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16S rRNA and transcriptome analysis revealed the regulatory mechanism of *Romboutsia lituseburensis* on serum immunoglobulin levels in geese

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ABSTRACT

Romboutsia is a dominant genus in the goose intestine. Recent studies have suggested that *Romboutsia lituseburensis* might regulate serum immunoglobulin levels in female geese, although the underlying mechanisms remain unclear. In this study, we administered *Romboutsia lituseburensis* (*R. lituseburensis*) orally to female geese, leading to successful colonization of the ileum. Subsequent analysis showed that the levels of IgM, IgA, and IgG in the serum significantly decreased after colonization ($P < 0.01$). 16S rRNA sequencing revealed that *R. lituseburensis* significantly altered the microbial composition and increased the relative abundance of *Jeotgalicoccus* ($P < 0.01$), *Turicibacter*, and *Bacillus* ($P < 0.05$) in the ileum. Transcriptome sequencing further identified 263 differentially expressed genes (**DEGs**) in the ileum (146 upregulated, 117 downregulated) and 725 DEGs in the spleen (300 upregulated, 425 downregulated). Kyoto Encyclopedia of Genes and Genomes (**KEGG**) pathway enrichment analysis revealed that these DEGs were enriched in 17 pathways in the ileum and 21 pathways in the spleen. Notably, the "Intestinal immune network for IgA production" pathway was significantly enriched in the spleen ($P < 0.05$). Further, Short Time-series Expression Miner (**STEM**) analysis grouped the DEGs in these 2 tissues into 49 clusters, with clusters 27 and 29 showing the highest significance and similar expression patterns. Pathway analysis confirmed that the "Intestinal immune network for IgA production" pathway was enriched in both clusters. Furthermore, a protein-protein interaction (**PPI**) network of these 2 clusters, along with correlation analysis between microbiota abundance and gene expression, highlighted *KEL, SERPING1, CALR*, and *OSTN* as key hub genes. Overall, *R. lituseburensis* significantly increased the abundance of *Jeotgalicoccus, Turicibacter*, and *Bacillus* in the ileum. Concurrently, it might downregulate the "Intestinal immune network for IgA production" pathway in the spleen (*CCR9, TNFRSF13B, AICDA*) via *KEL, SERPING1, CALR*, and *OSTN*, thereby contributing to the reduction of serum immunoglobulin levels. These findings offer new insights into how *R. lituseburensis* influences immune function in female geese and provide a theoretical basis for further research into its other physiological roles in geese.

Introduction

The intestinal microbiota is crucial for immune system development, nutrient absorption, and disease resistance enhancement (Shang, et al., 2018), and it profoundly impacts poultry production efficiency (Bae YeonJi, et al., 2017; Siegerstetter, et al., 2017). Notably, *Romboutsia*, a dominant genus in goose intestine (He, et al., 2024; Wen, et al., 2022), has recently attracted attention due to its species *Romboutsia lituseburensis* (*R. lituseburensis*), which has been suggested to influence the reproductive performance of female geese by modulating immune

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functions, including immunoglobulin levels (Ouyang, et al., 2024). However, the underlying mechanisms remain unclear. *R. lituseburensis* is closely associated with ileal immunity (Liu, et al., 2023) and development (Lee, et al., 2023). The ileum, a critical site for the final stages of food digestion and absorption (Hiżewska, et al., 2023), harbors a high density of immune cells, making it a key contributor to intestinal immunity (Liu, et al., 2023). The microbiota interacts with intestinal epithelial cells (Soderholm and Pedicord, 2019) and immune cells (Kayama, et al., 2020), influencing cell proliferation and barrier function (Mahapatro, et al., 2021), with these processes involving various immunoglobulins (Chen, et al., 2020). These interactions support intestinal homeostasis by fostering a robust immune system, which in turn affects poultry growth and development (Ptak, et al., 2015). Continuous bacterial treatments have been shown to induce transcriptional changes in the intestine, shedding light on microbiota-host regulatory mechanisms (van Baarlen, et al., 2009). Oral administration of *Lactobacillus* has been shown to not only impact metabolic and immune processes in the ileum but also potentially influence the crosstalk between immune cells and submucosal adipocytes (Hulst, et al., 2015). Moreover, the oral administration of *Lactobacillus* and *Bacillus* enhanced mucosal intestinal immunity in response to enteropathogens (Perdigon, et al., 1990) and helped maintain the integrity of the mucosal barrier in the ileum (Liu, et al., 2019). In addition, the spleen, as the largest secondary lymphoid organ in the body (Mebius, et al., 2004), plays a crucial role in the intestinal immune response (Wei, et al., 2021). The maturation of innate and adaptive immune responses at mucosal sites depends on microbial exposure (Smith, et al., 2007). The spleen provides precursor cells capable of producing SIgA for the intestines (Weiberg, et al., 2018), helping to maintain intestinal homeostasis. Correspondingly, the intestinal microbiota also influences spleen development (Fang, et al., 2022; Rosado, et al., 2018; Zhang, et al., 2023).

