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Intestinal microbiota-host crosstalk reveals mechanisms regulating residual feed intake in egg-type ducks

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Abstract

Background As an important economic trait in ducks, residual feed intake (RFI) may be influenced by the intestinal microbiota. However, the mechanisms underlying microbiota-host crosstalk remain unclear.

Results We analyzed the total egg number (TEN), total egg weight (TEW), total feed intake (TFI), feed conversion ratio (FCR), and RFI of 1,370 ducks over a 32-day period. Within the high-TEW ducks, 60 low RFI ducks and 60 high RFI ducks were selected to form the LH group (LH) and HH group (HH), respectively. The TFI, FCR, and RFI were significantly lower in the LH than in the HH ($P < 0.001$). Based on 16S rRNA sequencing, the duodenal microbiota in the LH showed a significantly higher evenness index compared to the HH ($P < 0.05$). Using linear discriminant analysis effect size (LEfSe), differential bacterial genera were identified in the duodenum and jejunum between groups (LDA > 3.5, $P < 0.05$). Functional prediction results indicated that, compared to the HH, the duodenal microbiota of the LH appeared to be more active in metabolism. Further analysis revealed four genera (*Paenibacillus*, *Kocuria*, *Corynebacterium*, and *Bacillus*) that showed significant differences in both the duodenum and jejunum (LDA > 3.5, $P < 0.05$). Their abundances in the LH were significantly higher than those in the HH ($P < 0.05$). Subsequently, 419 and 384 differentially expressed genes (DEGs) were identified in the duodenum and jejunum using DESeq2, respectively ($|Log_2FC| \geq 1$, $P < 0.05$). Functional enrichment analysis showed that 22 and 16 pathways were significantly enriched in the duodenum and jejunum ($P < 0.05$). Among them, five metabolism-related pathways, such as steroid biosynthesis, were co-enriched in both intestinal segments. We constructed a protein–protein interaction (PPI) network of DEGs from the five pathways and utilized the MCODE plugin to extract the top-ranking subnetwork. The expression levels of genes in this subnetwork (*HMGCS1*, *MSMO1*, *ACAT2*, *LSS*, *FDFT1*, *SQLE*, *IDI1*, *CYP51A1*) were significantly correlated with the abundance of key genera.

Conclusions *Paenibacillus*, *Kocuria*, *Corynebacterium*, and *Bacillus* were identified as key microbiota influencing feed efficiency in ducks. Their abundances were closely associated with the expression of *HMGCS1*, *MSMO1*, *ACAT2*, *LSS*, *FDFT1*, *SQLE*, *IDI1*, and *CYP51A1*. The specific mechanisms require further validation in future studies. These findings

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provide a preliminary investigation into the intestinal microbiota-host crosstalk affecting feed efficiency and offer novel perspectives for reducing RFI in egg-type ducks.

Keywords Egg-type ducks, Intestine, 16S rRNA sequencing, Transcriptome sequencing, RFI

Background

Feed represents the largest cost in poultry production, accounting for approximately 70% of total expenses [1]. Improving feed efficiency holds significant economic importance while simultaneously reducing organic waste. In livestock research, residual feed intake (RFI) is a key metric for feed efficiency. It represents the difference between actual and expected feed intake. Individuals with low RFI exhibit higher feed efficiency [2]. Selecting for low RFI can maintain lower energy intake without sacrificing production performance, thereby reducing feed consumption [3]. In ducks, the heritability estimate of RFI ranges from moderate (0.24) [4] to high (0.41) [5], suggesting strong potential for genetic improvement. Meanwhile, considering the genetic independence between RFI and production traits [6], we conducted RFI selection in a high-yield cohort to breed ducks that balance production performance and feed efficiency.

Feed efficiency is closely related to the digestive and absorptive capacity of the intestine [7]. Located in the upper gastrointestinal tract, the duodenum and jejunum are the major sites for chemical digestion and nutrient absorption, respectively [8, 9]. The microbiota residing in these segments play key regulatory roles in intestinal function, influencing host feed efficiency [10, 11]. Therefore, comparing the microbial composition of these two intestinal segments between low- and high-RFI individuals and identifying key microbiota will help elucidate the mechanisms by which microbiota regulate feed efficiency. However, in the context of differential feed efficiency, current research has largely focused on the ileum, cecum, and rectum [12–14], while the exploration of the duodenal and jejunal microbiota remains relatively insufficient.

Research has shown that the microbiota can influence the expression levels of host intestinal genes [15], serving as a crucial entry point for investigating microbiota-host crosstalk. Therefore, to elucidate the potential mechanisms by which key microbiota influence host feed efficiency, we selected high-egg-laying ducks exhibiting low- (LH) and high- (HH) RFI from a population of 1,370 ducks. By combining 16S rRNA sequencing and transcriptome sequencing (RNA-seq), we aimed to identify key microbiota in the duodenum and jejunum, as well as the core pathways and genes associated with them. The RFI-associated microbiota and genes identified in this study might serve as potential biomarkers for breeding feed-efficient egg-type ducks.

Methods

Experimental animals

A total of 1,370 experimental female ducks, 260 days of age, were from the sixth generation of the PM line of Minlong No. 1 egg-type ducks and were provided by the Shanma Duck Breeding Base (Longyan, China). The ducks were raised in accordance with the feeding and management protocols established by the base. During the raising process, all animals had free access to feed (Table 1) and water. Prior to laying, the ducks were transferred from net-floor rearing to individual cages. Natural lighting was applied during the growing period, and a 16L:8D photoperiod was used during the laying period.

