic acid metabolic process	115	9	0.77	0.04516

Note: "Annotated" denotes the number of genes annotated to the GO term; "Significant" denotes the number of differentially expressed genes (DEGs) annotated to the GO term; "Expected" denotes the expected number of DEGs annotated to the GO term; "KS" denotes the statistical significance of the enrichment of the GO term—the smaller the KS value, the more significant the enrichment.

PDK3, *LDHB*, and *SLC25A33*. There were 66 DEGs involved in GO terms that were significantly enriched between the refeeding and fasting groups, including *PANK1*, *HMGCS2*, *PCK1*, *HSD17B11*, *SOD1*, *ACACA*, *SLC25A33*, *CPT1A*, *FASN*, *HADHB*, *HADHA*, *ACOX1*, *LIPC*, *ACSL5*, *ME1*, and *LDHB*. In the comparison of the fasting group and the control group, 31 of the significantly enriched GO terms were related to lipid metabolism (*e.g*., cholesterol biosynthetic process, triglyceride metabolic process, fatty acid metabolic process, very-low-density lipoprotein particle remodeling, 3-hydroxyacyl-CoA dehydrogenase activity), mitochondria (*e.g*., mitochondrial inner membrane, mitochondrial outer membrane, mitochondrial pyruvate transport), and oxidative stress (*e.g*., superoxide dismutase activity and superoxide anion generation) (Table 4). In the comparison of the refeeding and fasting groups, 30 significantly enriched GO terms were also related to lipid metabolism, mitochondria, and oxidative stress (Table 5). These GO terms were similar to those identified in the comparison of the fasting and control groups.

Enrichment Analysis of KEGG Pathways

A total of 70 DEGs between the control group and the fasting group were enriched in 29 KEGG pathways (Supplementary Table 9). The top 20 KEGG pathways with the highest enrichment significance (Fig. 1A) and the DEGs involved in these pathways are listed in Table 6. Among the pathways, there were at least seven pathways closely related to fat metabolism, including fatty acid metabolism (the ratio of the number of DEGs enriched in the KEGG pathway to the number of all DEGs was 20.0%), fatty acid degradation (17.14%), PPAR signaling pathway (12.86%), biosynthesis of unsaturated fatty acids (8.57%), synthesis and degradation of ketone bodies (5.71%), fatty acid biosynthesis (5.71%), and adipocytokine signaling pathway (8.57%). The DEGs involved in these pathways included *EHHADH*, *PCK1*, *CPT1A*, *CPT1B*, *ACSL5*, *ACOX1*, *ACAA1*, *SCD*, and *ACSBG2*. There were at least three pathways closely related to amino acid/protein metabolism, including valine, leucine and isoleucine degradation (12.86%); tryptophan metabolism (10%); and arginine and proline metabolism (5.71%). The

Table 5. **Gene Ontology (GO) terms enriched with differentially expressed genes identified in the refeeding vs. fasting groups**