Despite the crucial role of the microbiota in poultry immune function, few studies have measured the impact of individual strains on immune parameters, intestinal microbiota, and the transcription of related tissues. In this study, female geese were orally administered *R. lituseburensis* to promote intestinal colonization, followed by an evaluation of serum immunoglobulin levels after colonization. To explore the underlying mechanisms, we utilized 16S rRNA and transcriptome sequencing to analyze changes in the ileal microbial composition as well as transcriptional alterations in the ileum and spleen. These findings will provide new insights into how *R. lituseburensis* influences immune function in female geese and provide a theoretical basis for further research into its other physiological roles in geese.

Materials and methods

Animal ethics statement

The animal handling protocols for this study were authorized by the Institutional Animal Care and Use Committee (**IACUC**) of Sichuan Agricultural University (Chengdu Campus, Sichuan, China), under License No. DKY20170913. A total of 90 adult female geese were sourced from the Waterfowl Breeding Experimental Base at Sichuan Agricultural University (Ya'an, Sichuan Province, China).

Antibiotic pre-treatment and oral administration of Romboutsia lituseburensis

Before the gavage of *R. lituseburensis*, all geese were orally administered 2 mL of an antibiotic mixture for 5 consecutive days to promote colonization of *R. lituseburensis*. The antibiotic mixture contained ampicillin (30 mg mL^{-1}), metronidazole (30 mg mL^{-1}), neomycin (30 mg mL^{-1}), and vancomycin (15 mg mL^{-1}). Following this, the control group (45 geese) received 2 mL of anaerobic saline orally for 14 consecutive days, while the experimental group (**Rl**) (45 geese) received 2 mL of *R. lituseburensis* strain (10^9 CFU mL^{-1}, Guangdong Microbial Culture Collection Center, No. 1.2509) orally for the same duration.

Sample collection and serum immunoglobulin levels assessment

Following 14 days of oral administration, blood samples were collected from the wing vein of 90 geese via venipuncture. The collected blood samples were then centrifuged at 4000 rpm for 5 min to separate the serum. The resulting serum was carefully collected and transferred to a clean tube for further analysis. Serum concentrations of IgA, IgG, and IgM were quantified using goose-specific immunoglobulin ELISA kits (Huding, Shanghai, China). Geese were randomly selected for slaughter, with 4 from the control group and 5 from the experimental group. The ileal contents were subsequently collected for 16S rRNA sequencing. Additionally, ileum and spleen tissues were collected, and 3 random samples from each group were selected for transcriptome sequencing.

16S rRNA sequencing and bioinformatics analysis

Following the manufacturer's instructions, microbial genomic DNA from the ileum was extracted using the E.Z.N.A. Stool DNA Kit (Omega Bio-Tek, Norcross, GA, USA). DNA concentration and purity were assessed using a NanoDrop2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), and DNA integrity was checked via 1 % agarose gel electrophoresis. Samples meeting the standards were used for subsequent experiments. The microbial 16S rRNA gene's V3-V4 hypervariable regions were amplified using ABI GeneAmp® 9700 (Life Technologies, Foster City, CA, USA) with primers 338-F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806-R (5′- GGACTACHVGGGTWTCTAAT-3′). PCR products were analyzed using 2 % agarose gel electrophoresis, and the products were quantified using the QuantiFluor™ -ST blue fluorescence quantification system (Promega, Madison, WI, USA). Subsequently, the products were mixed in the required proportions according to the sequencing demands for each sample. The resulting products were used to construct MiSeq libraries, which were then sequenced on the Illumina MiSeq platform (Illumina, San Diego, CA, USA). To ensure data quality, comprehensive quality control of the sequencing data was performed using fastp (Chen, et al., 2018) and FLASH (Magoč and Salzberg, 2011). Assembled reads were processed and assigned using QIIME2 software (Bolyen, et al., 2019). Amplicon sequence variants (**ASVs**) were identified using the denoise-paired method in DADA2. Taxonomic annotations were performed with the SILVA 138 database (Quast, et al., 2013), classifying sequences at the kingdom, phylum, class, order, family, and genus levels. Alpha and beta diversity metrics were calculated in QIIME2, and principal coordinate analysis (**PCoA**) was conducted based on Bray-Curtis distances. Microbial composition differences between groups were analyzed using Linear Discriminant Analysis Effect Size (**LEfSe**) (Segata, et al., 2011), applying selection criteria of LDA scores > 3 and $P < 0.05$.