Data collection and analysis

One day prior to the experiment, each duck was fasted for 12 h, and its initial body weight (iBW) was recorded. Over the subsequent 32 days, daily feed intake, egg production, and egg weight were monitored for each duck. At the end of the experiment, each duck was fasted for 12 h, and its final body weight (fBW) was recorded. Feed conversion ratio (FCR), metabolic body weight (MBW), and RFI were calculated [14]. In the following formula, μ represents the intercept, and a, b, and c are the regression coefficients (6.07, 0.43, and 0.42, respectively).

$$\mathrm{FCR} = \text{total feed intake (TFI)} / \text{total egg weight (TEW)}$$

$$\mathrm{MBW} = [(\mathrm{iBW} + \mathrm{fBW})/2]^{\wedge} 0.75$$

$$\mathrm{RFI} = \mathrm{TFI} - [\mu + \mathrm{a} \times \mathrm{MBW} + \mathrm{b} \times (\mathrm{fBW} - \mathrm{iBW}) + \mathrm{c} \times \mathrm{TEW}]$$

Sample collection

We sequentially analyzed various traits in 1,370 ducks, including iBW, body weight gain (i.e., fBW—iBW), TEW, and RFI. First, based on the mean ± 1 standard deviation of iBW and body weight gain, ducks with excessively fast or slow growth were removed, leaving 772 ducks. Next, their TEW was further analyzed, and ducks with TEW above the population mean (2279.72 g) were classified as the high-TEW subgroup ($n = 451$). Subsequently, 60 ducks were randomly selected from ducks with RFI < 0 ($n = 159$) and RFI > 0 ($n = 292$), respectively, forming the LH and HH groups. Ten healthy ducks with similar body conditions were randomly selected from each group. Following a 12-h fast, ducks were euthanized by carbon

Table 1 Dietary composition and nutrient levels

Item	Level
Crude protein (%)	≥ 17.5
Crude fiber (%)	≤ 6.0
Crude ash (%)	≤ 16.5
Calcium (%)	2.5—4.0
Total phosphorus (%)	0.4—1.2
Sodium chloride (%)	0.3—0.8
Moisture (%)	≤ 12.5
Lysine (%)	≥ 0.80
Methionine (%)	≥ 0.30

dioxide inhalation and cervical dislocation. Digestive contents from the duodenum and jejunum, along with intestinal tissue samples, were collected. The former were used for 16S rRNA sequencing, while the latter were employed for RNA-seq. The samples used for sequencing were shown in Table S1 and Table S2. The 16S rRNA sequencing data and RNA-seq data of the same duck could be matched one-to-one according to the "Sample ID" column.

16S rRNA sequencing and analysis

Total DNA was extracted using the DNA kit (Omega Bio-tek, GA, USA) according to the manufacturer's instructions. The V3-V4 hypervariable region was amplified with primers 338-F and 806-R. The resulting products were used to construct libraries, which were then sequenced on the Illumina NextSeq 2000 platform (Illumina, CA, USA).

Comprehensive quality control of the data was performed using fastp (0.20.1) [16] and FLASH (1.2.11) [17]. Assembled reads were processed and assigned using QIIME2 (2024.10) [18]. Amplicon sequence variants (ASVs) were obtained through denoising using the DADA2 (2024.10.0) [19]. Annotation was performed based on the SILVA ribosomal RNA gene database (138.2) [20]. Alpha and beta diversity metrics were calculated in QIIME2 [18], and principal coordinate analysis (PCoA) was performed based on Bray–Curtis distance. Linear discriminant analysis effect size (LEfSe) was used to analyze microbial composition differences between groups, with a screening criterion of LDA > 3.5 and $P < 0.05$. Microbial metabolic pathways were predicted using PICRUSt2 [21], followed by functional comparison with STAMP software (2.1.3) [22].

RNA-seq and analysis

Following the manufacturer's instructions, total RNA was extracted from duodenum and jejunum tissues using the Trizol (Invitrogen, Massachusetts, CA, USA). Libraries were prepared and sequenced on the DNBSEQ-T7 platform (MGI Tech Co., Shenzhen, China).

Clean reads were obtained using fastp [16]. HISAT2 (2.2.1) [23] was employed to map the clean reads to the duck reference genome (GCF_047663525.1), yielding SAM files. The output SAM file was converted to BAM format and sorted using SAMtools (1.13) [24]. Transcript expression levels were calculated for each transcript using featureCounts software (2.1.1) [25] and normalized using transcripts per million (TPM). Differentially expressed genes (DEGs) between groups were identified using DESeq2 (1.48.1) [26] with a screening criterion of $|\mathrm{Log_2FC}| \geq 1$ and $P < 0.05$. Kyoto Encyclopedia of Genes and Genomes (KEGG) functional enrichment analysis was performed using the online platform KOBAS 3.0 (http://bioinfo.org/kobas/) [27]. The STRING 12.0 database (https://cn.string-db.org/) [28] was used to construct the protein–protein interaction (PPI) network, visualized using Cytoscape software (version 3.10.2) [29]. The MCODE plugin within Cytoscape was employed to identify key subnetworks [30].

Statistical analysis

Before formal analysis, the normality of the data was evaluated using the Shapiro–Wilk test, supplemented by histograms and Q-Q plots. Student's *t*-test was applied to compare group differences for normally distributed traits, whereas the Mann–Whitney U test was used for traits that were not normally distributed. Spearman's correlation method was employed to assess correlations. Linear regression equations were fitted using SPSS 27.0 (IBM, Chicago, TL, USA). A *P*-value < 0.05 was considered statistically significant.

Results

Statistical results from 1,370 ducks and comparison of LH and HH groups

Descriptive statistical results for the entire population were shown in Table 2. During the 32-day measurement period, the population had an average TEN of 29.69 and an average TEW of 2100.02 g. Comparative analysis showed no significant differences in laying and growth performance between LH and HH. The LH exhibited significantly lower TFI, FCR, and RFI ($P < 0.001$) (Table 3).

