Gene expression profiling reveals candidate genes related to residual feed intake in duodenum of laying ducks^{1,2}

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ABSTRACT: Feed represents two-thirds of the total costs of poultry production, especially in developing countries. Improvement in feed efficiency would reduce the amount of feed required for production (growth or laying), the production cost, and the amount of nitrogenous waste. The most commonly used measures for feed efficiency are feed conversion ratio (FCR) and residual feed intake (RFI). As a more suitable indicator assessing feed efficiency, RFI is defined as the difference between observed and expected feed intake based on maintenance and growth or laying. However, the genetic and biological mechanisms regulating RFI are largely unknown. Identifying molecular mechanisms explaining divergence in RFI in laying ducks would lead to the development of early detection methods for the selection of more efficient breeding poultry.

The objective of this study was to identify duodenum genes and pathways through transcriptional profiling in 2 extreme RFI phenotypes (HRFI and LRFI) of the duck population. Phenotypic aspects of feed efficiency showed that RFI was strongly positive with FCR and feed intake (FI). Transcriptomic analysis identified 35 differentially expressed genes between LRFI and HRFI ducks. These genes play an important role in metabolism, digestibility, secretion, and innate immunity including *phospholipase c delta 4 (PLCD4)*, *ficolin 2 (FCN2)*, *trefoil factor 2 (TFF2)*, β -1, *3-Galactosyltransferase (B3GALT)*, and *fatty acid binding protein 1 (FABP1)*. These results improve our knowledge of the biological basis underlying RFI, which would be useful for further investigations of key candidate genes for RFI and for the development of biomarkers.

Key words: duck, duodenum, gene expression, metabolism, residual feed intake

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INTRODUCTION

Feed cost is a large component of the overall cost in all production settings, especially because the animals have a long production career (Willems et al., 2013). Breeding more efficient ducks would reduce the amount of feed required for laying, and the amount of nitrogenous waste, thereby enhancing profitability. Feed efficiency is often assessed as either the feed conversion ratio (FCR) or the residual feed intake (RFI; Koch et al., 1963; Case et al., 2012). Residual feed intake represents the amount of feed consumed that is not accounted for by the expected requirements of production (e.g., milk and egg production or body weight gain) and body weight main-

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tenance (Kennedy et al., 1993). The effectiveness of artificial selection for RFI has been fully demonstrated in mammals and avian breeds (Gilbert et al., 2007; Aggrey et al., 2010; Berry and Crowley, 2012). RFI is a heritable feed efficiency trait that allows an animal to be ranked based on its individual feed intake (FI) that is independent of its production traits (Herd and Arthur, 2009). Although traditional selection for RFI has made substantial genetic progress (Bezerra et al., 2013), it has not maintained the potential of current egg production of layers (Yuan et al., 2015a). Therefore, selection based on potential functional genes and genetic markers underlying RFI could be a promising alternative.

In the previous decades, quantitative trait loci (QTL) for many traits in chicken have been studied (Wolc et al., 2013). However, these QTL mapping studies and candidate gene approaches are insufficient because of the low power of linkage analyses and bias in the detection of biologically plausible candidates for complex traits (Tabor et al., 2002). Global transcript profiling is a logical approach to identify markers of RFI and would offer a new opportunity to decipher its underlying mechanism. To date, there has been no study on global gene expression profiling on RFI in ducks.

The objective of this study was to identify duodenum genes and pathways through transcriptional profiling in 2 extreme RFI phenotypes (HRFI and LRFI) of the duck population.

MATERIALS AND METHODS

The animal care and use protocol was approved by the Institutional Animal Care and Use Committee of the Zhejiang Academy of Agricultural Sciences and performed in accordance with the Regulations for the Administration of Affairs Concerning Experimental Animals.