Transcriptome sequencing and bioinformatics analysis

Following the manufacturer's instructions, total RNA was extracted from ileum and spleen using the MJZol Total RNA Extraction Kit (Majorbio, Shanghai, China). RNA purity and concentration were measured using a NanoDrop2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), and RNA integrity was assessed using the Agilent 5300 system (Agilent Technologies, Santa Clara, CA, USA), with an average RQN value of 9.38 across all samples. Libraries were prepared with the Illumina TruSeq™ RNA Sample Prep Kit and sequenced as 2 × 150 bp paired-end reads on the Illumina HiSeq™ platform . The RNA-seq data from this study were accessible under BioProject ID PRJNA1154399 at the the National Center for Biotechnology Information (**NCBI**). Low-quality reads were filtered with Fastp (Chen, et al., 2018), and clean reads were aligned to the unpublished goose reference genome (BioProject ID PRJNA801885) using HISAT2

(Kim, et al., 2015). The resulting SAM files were converted to BAM format and sorted with SAMtools. Gene expression levels were quantified using featureCounts (Liao, et al., 2014) and normalized to transcripts per million (**TPM**). DESeq2 (Love, et al., 2014) was utilized to identify differentially expressed genes (**DEGs**) between groups, using thresholds of $|\text{Log}_2(\text{FC})| \geq 1$ and $P < 0.05$. Gene Ontology (**GO**) and Kyoto Encyclopedia of Genes and Genomes (**KEGG**) functional enrichment analysis were performed using KOBAS (Bu, et al., 2021) (*Gallus gallus*, http://kobas.cbi.pku.edu.cn/kobas3/?t=1). Short Time-series Expression Miner (**STEM**) program was used to analyze gene expression patterns (Ernst and Bar-Joseph, 2006). The protein-protein interaction (**PPI**) network was retrieved from the STRING database (Szklarczyk, et al., 2019) (http://string-db.org/) and visualized using Cytoscape (Doncheva, et al., 2019).

Statistical analysis

Differences in the abundance of *Romboutsia* and serum immunoglobulin levels between groups were analyzed using the Student's *t* tests in SPSS 27.0. The results were visualized using GraphPad Prism 9 software. Correlations were assessed using Spearman's correlation coefficient. A *P*-value less than 0.05 was considered statistically significant.

Results and analysis

The effect of romboutsia lituseburensis on serum immunoglobulin levels

The 16S rRNA sequencing results of the ileum showed that the abundance of *Romboutsia* in the Rl group was significantly higher than that in the control group ($P < 0.05$) (Fig. 1A). As the abundance of *Romboutsia* significantly increased, a downward trend in serum immunoglobulins was observed. The levels of IgM, IgA, and IgG in the Rl group were significantly lower than those in the control group ($P < 0.01$) (Fig. 1B).

The effect of Romboutsia lituseburensis on ileal microbial structure

A total of 463 ASVs were identified, with 161 ASVs shared between the 2 groups, 72 ASVs unique to the control group, and 230 ASVs unique to the Rl group (Fig. 2A). These ASVs were annotated to 13 phyla, 21 classes, 45 orders, 80 families, and 123 genera.

At the phylum level, the dominant phyla in the control group were *Firmicutes* (88.85 %), *Actinobacteriota* (8.80 %), and *Proteobacteria* (2.16 %). In the Rl group, the dominant phyla were *Firmicutes* (87.50 %), *Actinobacteriota* (9.27 %), and *Bacteroidota* (1.79 %) (Fig. 2B). At the genus level, the top 3 genera in the control group were *Lactobacillus* (83.24 %), *Corynebacterium* (5.85 %), and *Staphylococcus* (2.39 %). Meanwhile, the most abundant genera in the Rl group were *Lactobacillus* (75.64 %), *Staphylococcus* (5.32 %), and *Corynebacterium* (2.60 %) (Fig. 2C). Alpha diversity analysis revealed that the Rl group had slightly higher Chao1, evenness, Faith-PD, observed features, and Shannon indices compared to the control group (Table 1). Additionally, Bray-Curtis-based PCoA results showed distinct clustering of microbial communities in the ileum within each group. The PERMANOVA test further indicated a significant difference in ileal microbial communities between the groups (pseudo-F = 2.72, $P = 0.016$) (Fig. 2D).

The LEfSe analysis indicated that, at the genus level, the Rl group had significantly higher abundances of *Romboutsia, Jeotgalicoccus, Turicibacter*, and *Bacillus* compared to the control group (LDA score > 3, $P < 0.05$) (Fig. 2E-F). Furthermore, *Romboutsia* exhibited a significant positive correlation with *Jeotgalicoccus* ($P < 0.01$), *Turicibacter*, and *Bacillus* ($P < 0.05$) (Fig. 2G).