Overview of 16S rRNA sequencing and ASVs identification

The sequencing report indicated satisfactory quality for further analysis (Table S1). A total of 840 and 1,084 ASVs were identified in the duodenum and jejunum, respectively, with 650 ASVs shared between them (Fig. 1A). In the duodenum, the LH and HH exhibited 650 and 719 ASVs, respectively, sharing 529 ASVs (Fig. 1B). In the jejunum, the LH contained 838 ASVs, and the HH contained 971 ASVs, with an overlap of 725 ASVs between groups (Fig. 1C).

Table 2 Statistical results from 1,370 ducks throughout the experimental period

Classification	Trait	Mean	Maximum	Minimum	SD	CV(%)
Laying performance	TEN	29.69	35.00	2.00	4.32	14.56
	TEW (g)	2100.02	2657.30	135.00	327.11	15.58
	AEW (g)	70.70	83.35	53.33	3.91	5.52
Growth performance	iBW (g)	1331.56	1910.00	970.00	133.27	10.01
	fBW (g)	1335.64	2051.00	991.00	132.76	9.94
Feed utilization	TFI (g)	4846.39	5265.00	3925.00	268.04	5.53
	FCR (g/g)	2.51	31.50	1.78	1.83	72.83
	RFI (g)	0.00	858.61	−905.29	213.72	-

Table 3 Comparison of LH and HH groups

Classification	Trait	LH ($n=60$)	HH ($n=60$)	*P* value
Laying performance	TEN	31.80 ± 0.63	31.70 ± 0.72	0.492
	TEW (g)	2346.30 ± 76.10	2331.22 ± 67.93	0.330
	AEW (g)	73.80 ± 2.40	73.57 ± 2.41	0.633
Growth performance	iBW (g)	1323.50 ± 59.68	1306.67 ± 58.79	0.122
	fBW (g)	1329.32 ± 62.14	1312.10 ± 60.63	0.127
Feed utilization	TFI (g)	4780.92 ± 156.68	5104.82 ± 56.22	$P<0.001$
	FCR (g/g)	2.04 ± 0.07	2.19 ± 0.06	$P<0.001$
	RFI (g)	−165.68 ± 131.27	177.62 ± 48.64	$P<0.001$

Comparative analysis of microbial composition

In the duodenum, a significant difference was observed in the evenness index, with the LH showing significantly higher evenness than the HH ($P<0.05$) (Table 4). PCoA based on the Bray–Curtis distance revealed similarities in microbial composition within each group. The first two principal coordinates explained 36.51% and 17.57% of the total variance, respectively. PERMANOVA further indicated a significant difference in microbial structure between the two groups (R2 = 0.154, F = 3.28, $P=0.01$) (Fig. 2A). In the LH, the top three dominant phyla were *Firmicutes* (38.42%), *Actinobacteriota* (29.16%), and *Proteobacteria* (28.29%). In the HH, they were *Firmicutes* (48.02%), *Campylobacterota* (23.15%), and *Actinobacteriota* (13.75%) (Fig. 2B). In the LH, the dominant genera were *Rothia* (12.37%), *Corynebacterium* (9.19%), and *Acinetobacter* (8.83%), whereas in the HH, they were *Helicobacter* (23.15%), *Romboutsia* (11.35%), and *Rothia* (6.74%) (Fig. 2C).

In the jejunum, no significant differences were observed in alpha diversity indices between the LH and HH (Table 4). Based on Bray–Curtis distance, PCoA showed the similarities within groups. The first two

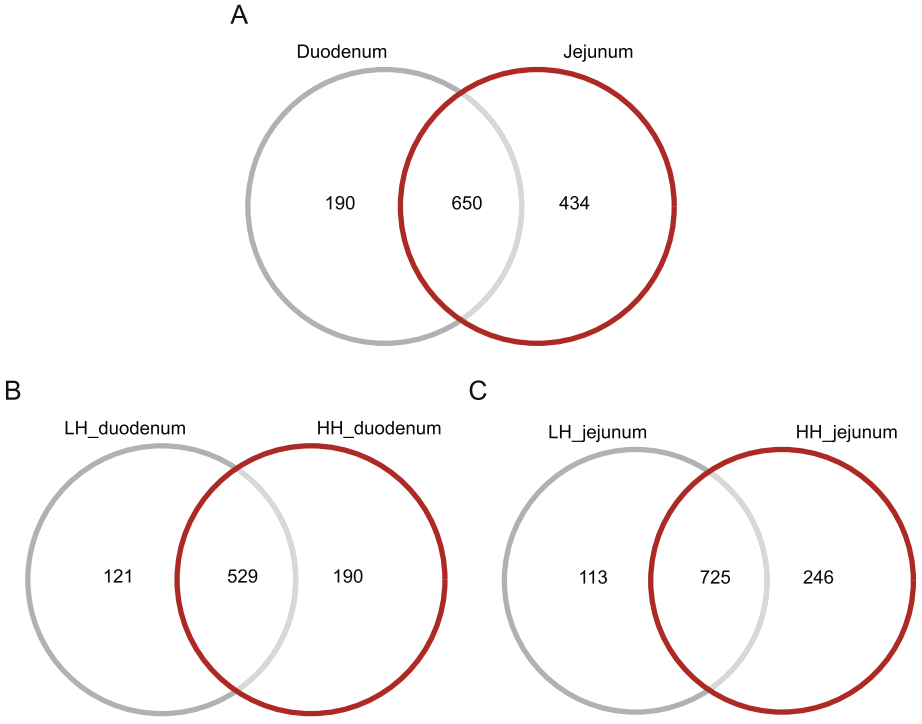

Fig. 1 Identification of unique and shared ASVs. **A** Duodenum versus jejunum. **B** Duodenum: LH group versus HH group. **C** Jejunum: LH group versus HH group