GO_ID	Term	Annotated	Significant	Expected	KS
GO:0006695	cholesterol biosynthetic process	9	2	0.12	0.00117
GO:0004613	phosphoenolpyruvate carboxykinase (GTP) activity	3	1	0.04	0.00431
GO:0019674	NAD metabolic process	9	1	0.12	0.00541
GO:0032787	monocarboxylic acid metabolic process	115	14	1.51	0.00772
GO:0006641	triglyceride metabolic process	22	4	0.29	0.00944
GO:0004784	superoxide dismutase activity	3	1	0.04	0.00968
GO:0019866	organelle inner membrane	83	5	0.85	0.01082
GO:0031966	mitochondrial membrane	100	6	1.02	0.01113
GO:0034370	triglyceride-rich lipoprotein particle remodeling	3	1	0.04	0.01161
GO:0034372	very-low-density lipoprotein particle remodeling	3	1	0.04	0.01161
GO:0005740	mitochondrial envelope	106	6	1.08	0.01403
GO:0005743	mitochondrial inner membrane	78	5	0.8	0.01824
GO:0016509	long-chain-3-hydroxyacyl-CoA dehydrogenase activity	2	2	0.03	0.01956
GO:0003857	3-hydroxyacyl-CoA dehydrogenase activity	2	2	0.03	0.01956
GO:0004030	aldehyde dehydrogenase [NAD(P)+] activity	4	1	0.05	0.02085
GO:0001676	long-chain fatty acid metabolic process	11	1	0.14	0.02126
GO:0006631	fatty acid metabolic process	76	8	1	0.02465
GO:0004022	alcohol dehydrogenase (NAD) activity	2	1	0.03	0.02739
GO:0039531	regulation of viral-induced cytoplasmic pattern recognition receptor signaling pathway	2	1	0.03	0.02758
GO:2001234	negative regulation of apoptotic signaling pathway	7	1	0.09	0.0279
GO:0003824	catalytic activity	2076	43	27.02	0.02951
GO:1903409	reactive oxygen species biosynthetic process	3	1	0.04	0.03145
GO:0050665	hydrogen peroxide biosynthetic process	3	1	0.04	0.03145
GO:0009743	response to carbohydrate	12	1	0.16	0.03298
GO:0010657	muscle cell apoptotic process	5	1	0.07	0.03472
GO:0006090	pyruvate metabolic process	24	5	0.32	0.03551
GO:0016616	oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor	38	7	0.49	0.03586
GO:0019752	carboxylic acid metabolic process	224	18	2.94	0.03609
GO:0071450	cellular response to oxygen radical	9	1	0.12	0.03778
GO:0071451	cellular response to superoxide	9	1	0.12	0.03778
GO:0019430	removal of superoxide radicals	9	1	0.12	0.03778
GO:0016614	oxidoreductase activity, acting on CH-OH group of donors	41	7	0.53	0.03798
GO:0090207	regulation of triglyceride metabolic process	9	1	0.12	0.03804
GO:0055114	oxidation-reduction process	177	14	2.33	0.03978
GO:0006848	pyruvate transport	2	1	0.03	0.04096
GO:0006850	mitochondrial pyruvate transport	2	1	0.03	0.04096
GO:0090181	regulation of cholesterol metabolic process	5	2	0.07	0.04249
GO:0009746	response to hexose	11	1	0.14	0.04386
GO:0009749	response to glucose	11	1	0.14	0.04386
GO:0034284	response to monosaccharide	11	1	0.14	0.04386
GO:0044429	mitochondrial part	130	6	1.33	0.04525
GO:0033158	regulation of protein import into nucleus, translocation	3	1	0.04	0.04557
GO:0004320	oleoyl-[acyl-carrier-protein] hydrolase activity	2	1	0.03	0.04657
GO:0016295	myristoyl-[acyl-carrier-protein] hydrolase activity	2	1	0.03	0.04657
GO:0016296	palmitoyl-[acyl-carrier-protein] hydrolase activity	2	1	0.03	0.04657
GO:0016297	acyl-[acyl-carrier-protein] hydrolase activity	2	1	0.03	0.04657
GO:0016406	carnitine O-acyltransferase activity	2	1	0.03	0.04664
GO:0006570	tyrosine metabolic process	5	1	0.07	0.04676
GO:0000303	response to superoxide	11	1	0.14	0.04792
GO:0000305	response to oxygen radical	11	1	0.14	0.04792
GO:0005741	mitochondrial outer membrane	19	2	0.19	0.04875

Note: “Annotated” denotes the number of genes annotated to the GO term; “Significant” denotes the number of differentially expressed genes (DEGs) annotated to the GO term; “Expected” denotes the expected number of DEGs annotated to the GO term; “KS” denotes the statistical significance of the enrichment of the GO term—the smaller the KS value, the more significant the enrichment.