Experimental Population and Sample Collection

A pure line of Shaoxing duck, maintained and selected for egg production and green shell for over 10 yr in the National Shaoxing Duck Conservation Farm, was used in this study. At 12 wk of age, 300 ducks were randomly placed in individual cages with ad libitum food and water. The feeding trial was conducted from 42 to 46 wk of age since this corresponds to the peak period of egg production and is the standard time used to assess feed efficiency in duck. FI was defined as the amount of distributed feed at the beginning of the week minus the remaining uneaten feed at the end of the week. Egg mass laid (EML), body weight (BW), and BW gain (difference between BW at the end of the test period and at the beginning of the test period, ΔW) were measured. During this period, birds were fed standard commercial

diets. We estimated the ducks' RFI as a linear function of FI, and its outputs, such as EML, metabolic mid-weight (MMW), ΔW , and maintenance requirements (BW^{0.75}) as (Yuan et al., 2015b; Zeng et al., 2016)

$$\text{MMW} = [(\text{initial weight} + \text{final weight})/2]^{0.75},$$

$$\text{RFI} = \text{FI} - (\mu + a \times \text{MMW} + b \times \Delta W + c \times \text{EML}),$$

where FI calculated feed intake over the test period; a, b, and c were partial regression coefficients; and μ represented the intercept.

At the end of the whole experimental period, we selected 6 samples consisting of 2 groups (3 biological replicates per group) to represent 2 distinct RFI performances (HRFI and LRFI). In particular, FI and RFI were significantly different between high and low RFI groups, but all other measured phenotypes were similar. Duodenal epithelial tissues were collected from each individual in all groups and stored at -80°C until analyzed.

RNA Isolation, Library Preparation, and Sequencing

Briefly, total RNA was extracted using Trizol reagent (Invitrogen, Carlsbad, CA) following the manufacturer's procedure. Total RNA quantity and purity were analyzed using Bioanalyzer 2100 RNA Nano 6000 Assay Kit (Agilent Technologies, Santa Clara, CA) and spectrophotometer (NanoVue; GE Healthcare, Piscataway, NJ). Only RNA samples with an RNA 260:280 ratio greater than 1.9 and RIN larger than 7 were used for cDNA library construction.

A total amount of 3 μg total RNA per sample was used as the initial material for RNA sample preparation. Ribosomal RNA was removed using an Epicentre Ribo-Zero Gold Kit (Epicentre Technologies, Madison, WI). Then, sequencing libraries with varied index labels were prepared with a NEBNextUltra Directional RNA Library Prep Kit for Illumina (New England BioLabs, Ipswich, MA) following the manufacturer's recommendations. The clustering of the index-coded samples was performed on a cBot cluster generation system using TruSeq PE Cluster Kit v3-cBot-HS (Illumina, San Diego, CA) according to the manufacturer's instructions. After cluster generation, the libraries were sequenced on an Illumina HiSeq 4000 platform and 150 bp paired-end reads were generated. The clean data were obtained by filtering the raw reads and removing polluted reads, low-quality reads, and reads with unknown bases accounting for more than 5%. Filtered reads were aligned to the duck genome by using TopHat (version 2.0.12; Trapnell et al., 2009) and mapped with Bowtie2 (version 2.2.3; Langmead et al., 2009).

Table 1. Descriptive statistics for residual feed intake (RFI)¹

Traits	HRFI ² (n = 30)	LRFI (n = 30)	Overall (n = 300)
FI (g/d)	177.2 ± 4.8 ^a	130.8 ± 13.1 ^b	162.2 ± 15.1 ^c
EML (g/d)	60.1 ± 4.9	63.7 ± 5.1	67.5 ± 7.1
BW(g)	1274.2 ± 177.9	1289.1 ± 113.5	1350.1 ± 123.6
ΔW (g/d)	3.2 ± 4.8	5.4 ± 5.3	1.5 ± 3.8
FCR	3.0 ± 0.4 ^a	2.0 ± 0.1 ^b	2.6 ± 0.3 ^c
RFI (g/d)	20.7 ± 2.4 ^a	-25.1 ± 5.3 ^b	0.0 ± 14.0 ^c

¹Values are expressed as mean ± SD.²HRFI = high RFI; LRFI = low RFI.^{a-c}Different lowercase letters indicate significant differences ($P < 0.05$) between any 2 groups.