Table 4 Alpha diversity analysis results

Tissue	Index	LH ($n=10$)	HH ($n=10$)	*P* value
Duodenum	Chao1	199.8 ± 77.41	256.20 ± 65.38	0.16
	evenness	0.75 ± 0.02	0.65 ± 0.10	0.01
	Faith_PD	10.72 ± 3.04	13.49 ± 2.68	0.06
	observed_features	199.8 ± 77.41	256.20 ± 65.38	0.16
	shannon	5.62 ± 0.63	5.13 ± 0.74	0.08
Jejunum	Chao1	284.20 ± 63.68	309.40 ± 116.83	0.79
	evenness	0.67 ± 0.13	0.71 ± 0.19	0.13
	Faith_PD	14.50 ± 3.06	15.92 ± 4.68	0.55
	observed_features	284.20 ± 63.68	309.40 ± 116.83	0.79
	shannon	5.50 ± 1.20	5.87 ± 1.74	0.26

principal coordinates explained 28.16% and 15.76% of the total variance, respectively. PERMANOVA indicated a significant difference in microbial composition between groups (R2 = 0.133, F = 2.751, $P = 0.003$) (Fig. 2D). In the LH, the dominant phyla were *Firmicutes* (54.11%), *Actinobacteriota* (30.33%), and *Campylobacterota* (8.45%), while the HH had *Firmicutes* (39.83%), *Proteobacteria* (20.46%), and *Campylobacterota* (13.14%) (Fig. 2E). In the LH, the dominant genera were *Rothia* (11.71%), *Corynebacterium* (11.39%), and *Helicobacter* (8.40%), whereas in the HH, they were *Helicobacter* (12.99%), *Romboutsia* (6.15%), and *Acinetobacter* (5.62%) (Fig. 2F).

Differential microbiota identification and predicted functional comparison

In the duodenum, based on LEfSe, five and four differential genera were identified in the LH and HH, respectively (LDA > 3.5, $P < 0.05$) (Fig. 3A). STAMP functional comparison results showed significant differences in organismal systems, metabolism, and genetic information processing between the two groups (Fig. 3B). The microbiota in the LH appeared to exhibit stronger metabolic capabilities.

In the jejunum, the results showed that eight and four differential genera were identified in the LH and HH, respectively (LDA > 3.5, $P < 0.05$) (Fig. 3C). STAMP functional comparison results in the jejunum indicated significant differences in environmental information processing between the two groups (Fig. 3D). The microbiota in the LH demonstrated stronger environmental information processing capabilities.

Further analysis revealed that four genera (*Paenibacillus*, *Kocuria*, *Corynebacterium*, and *Bacillus*) showed significant differences in both the duodenum and jejunum, with significantly higher abundances in the LH than the

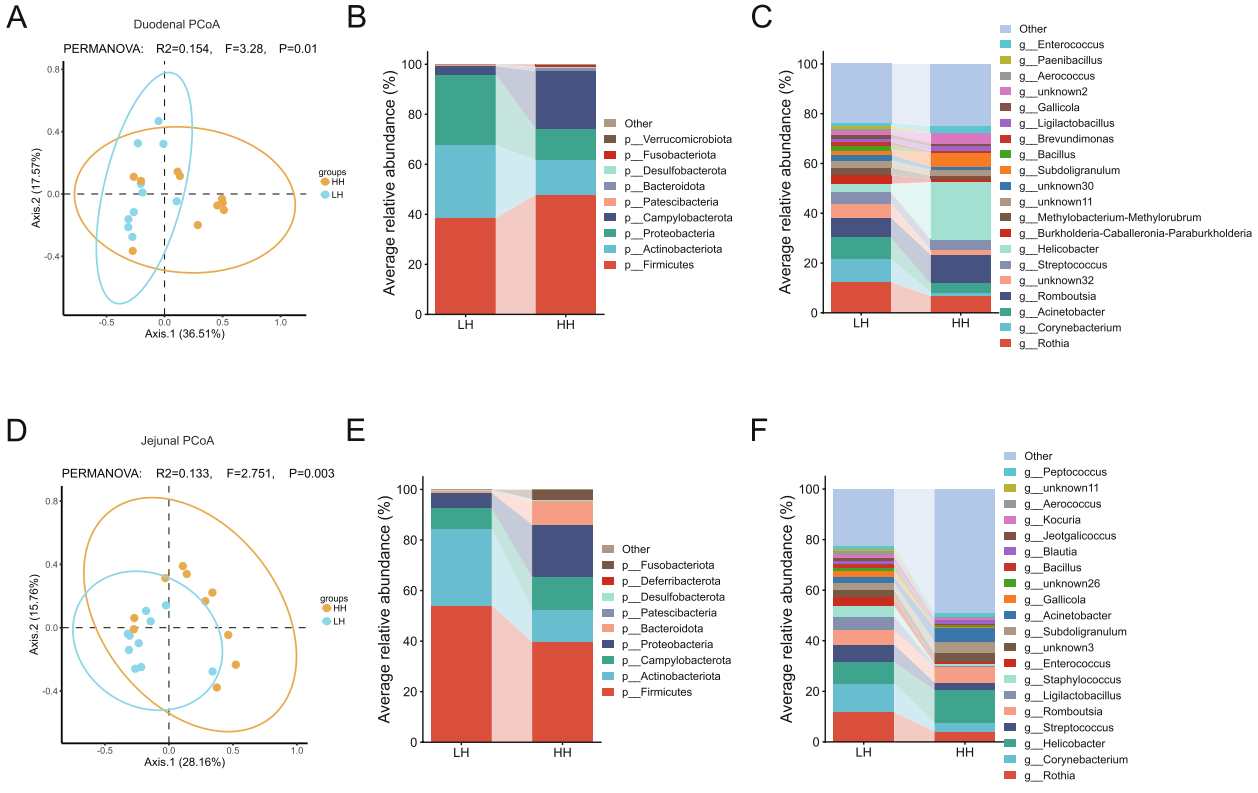

Fig. 2 Intergroup microbial composition comparison. **A** PCoA of the duodenum based on Bray–Curtis distance. **B** Microbial composition at the phylum level in the duodenum. **C** Microbial composition at the genus level in the duodenum. **D** PCoA of the jejunum based on Bray–Curtis distance. **E** Microbial composition at the phylum level in the jejunum. **F** Microbial composition at the genus level in the jejunum