RNA-Seq data quality control and differentially expressed genes identification

In this study, we constructed 12 cDNA libraries, generating a total of 153,092,418 raw reads (averaging 25,515,403 per sample) from the ileum and 152,488,547 raw reads (averaging 25,414,758 per sample) from the spleen. After stringent quality control, we obtained a total of 302,282,913 high-quality reads. The Q20 and Q30 values ranged from 97.90 % to 98.28 % and from 94.51 % to 95.32 %, respectively. Additionally, the average GC content and mapping rate were 47.44 % and 93.97 %. Overall, the sequencing quality met the requirements for subsequent analysis (Additional file: Table S1).

The results of principal component analysis (**PCA**) showed that there was some overlap in the ileal gene expression patterns between the control and Rl groups, but there were still differences. Meanwhile, the gene expression patterns in the spleen showed significant intergroup differences (Fig. 3A). Furthermore, we identified 263 DEGs in the ileum, including 146 upregulated genes and 117 downregulated genes. In the spleen, we identified 725 DEGs, comprising 300 upregulated genes and 425 downregulated genes (Fig. 3B). The expression patterns of DEGs in the ileum and spleen were shown in Fig. 3C and D.

Functional analysis of differentially expressed genes

A total of 199 GO terms were significantly enriched in the ileum ($P < 0.05$), among which T-tubule, protein-glutamine gamma-glutamyltransferase activity, and extracellular space showed the highest significance. The top 30 GO terms were shown in Fig. 4A, including the immune-related term "complement activation"(*C6* and *RGCC*). Additionally, 354 GO terms were significantly enriched in the spleen ($P < 0.05$), with the top 30 GO terms presented in Fig. 4C (Additional file:

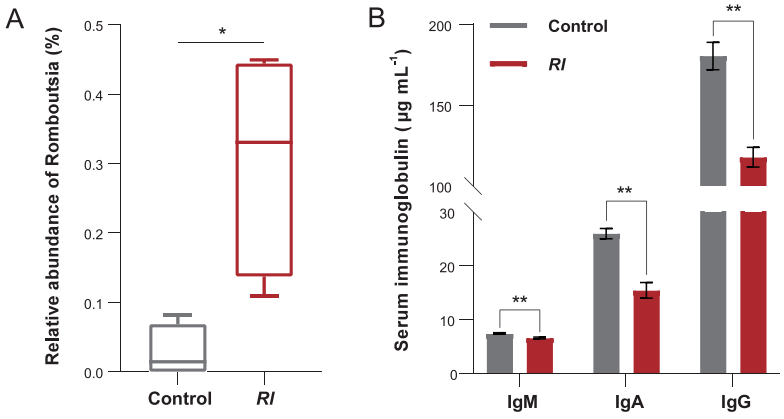

Fig. 1. Evaluation of *Romboutsia* abundance in the ileum (A) and serum immunoglobulin levels (B).