Differential Expression Analysis

The number of all clean reads for each gene in each sample was counted with HTSeq (version 0.6.0; Anders et al., 2015). The number of clean reads for each gene was calculated and normalized to reads per kilobase per million reads (RPKM) for gene expression analysis. Differential expression genes (DEG) were analyzed between 2 samples with biological replicates by DEGseq (version 1.16; Wang et al., 2010). To improve the credibility of DEG, the initial P -values were adjusted by the FDR (q) method. The expression fold change was calculated between groups. Genes with a $q \leq 0.05$ and $|\log_2 \text{ratio}| \geq 1$ were identified as DEG.

Quantitative Real-Time PCR Confirmations

To confirm our differential expression results, we conducted quantitative reverse transcription PCR (qRT-PCR) assays for 12 randomly selected DEG in the same RNA samples used for RNA-seq. A portion (1 µg) of the total RNA obtained from each extraction was reverse-transcribed in a 20-µL reaction volume by using the TransScript First-Strand cDNA Synthesis SuperMix (TransGen, Beijing, China) following the manufacturer's instructions. qRT-PCR was performed on an ABI 7500 (Applied Biosystems, Foster City, CA). Reactions were performed in a 20-µL reaction mixture containing 2 µL cDNA template, 0.4 µM forward/reverse primer, 10 µL 2× SYBR qPCR Mix, and 0.4 µL ROX reference dye (Takara, Osaka, Japan). The cycling protocol included an initial step at 94°C for 3 min, followed by 40 cycles of denaturation at 94°C for 10 s and annealing at 60°C for 30 s. Experiments for the detection of all the genes were performed in triplicates. The relative expression levels of the genes tested were calculated using the $2^{-\Delta\Delta C_t}$ method and were normalized by β -actin in each sample. We first tested the efficiency of using *glyceraldehyde 3 phosphate dehydrogenase* (*GAPDH*) and β -actin as controls and found that β -actin was more appropriate than

GAPDH for the current study. The data are expressed as means ± standard error. Results were considered statistically significant at $P < 0.05$. The primers used in this study are listed in the supplementary material (Table S1).

DEG Ontology Analysis

Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment of DEG was implemented by the hypergeometric test, where $q < 0.05$ was considered to be significantly enriched (Maere et al., 2005). The DEG were mapped onto the whole genome using Blast2GO in the GO enrichment analysis.

RESULTS

Phenotypic Aspects of Feed Efficiency

Descriptive statistics of RFI, reproductive traits, and feed efficiency traits are summarized in Table 1. Based on the performance in the RFI test, 30 animals with the LRFI and 30 animals with the HRFI were chosen for comparison. The mean FI value was 162.2 g/d, whereas the EML and BW were, respectively, approximately 67.5 g/d and 1,350.1 g. Difference analysis showed that FI and FCR in the HRFI group were significantly higher than those in the LRFI group. There were no differences in EML, BW, and ΔW between HRFI and LRFI ducks. On average, the HRFI group had higher RFI, FI, and FCR, indicating that HRFI ducks consumed significantly more feed to achieve similar gain as LRFI ducks.

Mapping Statistics Summary

To identify mRNA related to duck feed efficiency, we performed whole transcriptome RNA-Seq of the duodenal epithelial of HRFI and LRFI. The sequencing generated, on average, 111.0 million raw reads per sample (Table S2). After filtering out polluted reads, low-quality reads, and those with unknown bases accounting for more than 5% of the reads, as well as the rRNA mapping reads, the mean number of clean reads across the 2 groups was 107.2 million. The clean reads were mapped to the duck genome, and 69.2% of the reads were identified in the mapping data set. The efficient mapping on the duck genome demonstrated that the sets of reads from different groups were suitable for examining mRNA in the duck genome.