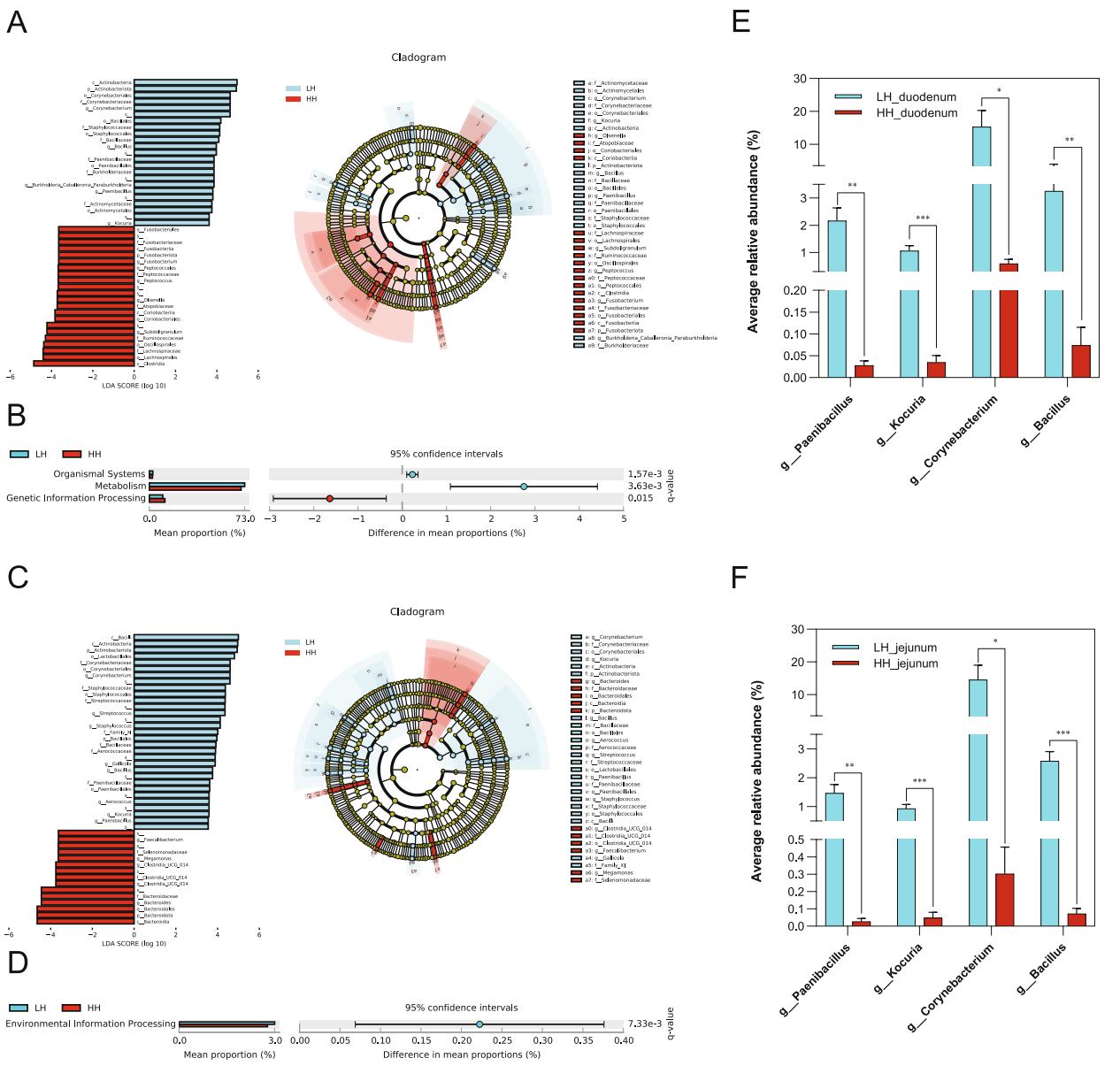

Fig. 3 Differential microbiota identification and function prediction in the duodenum and jejunum. **A** Differential microbiota in the duodenum and the corresponding cladogram. **B** Intergroup comparison of duodenal microbiota function. **C** Differential microbiota in the jejunum and the corresponding cladogram. **D** Intergroup comparison of jejunal microbiota function. **E** Abundance comparison of key genera in the duodenum. **F** Abundance comparison of key genera in the jejunum. Significance levels were denoted as follows: *** $P<0.001$, ** $P<0.01$, * $P<0.05$

HH. RNA-seq was performed on the duodenum and jejunum of five randomly selected ducks per group. In the duodenum, the abundances of *Paenibacillus*, *Kocuria*, *Corynebacterium*, and *Bacillus* were significantly higher in the LH than the HH (Fig. 3E). Similarly, in the jejunum, the abundances of these genera were also significantly higher in the LH than the HH (Fig. 3F).

Identification and functional analysis of DEGs

In this study, we constructed 20 cDNA libraries, generating 118.98 GB of raw data. Following stringent quality control, 118.64 GB of clean data were obtained. The Q20 and Q30 ranged from 98.63% to 98.80% and from 95.55% to 96.11%, respectively. The average mapping rate was 90.31%. The quality met the requirements for subsequent analysis (Table S2).

In the duodenum, 419 DEGs were identified, including 208 upregulated and 211 downregulated genes. In the jejunum, 384 DEGs were identified, comprising 217 upregulated and 167 downregulated genes ($|Log_2FC)|\geq 1$, $P<0.05$) (Fig. 4A). Among these, 95 genes were differentially expressed in both the duodenum and jejunum (Fig. 4B).