Table 6. **Top 20 Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways enriched with differentially expressed genes (DEGs) identified in the fasting vs. control groups**

KEGG pathway	Up-regulated DEGs	Down-regulated DEGs	P-value
Fatty acid metabolism	*EHHADH, CPT1A, PECR, ACAT1, CPT1B, HADHB, HADHA, ACSL5, ACOX1, ACAA1*	*ACACA, SCD, FASN, ACSBG2*	0
Fatty acid degradation	*EHHADH, CPT1A, ACAT1, CPT1B, HADHB, HADHA, ACSL5, ACOX1, ACAA1*	*ACSBG2, LOC106029566*	2.28E-13
Valine, leucine and isoleucine degradation	*EHHADH, HMGCS2, ACAT1, HADHB, HADHA, HMGCS1, ACAA1, HMGCL*		3.71E-08
PPAR signaling pathway	*EHHADH, PCK1, CPT1A, CPT1B, ACSL5, ACOX1, ACAA1*	*SCD, ACSBG2*	5.72E-07
Pyruvate metabolism	*PCK1, ACAT1, LDHB*	*ACACA, ACSS2, ME1*	1.05E-06
Butanoate metabolism	*EHHADH, HMGCS2, ACAT1, HADHA, HMGCS1, HMGCL*		1.75E-06
Tryptophan metabolism	*HHADH, ACAT1, HADHA*	*LOC106044188, LOC106044189, AFMID*	2.23E-06
Biosynthesis of unsaturated fatty acids	*LOC106040702, PECR, HADHA, ACOX1, ACAA1*	*SCD*	2.85E-06
Propanoate metabolism	*EHHADH, ACAT1, HADHA, LDHB*	*ACACA, ACSS2*	6.74E-06
Synthesis and degradation of ketone bodies	*HMGCS2, ACAT1, HMGCS1, HMGCL*		1.12E-05
Fatty acid biosynthesis	*ACSL5*	*ACACA, ACSBG2, FASN*	3.67E-05
Metabolism of xenobiotics by cytochrome P450		*LOC106044188, LOC106044189, LOC106029566, LOC106030435, LOC106030436*	8.92E-05
Glycolysis/Gluconeogenesis	*PCK1, LDHB*	*ACSS2, LOC106029566, LOC106030908*	0.000129
Peroxisome	*EHHADH, SOD1, PECR, ACSL5, ACOX1, ACAA, HMGCL*		0.000129
Retinol metabolism		*LOC106044188, LOC106044189, LOC106049006, LOC106049007, LOC106029566*	0.000143
Carbon metabolism	*EHHADH, ACAT1, HADHA*	*PGD, ACSS2, LOC106030908, ME1*	0.000162
Adipocytokine signaling pathway	*IRS4, PCK1, CPT1A, CPT1B, ACSL5*	*ACSBG2*	0.000495
Terpenoid backbone biosynthesis	*HMGCS2, ACAT1, HMGCS1*		0.001824
Pantothenate and CoA biosynthesis	*PANK1, LOC106032502, LOC106032503*		0.002598
Arginine and proline metabolism	*PYCR1, AZIN2*	*GATM*	0.005221

DEGs involved in these pathways included *STC2* and *AZIN2*. There were at least two pathways closely related to glucose metabolism, including pyruvate metabolism (10%) and glycolysis/gluconeogenesis (8.75%), and the DEGs involved in these pathways included *PCK1*, *ACAT1*, *LDHB*, *ACSS2*, and *ME1*.