Differentially Expressed Genes Related to Feed Efficiency

In total, we identified 35 significant DEG between the LRFI and HRFI groups, with 33 of these being an-

Table 2. Differentially expressed genes in duodenal epithelial of HRFI and LRFI laying ducks

Ensemble ID	Gene symbol	LRFI count normalize ¹	HRFI count normalize ¹	Log ₂ FC(LRFI/HRFI) ²	q-value ³
ENSAPLG00000001594	MOG	238.45	0.85	8.13	1.15E-21
ENSAPLG00000004043	PLCD4	23.59	0.29	6.35	7.63E-05
ENSAPLG00000008079	CHIA	245.62	3.44	6.16	2.42E-07
ENSAPLG00000000407	LOC100858687	776.49	19.09	5.35	4.32E-25
ENSAPLG000000009361	CACNB4	1099.23	30.74	5.16	8.47E-18
ENSAPLG000000006170	RPL7	1882.07	71.77	4.71	2.22E-05
ENSAPLG000000003957	VTG	80.64	3.46	4.54	4.44E-08
ENSAPLG000000007558	FCN2	256.48	11.38	4.50	1.66E-12
ENSAPLG000000013538	RPL19	1300.69	63.52	4.36	1.20E-06
ENSAPLG000000001196	FABP1	48153.07	2644.47	4.19	7.20E-13
ENSAPLG000000002559	AKR1	604.09	45.66	3.73	6.02E-14
ENSAPLG000000005067	SLC27A6	177.72	13.89	3.68	6.63E-05
ENSAPLG000000006922	STMN1	167.88	16.50	3.35	5.58E-07
ENSAPLG000000011205	SLC34A	144.31	20.61	2.81	4.35E-05
ENSAPLG000000000274	/	398.24	73.35	2.44	2.56E-06
ENSAPLG000000007143	NDUFB9	12989.27	2603.90	2.32	9.61E-07
ENSAPLG000000002946	GALNT8	953.00	2658.69	-1.48	1.31E-05
ENSAPLG000000011151	RPL44	1897.88	5918.45	-1.64	7.77E-06
ENSAPLG000000001226	NDUFV3	797.22	2623.59	-1.72	6.60E-07
ENSAPLG000000012758	LOC102054988	85.36	325.21	-1.93	3.59E-05
ENSAPLG000000002315	H2A	412.80	1750.59	-2.08	3.41E-09
ENSAPLG000000012099	LOC102104598	479.67	2148.11	-2.16	2.16E-06
ENSAPLG000000014394	TNFRSF6B	117.90	567.62	-2.27	6.24E-08
ENSAPLG000000014340	LOC100190186	670.69	4737.36	-2.82	2.00E-11
ENSAPLG000000001620	/	12.84	119.91	-3.22	3.00E-07
ENSAPLG000000011750	B3GALT5	48.91	652.47	-3.74	1.05E-06
ENSAPLG000000005304	SAA	27.21	872.39	-5.00	5.07E-31
ENSAPLG000000009680	SLC50A1	3.55	117.22	-5.04	2.78E-07
ENSAPLG000000008835	TFF2	9.90	604.05	-5.93	1.26E-06
ENSAPLG000000011115	SAMD3	0.39	51.22	-7.05	5.05E-09
ENSAPLG000000004628	MHC_I	0	883.58	/	1.92E-61
ENSAPLG000000009613	B3GNT3	0	269.85	/	1.28E-33
ENSAPLG000000013605	BTN3A3	0	121.87	/	1.43E-18
ENSAPLG000000001972	NUDT8	0	74.70	/	4.08E-13
ENSAPLG000000001035	BTN1A1	0	47.91	/	1.54E-09

¹LRFI = low residual feed intake; HRFI = high residual feed intake.

²FC = fold change.