In the duodenum, 22 KEGG pathways were significantly enriched, of which 13 were related to metabolism ($P < 0.05$) (Fig. 4C). In the jejunum, 16 KEGG pathways were significantly enriched, with six associated with metabolism ($P < 0.05$) (Fig. 4D). Among them, five metabolism-related pathways were co-enriched in both intestinal segments: steroid biosynthesis, metabolic pathways, terpenoid backbone biosynthesis, nitrogen metabolism, and glycine, serine and threonine metabolism (Fig. 4E).

Prediction of intestinal microbiota-host crosstalk mechanisms

Using the DEGs from both intestinal segments that were enriched in the five common pathways, we constructed a PPI network and subsequently identified key subnetworks with MCODE (Fig. 5A). As shown in Fig. 5B, the top-ranking subnetwork comprised nine genes, including *HMGCS1*, *MSMO1*, *ACAT2*, *LSS*, *FDFT1*, *SQLE*, *IDI1*, *CYP51A1*, and *HMGCS2*. Of these, *HMGCS1*, *MSMO1*, *ACAT2*, *LSS*, *FDFT1*, *SQLE*, *IDI1*, and *CYP51A1* were differentially expressed in the duodenum. Their expression levels were significantly correlated with the abundances of the key genera in the duodenum (Fig. 5C). *HMGCS2*, *SQLE*, *IDI1*, and *CYP51A1* were differentially expressed in the jejunum. Excluding *HMGCS2*, all were significantly correlated with the abundance of key genera in the jejunum (Fig. 5D). Notably, *SQLE*, *IDI1*, and *CYP51A1* were differentially expressed in both intestinal segments, with significant correlations to the key genera.

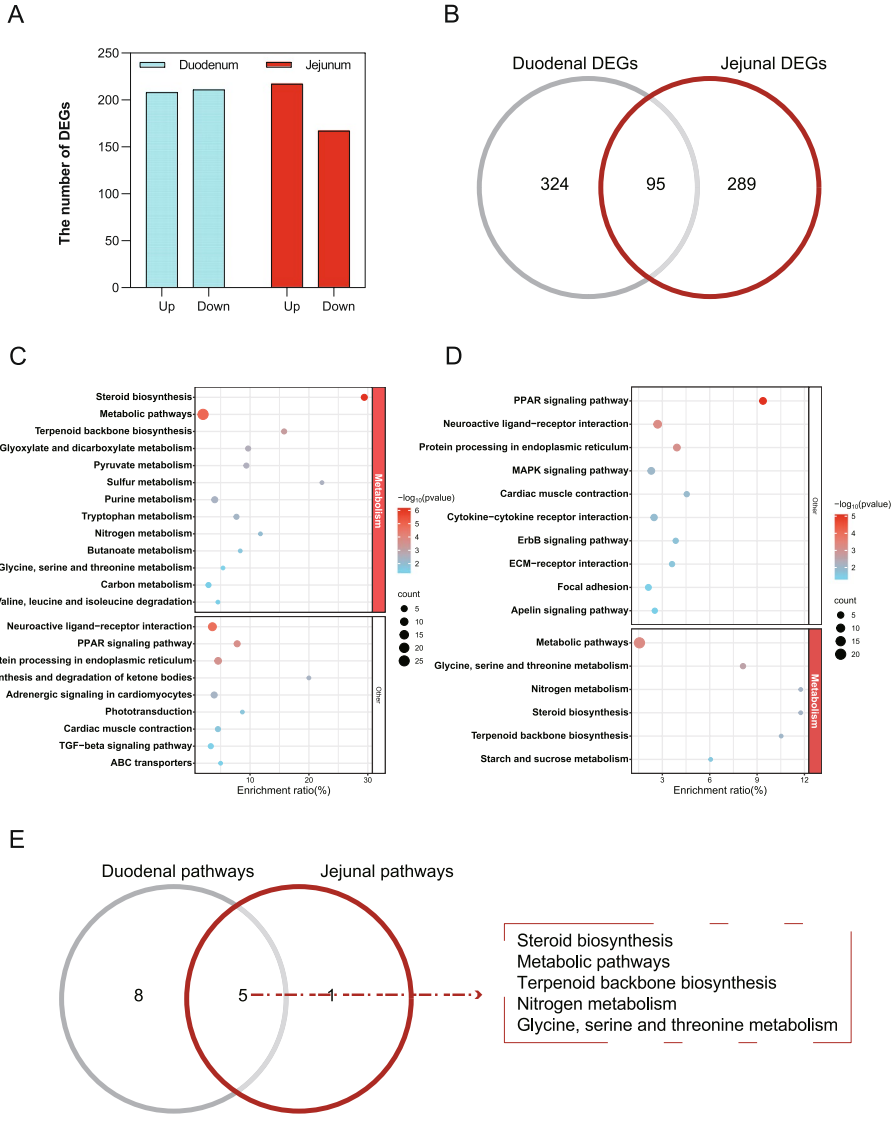

Fig. 4 Identification and functional analysis of DEGs. **A** Number of DEGs identified in the duodenum and jejunum. **B** Identification of unique and shared DEGs. **C** KEGG pathways significantly enriched in the duodenum. **D** KEGG pathways significantly enriched in the jejunum. **E** Co-enriched metabolism-related pathways in the duodenum and jejunum