Similarly, 218 DEGs between the fasting and refeeding groups were enriched in 31 pathways (Supplementary Table 10). The top 20 KEGG pathways with the highest enrichment significance (Fig. 1B) and the associated DEGs are listed in Table 7. Among these pathways, at least seven pathways were closely related to fat metabolism, including the adipocytokine signaling pathway (5.96%), PPAR signaling pathway (5.96%), fatty acid metabolism (5.05%), fatty acid biosynthesis (2.75%), biosynthesis of unsaturated fatty acids (3.67%), fatty acid elongation (2.29%), and fatty acid degradation (2.75%). The DEGs involved in these pathways included *ACSBG2*, *IRS1*, *SCD*, *FABP7*, *ACACA*, *FASN*, *ELOVL5*, *IRS4*, *PCK1*, *CPT1A*, *G6PC*, *ACACB*, *ACSL5*, *PPARGC1A*, *ACSL1*, *PRKAG2*, and *AKT1*. At least six pathways were closely related to glucose metabolism, including the insulin signaling pathway (8.72%), starch and sucrose metabolism (4.59%), FoxO signaling pathway (6.88%), amino sugar and nucleotide sugar metabolism (2.75%), galactose metabolism (2.29%), and glycolysis/gluconeogenesis (3.21%). The DEGs involved in these pathways included *PPP1R3C*, *CRK*, *PIK3R1*, *ACACA*, *GINS4*, *FASN*, *IRS1*, *PGM2*, *UGDH*, *GBE1*, *UGP2*, *CCNB1*, *SGK1*, *IGF1*, *GFPT1*, *PCK1*, *G6PC*, *PGM3*, *IRS4*, *ACACB*, *PPARGC1A*, *PRKAG2*, *AKT1*, and *GYS2*. There were at least three pathways closely related to amino acid/protein metabolism, including alanine, aspartate, and glutamate metabolism (2.29%) and protein processing in the endoplasmic reticulum (6.42%). The DEGs involved in these pathways included *STC2*, *HGD*, *AFMID*, *TGM2*, *SH3RF1*, *LOC106032502*, and *AZIN2*. The critical pathways with differences in the control vs. fasting and fasting vs. refeeding comparisons (selected from the top 20 KEGG pathways) are listed in Supplementary Table 11.

In addition, fluorescent qPCR with *GAPDH* and β-actin as internal reference genes was used to verify the differential

Table 7. **Top 20 Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways enriched with differentially expressed genes (DEGs) identified in the refeeding vs. fasting groups**

KEGG pathway	Up-regulated DEGs	Down-regulated DEGs	P-value
Insulin signaling pathway	*PPP1R3C, CRK, PIK3R1, ACACA, GINS4, FASN, LOC106030908, SHC4, IRS1*	*IRS4, PCK1, G6PC, LOC106029669, ACACB, PPARGC1A, PRKAG2, AKT1, GYS2*	7.32E-06
Starch and sucrose metabolism	*PGM2, UGDH, GBE1, UGP2, LOC106030908*	*AGL, G6PC, LOC106029669, ENPP1, GYS2*	9.47E-06
Adipocytokine signaling pathway	*ACSBG2, IRS1*	*IRS4, PCK1, CPT1A, G6PC, LOC106029669, ACACB, ACSL5, PPARGC1A, ACSL1, PRKAG2, AKT1*	1.16E-05
PPAR signaling pathway	*LOC106039033, SCD, ACSBG2, FABP7*	*PCK1, GK, CPT1A, PLIN2, ACSL5, ACOX3, ACSL1, PLIN1*	1.16E-05
Fatty acid biosynthesis	*ACACA, FASN, ACSBG2*	*ACACB, ACSL5, ACSL1*	1.70E-05
Fatty acid metabolism	*ACACA, SCD, ACSBG2, ELOVL5, FASN*	*ELOVL2, CPT1A, PECR, ACSL5, ACOX3, ACSL1*	1.76E-05
Biosynthesis of unsaturated fatty acids	*SCD, LOC106048282, ELOVL5*	*ELOVL2, LOC106040702, LOC106040707, PECR, ACOX3*	2.97E-05
FoxO signaling pathway	*CCNB1, SGK1, IGF1, IRS1*	*IRS4, EGF, LOC106040363, PCK1, G6PC, LOC106029669, FBXO25, PRKAG2, AKT1, FBXO32*	0.001866
Peroxisome	*LOC106048282, --, PRDX1*	*PECR, CRAT, ACSL5, ACOX3ACSL1, PEX11A, HMGCL*	0.002743
Fatty acid elongation	*ELOVL5*	*ELOVL2, LOC106040702LOC106040707, ELOVL1*	0.006513
Alanine, aspartate and glutamate metabolism	*GPT2, ABAT, GFPT1, GLUL, LOC106037044*		0.023846
Amino sugar and nucleotide sugar metabolism	*PGM2, UGDH, UGP2, GFPT1, LOC106030908*	*PGM3*	0.021594
Carbon metabolism	*PGK1, GPT2, PGD, MDH1, GLDC, TALDO1, CS, G6PD, LOC106030908, ME1*	*gene12631*	0.010117
Fatty acid degradation	*ACSBG2, LOC106029566*	*CPT1A, ACSL5, ACOX3, ACSL1*	0.009729
Galactose metabolism	*PGM2, UGP2, LOC106030908*	*G6PC, LOC106029669*	0.014311
Glycolysis / Gluconeogenesis	*PGK1, PGM2, LOC106029566, LOC106030908*	*PCK1, G6PC, LOC106029669*	0.014311
Glyoxylate and dicarboxylate metabolism	*MDH1, GLDC, GLUL, CS, LOC106037044*		0.010688
Metabolism of xenobiotics by cytochrome P450	*LOC106044188, LOC106044189, LOC106029566, LOC106030436, LOC106030438*		0.014311
Phagosome	*LOC106045884, LOC106029513, CANX, TUBB4B, SEC61A1*	*CTSL, ITGB5, PIKFYVE, SFTPD*	0.019045
Protein processing in endoplasmic reticulum	*HYOU1, HSPA5, RRBP1, ERO1B, PDIA4, LOC106029513, PDIA6, DERL3, CANX, HSP90B1, VIMP, SEC61A1*	*EIF2AK3, UBE2J1*	0.009977