³q-value = FDR-adjusted *P*-value.

notated genes (Table 2). Sixteen DEG were upregulated in the LRFI ducks and the other 19 were downregulated compared with HRFI ducks (Table 2 and Fig. S1). Among the DEG, the highest fold change estimate was for the *myelin oligodendrocyte glycoprotein (MOG)* gene, involved in cell adhesion, which was upregulated in the more efficient LRFI ducks. On the other hand, the lowest fold change estimate was *sterile α motif (SAMD3)*, involved in aiding the cell to respond to nitrogen starvation (Grimshaw et al., 2004), which was downregulated in the LRFI ducks. Interestingly, there were 5 genes that have zero expression in LRFI named *MHC_I (major histocompatibility complex I)*, *B3GNT3* (β -1,3-*N*-Acetylglucosaminyltransferase 3), *BTN3A3*

(*butyrophilin Subfamily 3 Member A3*), *NUDT8 (nudix Hydrolase 8)*, and *BTN1A1 (butyrophilin Subfamily 1 Member A1)*. To validate DEG obtained through RNA-Seq, a total of 12 genes were selected randomly out of the transcripts having twofold or greater differential expression using the same RNA samples that their qRT-PCR analysis was performed on. The comparative results of the fold changes by qRT-PCR were shown in Fig. 1 and Table 3. For 12 chosen genes, 9 showed the consistent expression patterns between RNA-Seq and qRT-PCR results. After excluding 3 genes with opposite expression levels, the computational and experimental fold changes in our study also showed positive correlation with $R^2 = 0.9321$.

Figure 1. Validation of differentially expressed genes (DEG) in duodenum from HRFI and LRFI ducks. X-axis represents 8 selected genes for qRT-PCR assays and Y-axis represents the $\log_2$ (fold change) derived from RNA-Seq and qRT-PCR analysis. FC = fold change (LRFI/HRFI).

Functional Annotation of Differential Expressed Genes

To determine the associated functional categories and pathways of the 35 DEG in the duodenal epithelial between the HRFI and LRFI groups, we used Blast2GO to annotate their functions. The significantly enriched GO terms are shown in Fig. 2 and Table S3. From GO, the main processes that distinguish LRFI and HRFI are cellular process (16 genes), biological regulation (12 genes), single-organism process (16), and metabolic process (13 genes; Fig. 2 and Table S3). The relevant molecular functions from the annotation analysis are binding (13 genes), transporter (5 genes), and catalytic (9 genes; Fig. 2 and Table S3). The KEGG pathways analysis revealed 6 overrepresented pathways, including ribosome, PPAR signaling pathway, MAPK signaling pathway, metabolic pathways, glycosphingolipid biosynthesis, and pentose and glucuronate interconversions. (Table 4).

DISCUSSION

Using a global transcriptome analysis, we identified 35 genes that were differentially expressed in the duodenum of LRFI and HRFI ducks. To our knowledge, the work presented here is the first RNA-Seq analysis of RFI during the laying period in ducks. However, the number of DEG was not high. First, the duck genome sequence was not intact. Second, the experimental population is a pure line of Shaoxing laying ducks with lower genetic variation at a global level (Coble et al., 2014). Moreover, the number of DEG was also greatly influenced by different detection algorithms and biological replicates (Rapaport et al., 2013; Liu et al., 2014; Yi et al., 2015).

Among these DEG, 9 genes identified, including *phospholipase c delta 4 (PLCD4)*, *ficolin 2 (FCN2)*, *Chitinase (CHIA)*, *trefoil factor 2 (TFF2)*, *polypeptide*

Table 3. Comparison of the RNA-seq expression data and real-time PCR results for four selected genes

Gene	RNA-seq		Real-time PCR	
	Count mean (LRFI/HRFI)	<i>q</i> -value ¹	Fold change ² (LRFI/HRFI)	<i>P</i> -value ³
MHC_I	0/883.58	1.92E-61	172.82	0.01
B3GNT3	0/269.85	1.28E-33	21.72	0.02
BTN3A3	0/121.87	1.43E-18	0.82	0.42
NUDT8	0/74.70	4.08E-13	8.72	0.03

¹*q*-value = FDR-adjusted *P*-value.

²Relative expression of genes was normalized to the control gene β -actin using the $2^{-\Delta\Delta Ct}$ method.

³*P* < 0.05 was considered as significant.