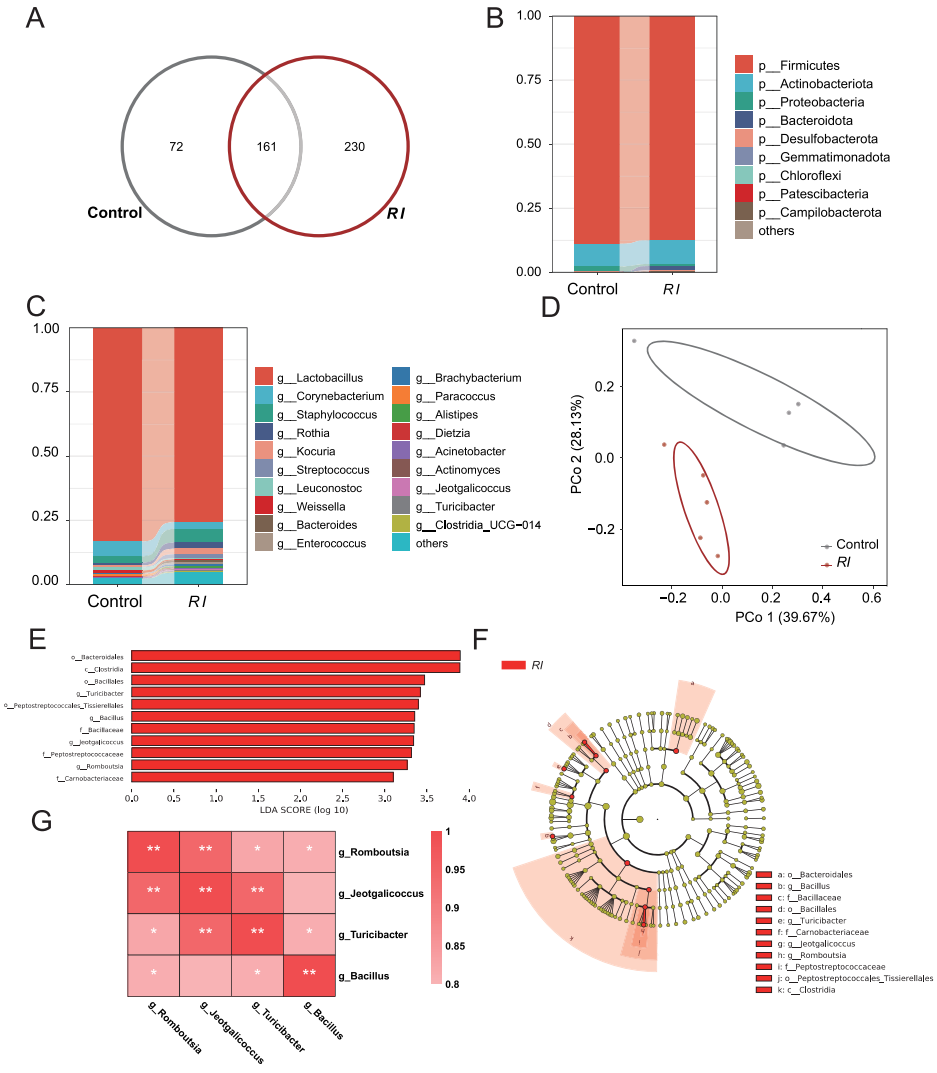

Fig. 2. ***16S rRNA sequencing analysis.*** (A) Venn diagram of ASVs. (B) Microbial composition at the phylum level. (C) Microbial composition at the genus level. (D) PCoA based on Bray-Curtis distances. (E) Differential microbiota between groups. (F) Cladogram of differential microbiota. (G) Correlation analysis of differential genera.

Table 1
Alpha diversity analysis results

Indices	Control	Rl	*P*-value
Chao1	81.5 ± 52.87	150.8 ± 53.20	0.085
evenness	0.45 ± 0.15	0.56 ± 0.08	0.221
Faith-PD	4.34 ± 1.82	7.88 ± 2.47	0.142
observed features	81.5 ± 52.87	150.8 ± 53.20	0.085
Shannon	2.87 ± 1.41	4.03 ± 0.78	0.142

Table S2).

KEGG enrichment analysis showed that 17 pathways were enriched in the ileum (Fig. 4B) and 21 pathways were enriched in the spleen (Fig. 4D). In the ileum, the enriched pathways were primarily related to environmental information processing, including ECM-receptor interaction, neuroactive ligand-receptor interaction, apelin signaling pathway, MAPK signaling pathway, calcium signaling pathway, and VEGF signaling pathway. Additionally, pathways associated with metabolism, such as arginine biosynthesis, tyrosine metabolism, metabolic pathways, arginine and proline metabolism, and arachidonic acid metabolism, were also identified. Furthermore, immune system-related pathways, including the Toll-like receptor signaling pathway, were significantly enriched. In the spleen, most pathways were also related to metabolism. Notably, the pathway "Intestinal immune network for IgA production," which is directly associated with serum immunoglobulin levels, was enriched (*CCR9, TNFRSF13B*, and *AICDA*).

Short Time-series Expression Miner analysis

Through STEM analysis, the DEGs in the ileum and spleen were divided into 49 clusters, with genes in each cluster showing similar expression patterns. Among them, a total of 9 significant clusters were presented in Fig. 5A. Notably, clusters 27 and 29 exhibited the highest significance and displayed similar expression patterns (Fig. 5A),