expression of nine DEGs involved in lipid metabolism identified from the transcriptome analysis, with a focus on those involved in the PPAR signaling pathway: *ACSBG2*, *ACOX1*, *CPT1A*, *ACSL5*, *ACAA1*, *CPT1B*, *EHHADH*, *SCD*, and *PCK1*. Among these nine genes, the qPCR results showed that the trends in the expression of eight DEGs were consistent with the results from the transcriptome sequencing analysis, with only an inconsistent trend found in the expression of *CPT1B* (Fig. 2A, 2B, Supplementary Fig. 3). These results indicated the overall good reliability of the transcriptome sequencing analysis.

Discussion

The liver is an important organ for the metabolism and distribution of nutrition in animals, and its transcriptome can be easily altered by variations in nutrition and energy sources. The liver serves as the main site for fatty acid synthesis in avian species as opposed to the adipose tissue playing this role in mammals (Goodridge, 1968; O'Hea and Leveille, 1969; Yeh and Leveille, 1973); thus, the avian liver may play a more important role in lipid metabolism. In mammals, lipid metabolism is regulated by PPARα, PPARγ, SREBP1, SREBP2, and NR1H3, which are critical transcrip-

tion factors for the regulation of fatty acid beta-oxidation, fatty acid synthesis, and cholesterol metabolism (Shchelkunova *et al*., 2013). Similarly, previous studies have indicated that PPARα plays a key role in the up-regulation of fatty acid beta-oxidation, whereas PPARγ is the key regulator of fatty acid synthesis in the chicken (Désert *et al*., 2008; Navidshad and Royan, 2015). Consistently, Désert *et al*. (2008) identified a potential PPARα response element located in *ACOX1, CPT1,* and *HMGCS2*, and the mRNA expression levels of *ACOX1* and *CPT1* were significantly associated with that of *PPARα.* Moreover, SREBP1 is an important transcription factor involved in lipogenesis, whereas SREBP2 regulates cholesterol synthesis. Désert *et al*. (2008) also observed a significant correlation between *SREBP1* mRNA levels and those of its putative target genes *ACLY, ACACA, FASN*, and *SCD*, which are known to be involved in lipogenesis. Compared to that in the chicken, there has been much less research focused on lipid metabolism in the goose liver. Considering the unique features of lipid metabolism in geese, it is uncertain whether these critical transcription factors also play an important role in the regulation of fatty acid beta-oxidation, fatty acid synthesis, and cholesterol metabolism in the goose liver.