N-acetylgalactosaminyltransferase 8 (GALNT8), *aldehyde reductase (AKR1)*, *NADH:ubiquinone oxidoreductase subunit (B9NDUFB9)*, *B3GNT3*, and *calcium voltage-gated channel auxiliary subunit β 4 (CACNB4)*, are directly or indirectly modulated involved in metabolism and secretion. *PLCD4* is one of the delta-type PLC isozymes, hydrolysis of phosphatidylinositol 4,5-bisphosphate (PI(4,5)P₂) by phospholipase C (PLC; PI-PLC; E.C.3.1.4.11) is a critical step for generation of 2 s messengers, inositol 1,4,5-bisphosphate (Ins(1,4,5)P₃), and diacylglycerol (DAG). Previous studies have shown that *PLCD4* has a functional role in sperm at the early step of fertilization (Fukami et al., 2003). It was observed that the calcium transients in eggs associated with fertilization were absent or delayed when used *PLCD4*^{-/-} sperm. Our genome-wide association studies (GWAS) have found that *PLCD4* was a candidate gene for RFI in ducks. These results showed that *PLCD4* might have a specific role in feed efficiency, which needs further study.

The innate immune system is an important subsystem of the overall immune system that comprises the cells and mechanisms that defend the host from infection by other organisms (Kim, 2016). As part of the innate immune system, endotoxins are recognized by cells expressing the pattern recognition receptor toll-like receptor 4 (TLR4). These findings are extremely relevant to feed efficiency because once in circulation, endotoxins are detoxified or deactivated by immune cells. Failure to do so results in an increased concentration of endotoxins leading to local and systemic inflammation, which has been shown to antagonize growth and performance of livestock because nutrients are redirected toward immunity rather than anabolic processes (Johnson, 1997). Paradis et al. (2015) have shown that several genes involved in innate immune were differentially expressed in the liver between HRFI and LRFI heifers. In our study, *FCN2* mRNA abundance was found to be higher in LRFI ducks. It has been reported that low *FCN* is associated with prematurity, low birth weight, and infection in neonates (St. Swierzko et al., 2009). These results suggest that LRFI ducks may have a

Figure 2. Function classifications of gene ontology (GO) terms of differentially expressed genes (DEG) in duodenum from HRFI and LRFI ducks. The results are summarized in 3 main categories: biological process, cellular component, and molecular function. The x axis indicates the subcategories, and the y axis indicates the percent (number) of genes in the same category.

stronger or healthier innate immunity, spending less energy to combat systemic inflammation, leaving more energy for growth and performance, such as laying. However, further investigation will be needed.

Gastric acid, gastric juice, or stomach acid is a digestive fluid, formed in the stomach and is composed of hydrochloric acid (*HCL*). Under normal circumstance, it plays a key role in digestion of proteins by activating digestive enzymes and making ingested proteins unravel so that digestive enzymes break down the long chains of amino acids. The highly acidic environment in the stomach and intestinal tract causes proteins from food to lose their characteristic folded structure (or denature; Schubert, 2015). TFF are members of a unique family of proteins characterized by 1 or more 3-looped structural motifs (trefoil or P-motif) held together by disulphide bonds. Evidence has been shown that *TFF2* can protect the mucosa from insults, stabilize the mucus layer, affect healing of the epithelium, and its encoded protein inhibits gastric acid secretion (Baus-Loncar et al., 2005). In our study, we found that the *TFF2* genes were significantly higher in HRFI ducks, suggesting that the secretion of gastric acid was inhibited. Based on these results, one can hypothesize that those ducks that were divided into LRFI groups have relatively more gastric acid secretion, leading to higher digestion and absorption of feed, therefore having a better feed efficiency. Alternatively, maybe HRFI ducks have higher expression of *TFF2* because of higher gastric acid and then more damage to their gut and decreasing feed efficiency.