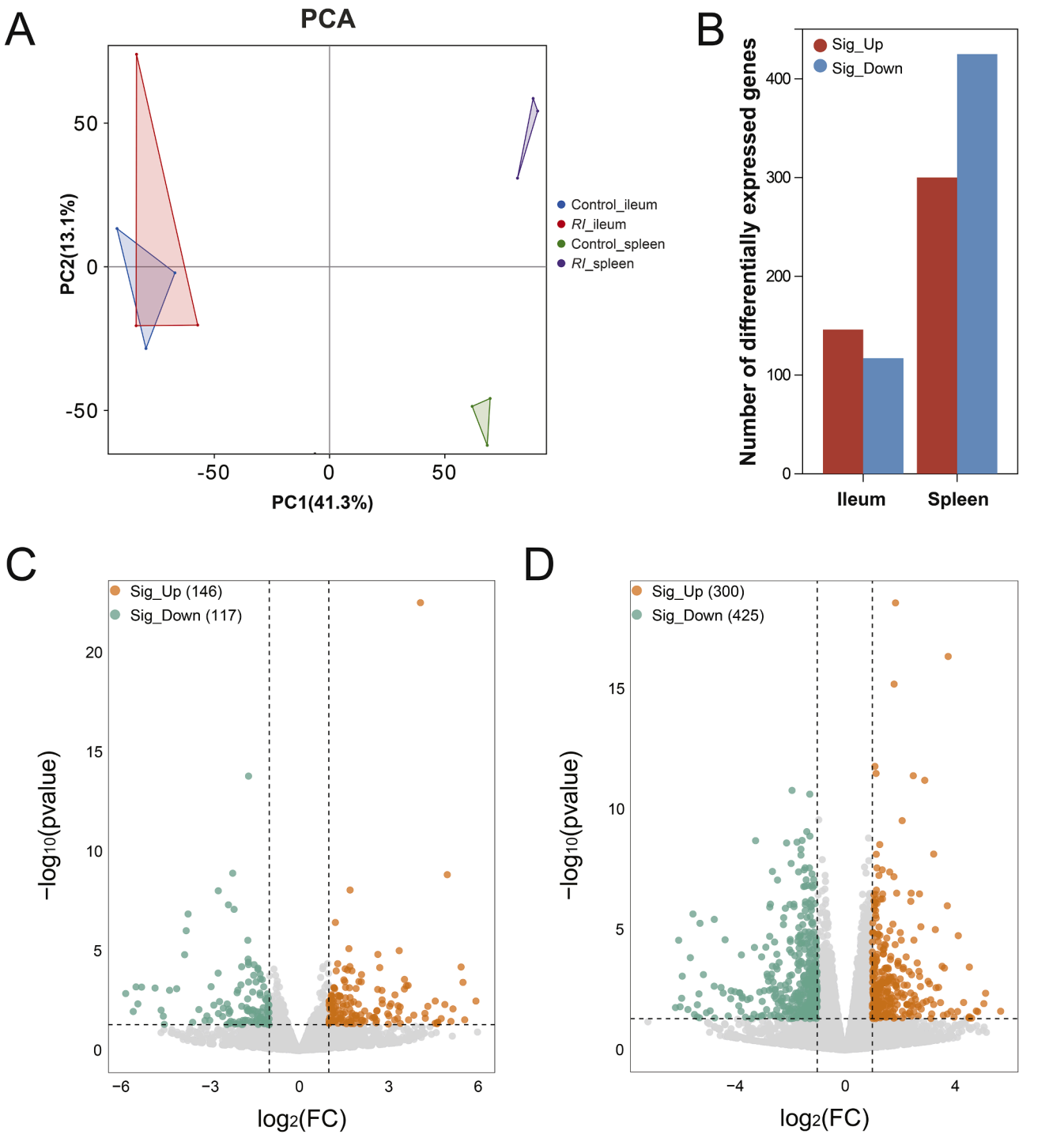

Fig. 3. ***Transcriptome sequencing analysis.*** (A) PCA of transcriptome profiling. (B) DEGs identification. (C) Ileal DEGs volcano map. (D) Splenic DEGs volcano map.

suggesting they may be involved in similar functions. The KEGG enrichment analysis revealed that 13 and 6 signaling pathways were significantly enriched in clusters 27 and 29, respectively ($P < 0.05$). The "Intestinal immune network for IgA production" pathway was enriched in both clusters (Fig. 5B and C).

Identification of key hub genes

We constructed a PPI network using DEGs from clusters 27 and 29. The color of each circle indicated the tissue in which the corresponding gene was differentially expressed. (Fig. 6A). *KEL, SERPING1, CALR, SERPINB10, TGM3, CCL4, EOMES, GVINP1*, and *OSTN* were differentially expressed in both the ileum and spleen. To reveal the potential regulatory relationships between *R. lituseburensis*, ileal microbiota, ileal genes, splenic genes, and serum immunoglobulins, we performed a Spearman correlation analysis. As shown in Fig. 6B, *R. lituseburensis* significantly upregulated the abundance of *Jeotgalicoccus* ($P < 0.01$), *Turicibacter*, and *Bacillus* ($P < 0.05$) in the ileum. Meanwhile, *KEL, SERPING1, CALR*, and *OSTN* were not only differentially expressed in both tissues, but their expression levels were also significantly correlated with the relative abundance of *Romboutsia*.