Experimental chicken models of fasting and refeeding are widely used in research. We also used these models to identify the genes and pathways that were affected by fasting and refeeding in the livers of 70-day-old geese. When the geese were fasted for 24 h, the changes in the liver transcriptome were mainly reflected in metabolic processes, especially in the pathways related to fat metabolism such as fatty acid degradation, the PPAR signaling pathway, biosynthesis of unsaturated fatty acids, and the synthesis and degradation of ketone bodies. The changes in these pathways were reflected by the up-regulated expression of *CPT1A*, *ACAT1*, *CPT1B*, *HADHB*, *HADHA*, *ACSL5*, *ACOX1*, *ACAA1*, and *PCK1,* and the down-regulated expression of *SCD*, *ACACA*, and *FASN*. This indicates an increase in fatty acid degradation and ketone body production in the goose liver during fasting. Among these pathways, the PPAR and SREBP signaling pathways may play an important role in the response of the goose liver to fasting and refeeding. This is based on the finding of induced expression of *CPT1A*, *CPT1B*, *ACSL5*, and *ACOX1*, the downstream genes of PPARα, whereas the expression of *SCD*, *ACACA*, and *FASN*, the downstream genes of SREBP, was inhibited by fasting, consistent with previous reports (Brandt *et al*., 1998; Yeon *et al*., 2004; Morais *et al*., 2007; Rakhshandehroo *et al*., 2007). Moreover, fatty acid oxidation is carried out in the mitochondrion, as the main site for the production of reactive oxygen species. Therefore, it is reasonable that the GO terms enriched with DEGs were not only related to fatty acid metabolism but also to mitochondria (*e.g*., mitochondrial inner membrane, mitochondrial outer membrane, mitochondrial pyruvate transport) and oxidative stress (*e.g*., superoxide dismutase activity and superoxide anion generation). The oxidative stress-related genes that were differentially expressed in a fasting condition included *MPC1*, *SOD1*, *CPT1A*, *HADHB*, *HADHA*, and *ACSL5*. In addition, GO terms that regulate fat metabolism (*e.g*., cholesterol biosynthetic process) were enriched. Although the changes in the liver transcriptome in response to fasting were mainly related to fat metabolism, there were also some enriched pathways or GO terms related to amino acid, protein, and carbohydrate metabolism. Fat metabolism, protein metabolism, and carbohydrate metabolism all involve a series of enzyme catalytic reactions, which may explain why the DEGs were largely enriched in the secondary-tier GO function category "catalytic activity."

When an animal is refed for a period of time after fasting, the glucose level in the blood rises sharply, leading to the release of insulin, which in turn promotes the absorption of glucose by the liver and accelerates glucose conversion into fatty acids, most of which are transported to the adipose tissue for storage. In this study, the comparison between the refeeding and fasting groups indicated that the changes in the liver transcriptome were not only reflected in fat metabolism but also in the pathways related to carbohydrate metabolism. The majority of the fat metabolism-related pathways that were enriched after refeeding were those involved in fatty acid synthesis (*e.g*., fatty acid elongation), with fewer enriched pathways involved in the synthesis and degradation of ketone bodies, indicating a decrease in fatty acid oxidation and an increase in synthesis. The DEGs involved in these pathways included the down-regulated genes *CPT1A*, *ACSL5*, *ACOX3*, and *ACSL1*, as well as the up-regulated genes *FASN*, *ACACA*, and *SCD*. Consistently, the enriched GO terms included cholesterol biosynthetic process, triglyceride metabolic process, fatty acid metabolic process, very-low-density lipoprotein particle remodeling, and 3-hydroxyacyl-CoA dehydrogenase activity, all of which are related to the synthesis of fatty acids. In addition, GO terms that are associated with the regulation of fat metabolism (*e.g*., cholesterol biosynthetic process, regulation of cholesterol metabolic process) were also enriched after refeeding. Among the pathways related to carbohydrate metabolism, the insulin signaling pathway and FoxO signaling pathway may play important roles, characterized by the up-regulated genes *PPP1R3C*, *CRK*, *PIK3R1*, *IRS1*, *SGK1*, and *IGF1*, indicating that carbohydrate synthesis was significantly enhanced by refeeding.