We have also found an increased mRNA abundance for Beta-1,3-Galactosyltransferase (*B3GALT*) in HRFI ducks, and this is consistent with the results from Tizioto et al. (2016), who showed upregulation of *B3GALT* in HRFI steers. *B3GALT* is involved in fucose metabolic processes and functions as an O-glucosyltransferase, contributing to the elongation of O-fucosylglycan. Fucose is a component of innate immunity glycoproteins (mucins) produced by the intestinal mucosa and in saliva to help maintain the integrity of the mucosal barrier (Hooper et al., 1999). “Fucose sensing” has been identified as an important cross-talk between the intestinal microbiome and host tissues in studies with mice (Hoorens et al., 2011) and rabbits (Pacheco et al., 2012). Genes involved in fucose metabolism have previously been identified as playing a role in feed efficiency (Roehe et al., 2016). The upregulation of *B3GALT* may provide a mechanism for fucose with supply and so affect the development of the microbiome in intestine.

We also found the ribosome biogenesis pathway to be enriched for DEG in our study. Studies have revealed that the ribosome has an essential role in the regulation of cell proliferation and growth (Kirm-Safran et al., 2007) and homeostasis in mammalian organisms (Teng et al., 2013). Teng et al. (2013) have shown that mutations and deletions of ribosome biogenesis related genes result in pathologies known as ribosomopathies, which are associated with growth retardation and malformation. Furthermore, ribosome biogenesis may have an essential

Table 4. Enriched KEGG pathway-based sets of differentially expressed gene (DEG) in duck duodenal epithelial between the HRFI and LRFI groups

Term	P-value	Genes
Ribosome biogenesis	0.01	SAMD3, RPL7, RPL19
PPAR signaling pathway	0.01	FABP1, SLC27A6
MAPK signaling pathway	0.02	CACNB4, STMN1
Metabolic pathways	0.05	PLCD4, GALNT8, AKR1, NDUFB9, B3GNT3
Glycosphingolipid biosynthesis	0.05	B3GNT3
Pentose and glucuronate interconversions	0.05	AKR1

role in the regulation of skeletal muscle mass (Chaillou et al., 2014). Our results are consistent with that of Tizioto et al. (2016), who identified several ribosome biogenesis related genes have been differentially expressed between LRFI and HRFI steers (Tizioto et al., 2016).

It is well known that lipid metabolism is critically important in determining lipid deposition. In this study, several genes involved in the PPAR pathway (FABP1 and SLC27A6) were significantly changed in the duodenum of HRFI and LRFI ducks. The PPAR pathway is essential for lipometabolism and adipocyte differentiation in vertebrates (Li and Glass, 2004). Cui et al. (2012) suggested that the deposition of intramuscular fat in chicken is associated with the PPAR pathway. In our study, we found that fatty acid binding protein 1 (FABP1) and SLC27A6 were upregulated in the duodenum of LRFI ducks compared with HRFI ducks. The FABP gene family is thought to function as a transport protein for mitochondrial oxidation and lipid storage (Jordal et al., 2006). The high expression level of FABP genes were also found in jejunum in the low FCR ducks (Zhu et al., 2015). Additionally, some results have shown that several body fat traits were positively related to RFI performance (Hoque et al., 2009). The upregulation of FABP1 and SLC27A6 in the LRFI group may lead to decreased feed intake, high-efficiency energy utilization, and fewer energy costs by modulating fatty acid through mitochondrial oxidation and lipid storage (Jordal et al., 2006).

In conclusion, we performed global gene expression profiling to elucidate the underlying genetic mechanisms of RFI in laying ducks. The results identified a total of 35 DEG associated with RFI. Among them, 16 DEG were upregulated in the LRFI ducks and the other 19 were downregulated compared with HRFI ducks. These genes play an important role in metabolism, digestibility, secretion, and innate immunity. The results of this study, together with previous research, provide a more comprehensive understanding of gene expression in the duodenum of animals genetically divergent for RFI. However, it will be important to test if these genes can also be used to predict feed efficiency in large com-

mercial duck populations or in other breeds. Further study and experiments are necessary before these results can be used for prediction of continuous phenotypes in animal production and breeding.

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