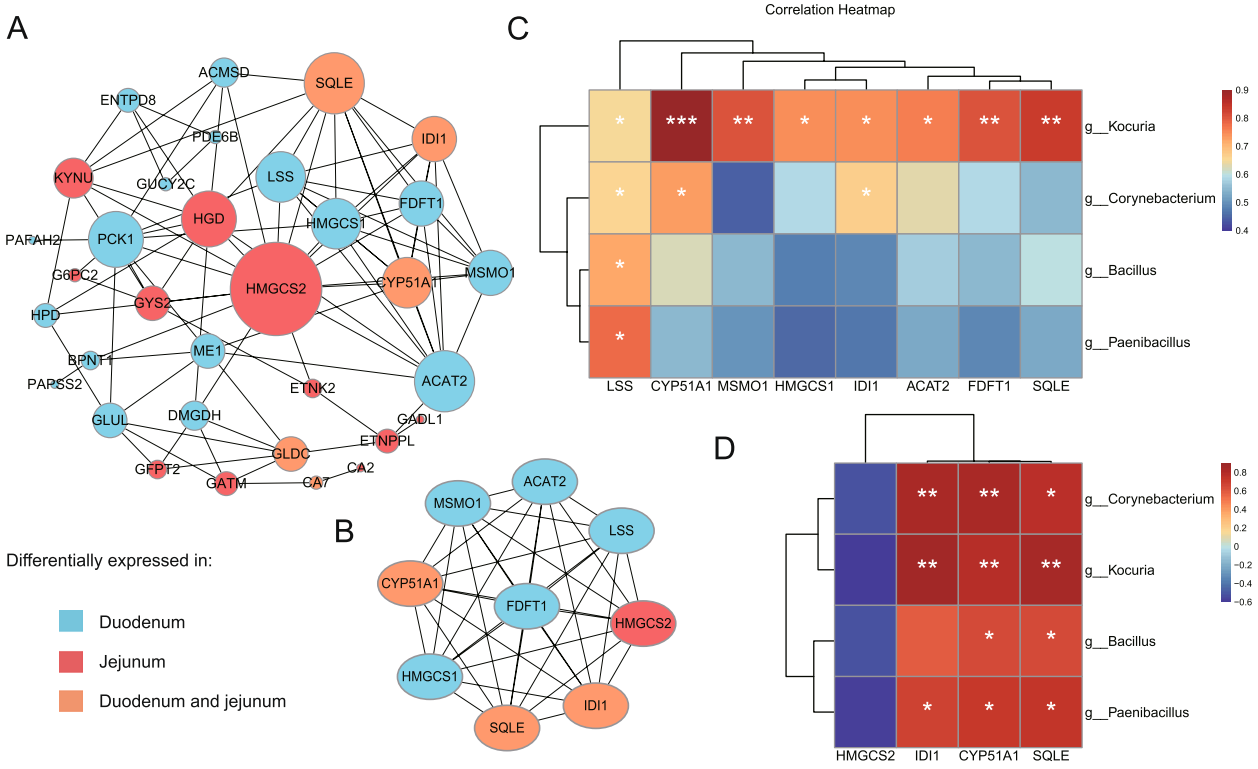

Fig. 5 Crosstalk mechanism prediction. **A** A PPI of DEGs enriched in the five pathways. Color indicated the source of the DEGs. **B** Top-ranking subnetwork extracted by MCODE. **C** Heatmap of correlation between key genera and core genes in the duodenum. **D** Heatmap of correlation between key genera and core genes in the jejunum. Significance levels were denoted as follows: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$

Discussion

RFI is widely used in the livestock industry to evaluate feed efficiency [31, 32]. In our study, low RFI was accompanied by low TFI and FCR, consistent with previous research in poultry [33, 34]. These findings indicated that breeding egg-type ducks with low RFI not only reduced feed consumption but also lowered FCR. Therefore, selecting for RFI is an effective approach to reducing costs and increasing benefits.

Based on 16S rRNA sequencing of duodenal and jejunal digesta, we identified that 77.38% of ASVs in the duodenum were also present in the jejunum. This aligned with previous studies indicating microbial composition similarities between the duodenum and jejunum [35]. Furthermore, we identified four genera differentially abundant in both intestinal segments, all of which exhibited significantly higher abundance in the LH compared to the HH (*Paenibacillus*, *Kocuria*, *Corynebacterium*, and *Bacillus*).

Paenibacillus species are of significant relevance to human, animal, and plant, as well as to the environment [36]. *Paenibacillus* strains can produce exo-polysaccharides with distinct characteristics that exhibit various biological activities, such as antioxidant properties [37]. In chickens, dietary supplementation with *P. xylanexedens* improved intestinal morphology, enhanced immune response, and improved growth performance [38]. In addition, the supplementation significantly reduced FCR [39]. *P. polymyxa* [40–42], *P. konkukensis* [43] and *P. lentus* [44] have also been reported to show potential as probiotics. In contrast, *Kocuria* has been less studied. One study observed a higher abundance of *Kocuria* in the intestines of high egg laying ducks [45]. After controlling for non-significant differences in laying performance between groups, *Kocuria* remained a key genus associated with high feed efficiency, suggesting its positive effects on both production performance and feed efficiency in ducks. *Corynebacterium* can metabolize various carbohydrates and produce organic acids (e.g., lactate and succinate) [46]. It was reported as a dominant genus in all intestinal parts of chickens (duodenum, jejunum, ileum, cecum, and colon), indicating its crucial role in chicken health and growth [47]. Furthermore, *Corynebacterium* was more abundant in the intestines of high feed efficiency (low RFI) chickens [10], which was consistent with our results. Building on this, we found that its abundance was positively correlated with the expression levels of *LSS*, *CYP51A1*, *IDI1*, and *SQLE*, suggesting a potential role in steroid metabolism. It was also reported to be associated with abdominal fat deposition and egg production in ducks [45, 48], and could be used as a feed additive for poultry [49]. Members of *Bacillus*

can bring health benefits to the host and are considered promising probiotics. The high stability of their spores can resist high temperatures during feed processing and harsh environments in the gastrointestinal tract [50]. In broiler chickens, *B. coagulans* has been found to optimize FCR and microbial composition [51]. Moreover, *B. subtilis* was reported to improve growth performance [52]. Similar results were also observed in ducks [53]. Overall, our study identified the key roles of *Paenibacillus*, *Kocuria*, *Corynebacterium*, and *Bacillus* in influencing duck feed efficiency, but specific species remain unidentified. Further validation is needed in subsequent research.