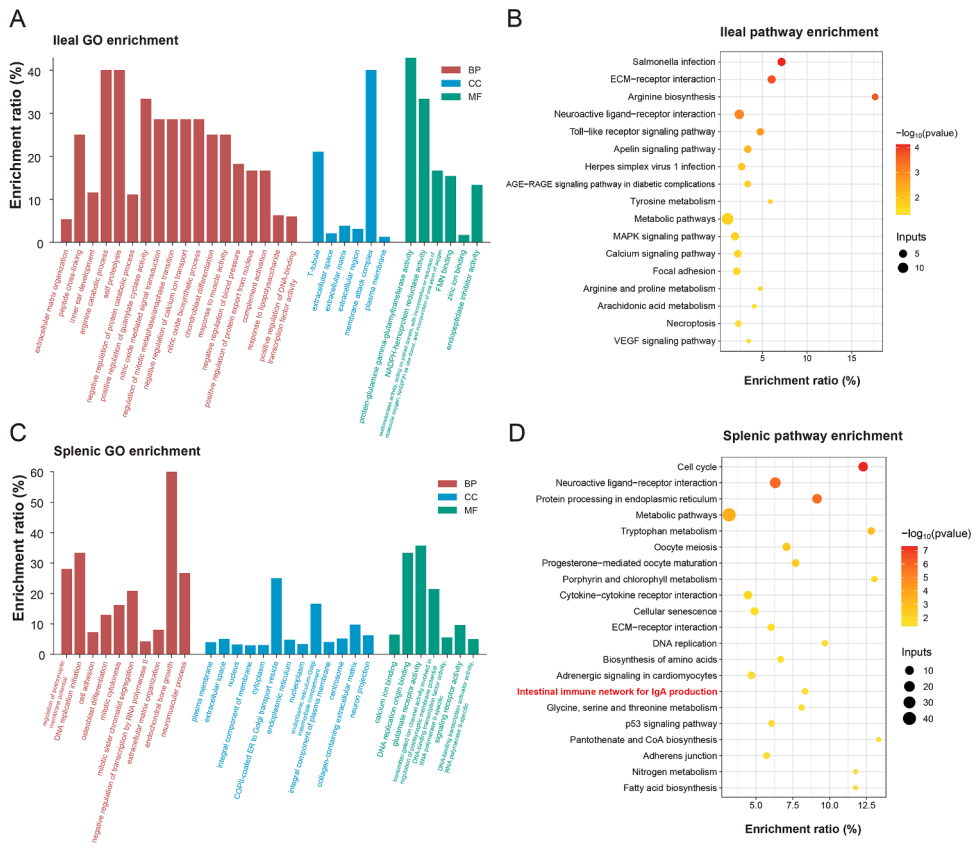

Fig. 4. ***Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) functional enrichment analysis.*** (A) Top 30 GO terms enriched by DEGs in the ileum. (B) Significantly enriched KEGG pathways in the ileum. (C) Top 30 GO terms enriched by DEGs in the spleen. (D) Significantly enriched KEGG pathways in the spleen.