It is interesting that the DEGs and enriched pathways identified in the refeeding vs. fasting model comparison partially overlapped with those identified in a previous goose overfeeding vs. normal feeding model comparison (Liu *et al*., 2016), especially those related to the metabolism of carbohydrates, lipids, and amino acids. However, the expression pattern of the DEGs identified in the refeeding vs. fasting and the overfeeding vs. normal-feeding comparisons was in contrast to that revealed by the fasting vs. normal-feeding model comparison. For example, overfeeding promoted fatty acid synthesis (as indicated by the up-regulated expression of *GPI, PDH, CS, ACLY, ACC, FASN, SCD,* and *DGAT2*) and suppressed lipid packing and release (as indicated by the down-regulated expression of *APOB26* and

LPL27), whereas fasting promoted fatty acid oxidation and suppressed fatty acid synthesis as mentioned above. In addition, there are some striking differences between the overfeeding model and the fasting and refeeding models. In particular, in the later stage of overfeeding, the liver transcriptome was characterized by DEGs that were mainly enriched in the "cell growth and death" and "immune diseases" pathways, which were not revealed by the fasting and refeeding model. Overfeeding can induce dramatic growth of the goose liver, which leads to a fatty liver weight (about 800−1200 g) that is 10-times heavier than the normal liver (approximately 80−120 g). Overfeeding can also cause severe steatosis (about 60% fat in the liver) without any overt pathological symptoms, which suggests that geese may have a protective mechanism for preventing severe steatosis-associated inflammation and injury from a fatty liver. Therefore, the differences between the models are likely due to the dramatic cell growth, accompanied by suppressed inflammation and injury in the goose fatty liver. Indeed, the unique finding revealed by the overfeeding model was that complement genes, the key components of the immune response and inflammation, were entirely suppressed in the goose fatty liver compared with the normal liver.

In summary, when a goose is fasted, the response of the liver transcriptome is mainly related to fat metabolism; that is, fasting promotes fatty acid oxidation and ketone body synthesis, and inhibits fatty acid and cholesterol synthesis. When the goose is refed, the response of the liver transcriptome is not only related to fat metabolism (*i.e.*, refeeding promotes fatty acid and cholesterol synthesis, and inhibits fatty acid oxidation and ketone body synthesis) but is also related to carbohydrate metabolism. These results are consistent with those of previous studies, indicating the feasibility of the fasting and refeeding model in goose nutritional studies. In addition, this study revealed the response of the goose liver transcriptome to drastic changes in nutritional status, and obtained a batch of DEGs with enriched GO terms and KEGG pathways. These data can therefore provide a good foundation for further investigations of the mechanism underlying the regulation of nutrition on animal growth and development, production performance, and disease occurrence.

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Conflict of Interest

The authors declare no conflict of interest.

References

American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care, 37: S81−90. 2014.

Brandt JM, Djouadi F and Kelly DP. Fatty acids activate transcription of the muscle carnitine palmitoyltransferase I gene in cardiac myocytes via the peroxisome proliferator-activated receptor alpha. Journal of Biological Chemistry, 273: 23786−23792. 1998.

Buzzetti E, Pinzani M and Tsochatzis EA. The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). Metabolism, 65: 1038−1048. 2016.

Cogburn LA, Trakooljul N, Wang X, Ellestad LE and Porter TE. Transcriptome analyses of liver in newly-hatched chicks during the metabolic perturbation of fasting and re-feeding reveals THRSPA as the key lipogenic transcription factor. BMC Genomics, 21: 109. 2020.

Desert C, Baéza E, Aite M, Boutin M, Cam AL, Montfort J, Houee-Bigot M, Blum Y, Roux PF, Hennequet-Antier C, Berri C, Metayer-Coustard S, Collin A, Allais S, E. Bihan L, Causeur D, Gondret F, Duclos MJ and Lagarrigue S. Multi-tissue transcriptomic study reveals the main role of liver in the chicken adaptive response to a switch in dietary energy source through the transcriptional regulation of lipogenesis. BMC Genomics, 19: 187. 2018.

Désert C, Duclos MJ, Blavy P, Lecerf F, Moreews F, Klopp C, Aubry M, Herault F, Roy PL, Berri C, Douaire M, Diot C and Lagarrigue S. Transcriptome profiling of the feeding-to-fasting transition in chicken liver. BMC Genomics, 9: 611. 2008.

Fournier E, Peresson R, Guy G and Hermier D. Relationships between storage and secretion of hepatic lipids in two breeds of geese with different susceptibility to liver steatosis. Poultry Science, 76: 599−607. 1997.