Previous studies showed that microbiota could affect host gene expression, thereby influencing intestinal function [54, 55]. To further elucidate the mechanisms underlying the effects of key genera on the host, we performed RNA-seq on duodenal and jejunal tissues from both LH and HH ducks. In the matched digestive samples, the abundance of these key genera was significantly higher in the LH than the HH. Based on the RNA-seq results, enrichment analysis revealed that more than half (59.09%) of the KEGG pathways in the duodenum were related to metabolism. In the jejunum, the proportion was 37.5%. These results suggested a potential impact of differences in the abundance of key genera on host intestinal metabolism. In both intestinal segments, the enrichment of five pathways (steroid biosynthesis, metabolic pathways, terpenoid backbone biosynthesis, nitrogen metabolism, and glycine, serine and threonine metabolism) indicated a close relationship between this impact and steroid/amino acid metabolism. Steroid metabolism is crucial for ovarian/follicular development [56, 57] and yolk deposition [58] in poultry, profoundly influencing laying performance. Meanwhile, egg albumen formation relies on substantial protein synthesis, which involves multiple amino acid metabolic pathways [59]. Therefore, we speculated that higher abundances of key genera were associated with greater utilization efficiency of these nutrients in ducks.

Furthermore, we identified eight core genes that were likely to be regulated, including *HMGCS1*, *MSMO1*, *ACAT2*, *LSS*, *FDFT1*, *SQLE*, *IDI1*, and *CYP51A1*. 3-hydroxy-3-methylglutaryl-CoA synthase 1 (*HMGCS1*) participates in cytosolic cholesterogenesis [60], and its expression level is closely associated with the animal energy status [61]. As a typical enzyme in cholesterol biosynthesis, MSMO1 catalyzes the demethylation of C4-methylsterol [62]. Previous reports indicated that *MSMO1* was a key gene affecting lipid deposition in chickens [63] and geese [64]. It is worth noting that reducing lipid deposition is one of the ways to improve feed efficiency [65]. The gene *ACAT2* encodes acetyl-CoA acetyltransferase 2. This enzyme was reported to oxidize acetoacetyl-CoA to acetyl-CoA. The resulting acetyl-CoA could then be directed into the TCA cycle to generate energy or toward anabolic reactions that support muscle growth [66]. Also, the lanosterol synthase (*LSS*) enzyme is involved in the synthesis of cellular cholesterol, catalyzing the conversion of (S)−2,3-oxidosqualene to lanosterol [67]. *FDFT1*, *SQLE*, *IDI1*, and *CYP51A1* are also involved in key steps of cholesterol biosynthesis [68–70]. In chickens, *SQLE* is associated with intracellular triglyceride and total cholesterol levels [71]. Consistent with our findings, *ACAT2* and *SQLE* showed differential expression in the duodenum between low- and high- RFI chickens [72]. In addition, previous studies reported that *MSMO1*, *ACAT2*, and *SQLE* were involved in the differential regulation of lipid metabolism in ducks under feed restriction [73]. Overall, the core genes were primarily related to steroid metabolism. It is worth noting that the impact of microbiota on metabolism is systemic [74]. Our research was only a preliminary exploration, and more rigorous validation is needed in the future.

Conclusion

Paenibacillus, *Kocuria*, *Corynebacterium*, and *Bacillus* were identified as key microbiota influencing feed efficiency in ducks. Their abundances were closely associated with the expression of *HMGCS1*, *MSMO1*, *ACAT2*, *LSS*, *FDFT1*, *SQLE*, *IDI1*, and *CYP51A1*. The specific mechanisms require further validation in future studies. These findings provide a preliminary investigation into the intestinal microbiota-host crosstalk affecting feed efficiency and offer novel perspectives for reducing RFI in egg-type ducks.

Abbreviations

RFI	Residual feed intake
TEN	Total egg number
TEW	Total egg weight
TFI	Total feed intake
FCR	Feed conversion ratio
LEfSe	Linear discriminant analysis effect size
DEGs	Differentially expressed genes
KEGG	Kyoto Encyclopedia of Genes and Genomes
PPI	Protein-protein interaction
RNA-seq	Transcriptome sequencing
iBW	Initial body weight
fBW	Final body weight
MBW	Metabolic body weight
ASVs	Amplicon sequence variants
PCoA	Principal coordinate analysis
TPM	Transcripts per million
AEW	Average egg weight
NCBI	National Center for Biotechnology Information

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12864-026-12865-x.

Supplementary Material 1. Table S1 Basic information of 16S rRNA sequencing.

Supplementary Material 2. Table S2 Basic information of RNA-seq.

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Authors' contributions
ZYH: Conceptualization, Methodology, Formal analysis, Writing—Original Draft; XLC: Methodology, Formal analysis, Investigation; SYR: Formal analysis, Investigation; RLL: Investigation, Supervision; HPC: Investigation, Data Curation; KXC: Investigation; XLY: Investigation; JWH: Visualization; HH: Supervision, Visualization; LL: Project administration; HHL: Resources; JWW: Conceptualization, Funding acquisition, Writing—Review & Editing.

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Data availability
The 16S rRNA sequencing and RNA-seq data from this study are available in the National Center for Biotechnology Information (NCBI) under BioProject ID: PRJNA1379160 (https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA1379160).

Declarations

Ethics approval and consent to participate
All animal handling procedures in this study were approved by the Institutional Animal Care and Use Committee (IACUC) of Sichuan Agricultural University (Chengdu, China) (Approval No. DKY20170913).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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