Fujita S, Yamaguchi M, Hiramoto D, Saneyasu T, Honda K and Kamisoyama H. Effects of fasting and refeeding on the mRNA levels of insulin-like growth factor-binding proteins in chick liver and brain. Journal of Poultry Science, 55: 269−273. 2018.

Goodridge AG. Metabolism of glucose-U-14C in vitro in adipose tissue from embryonic and growing chicks. American Journal of Physiology, 214: 897−901. 1968.

Ji B, Ernest B, Gooding JR, Das S, Saxton AM, Simon J, Dupont J, Métayer-Coustard S, Campagna SR and Voy BH. Transcriptomic and metabolomic profiling of chicken adipose tissue in response to insulin neutralization and fasting. BMC Genomics, 13: 441. 2012.

Leveille GA, Romsos DR, Yeh Y and O'Hea EK. Lipid biosynthesis in the chick. A consideration of site of synthesis, influence of diet and possible regulatory mechanisms. Poultry Science, 54: 1075−1093. 1975.

Li Q, Li J, Lan H, Wang N, Hu X, Chen L and Li N. Effects of fasting and refeeding on expression of MAFbx and MuRF1 in chick skeletal muscle. Science China-Life Sciences, 54: 904−907. 2011.

Liu L, Zhao X, Wang Q, Sun X, Xia L, Wang Q, Yang B, Zhang Y, Montgomery S, Meng H, Geng T and Gong D. Prosteatotic and Protective Components in a Unique Model of Fatty Liver: Gut Microbiota and Suppressed Complement System. Scientific Reports, 6: 31763. 2016.

Morais S, Knoll-Gellida A, Andre M, Barthe C and Babin PJ. Conserved expression of alternative splicing variants of peroxisomal acyl-CoA oxidase 1 in vertebrates and developmental and nutritional regulation in fish. Physiological Genomics, 28: 239−252. 2007.

Navidshad B and Royan M. Ligands and regulatory modes of peroxisome proliferator-activated receptor gamma (PPARγ) in avians. Critical Reviews in Eukaryotic Gene Expression, 25:

287−292. 2015.
O'Hea EK and Leveille GA. Lipid biosynthesis and transport in the domestic chick (Gallus domesticus). Comparative Biochemistry and Physiology, 30: 149−159. 1969.
Rakhshandehroo M, Sanderson LM, Matilainen M, Stienstra R, Carlberg C, Groot PJ, Muller M and Kersten S. Comprehensive analysis of PPARalpha-dependent regulation of hepatic lipid metabolism by expression profiling. PPAR Research, 2007: 26839. 2007.
Shchelkunova TA, Morozov IA, Rubtsov PM, Bobryshev YV, Sobenin IA, Orekhov AN, Andrianova IV and Smirnov AN. Lipid regulators during atherogenesis: expression of LXR, PPAR, and SREBP mRNA in the human aorta. PLoS One, 8: e63374. 2013.
Streba LA, Carstea D, Mitrut P, Vere CC, Dragomir N and Streba CT. Nonalcoholic fatty liver disease and metabolic syndrome: a concise review. Romanian Journal of Morphology and Embryology, 49: 13−20. 2008.
Wang Y, Buyse J, Courousse N, Tesseraud S, Métayer-Coustard S, Berri C, Schallier S, Everaert N and Collin A. Effects of sex and fasting/refeeding on hepatic AMPK signaling in chickens (Gallus gallus). Comparative Biochemistry and Physiology A-Molecular and Integrative Physiology, 240: 110606. 2020.
Yeh SJ and Leveille GA. Significance of skin as a site of fatty acid and cholesterol synthesis in the chick. Proceedings of the Society for Experimental Biology and Medicine, 142: 115−119. 1973.
Yeon JE, Choi KM, Baik SH, Kim KO, Lim HJ, Park KH, Kim JY, Park JJ, Kim JS, Bak YT, Byun KS and Lee CH. Reduced expression of peroxisome proliferator-activated receptor-alpha may have an important role in the development of non-alcoholic fatty liver disease. Journal of Gastroenterology and Hepatology, 19: 799−804. 2004.