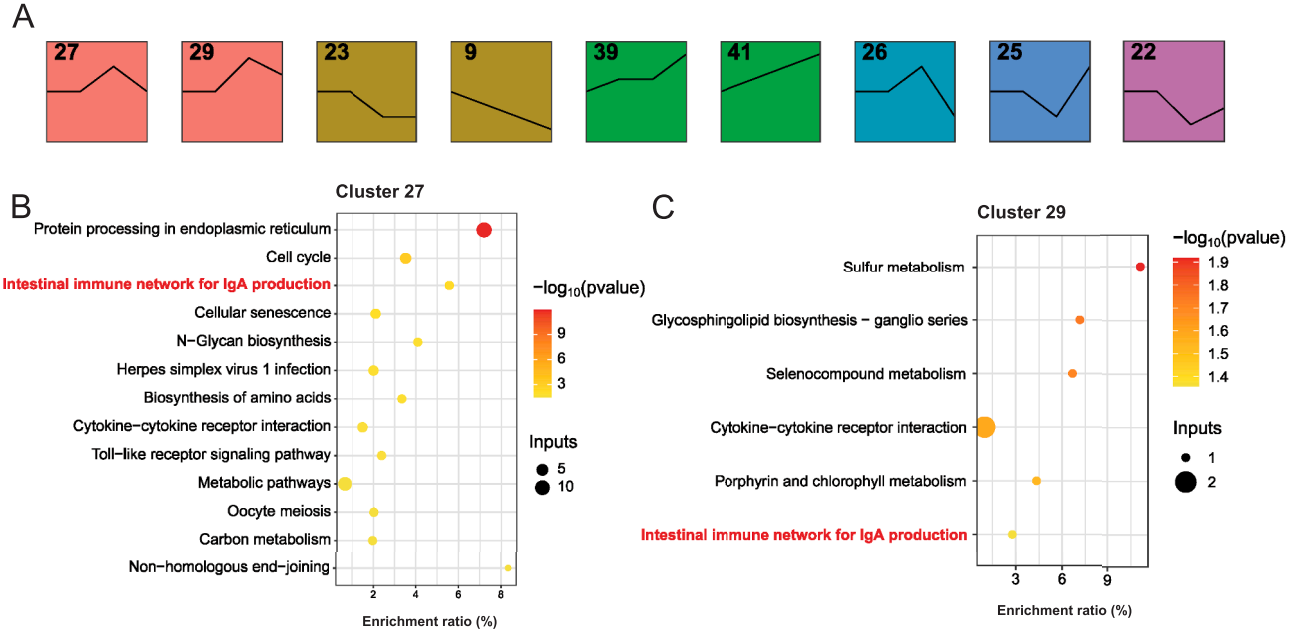

Fig. 5. ***Short Time-series Expression Miner (STEM) analysis.*** (A) Significant clusters. (B) KEGG pathways significantly enriched in cluster 27. (C) KEGG pathways significantly enriched in cluster 29.

Discussion

Romboutsia is commonly found in the intestines of mammals and was recently discovered in poultry (Glendinning, et al., 2019; Johnson, et al., 2019; Qiao, et al., 2019). As the dominant genus in the goose intestine, it may be of significant importance to geese (He, et al., 2024; Wen, et al.,

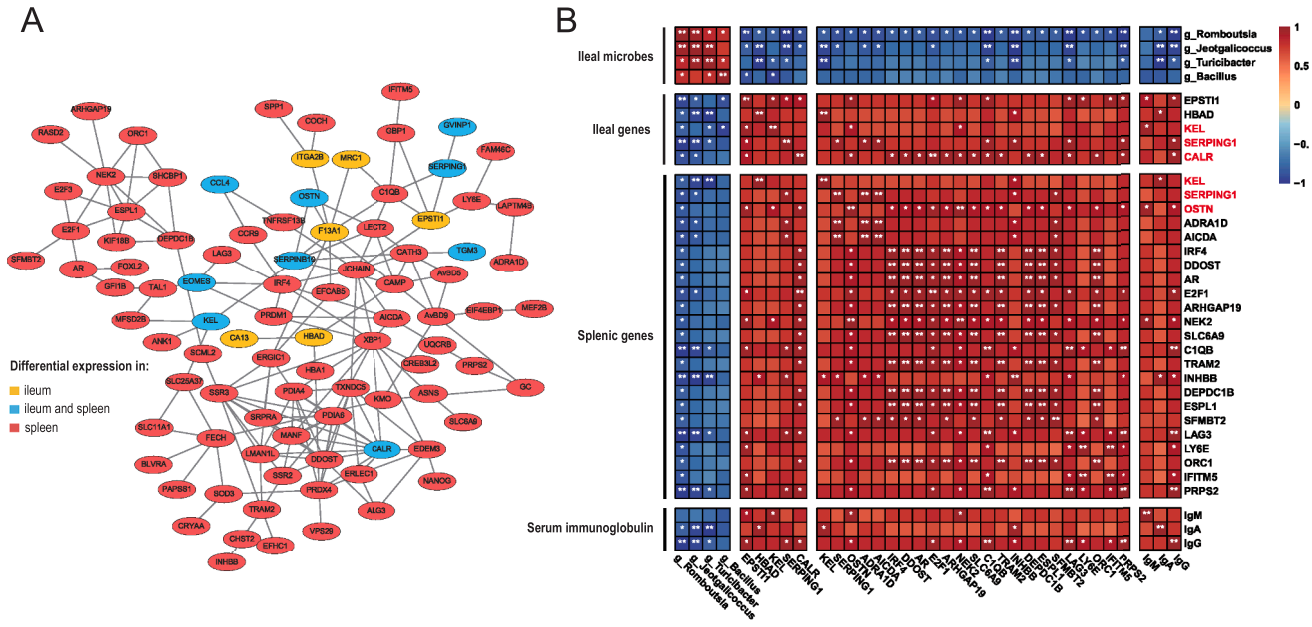

Fig. 6. ***Identification of key hub genes.*** (A) The PPI network of DEGs in clusters 27 and 29. (B) Spearman correlation analysis.

2022). In this study, *R. lituseburensis* significantly reduced the levels of IgM, IgA, and IgG in goose serum, indicating a potential negative regulatory effect on immune function.

To explore the underlying regulatory mechanism, we first performed 16S rRNA sequencing on ileal contents. *R. lituseburensis* significantly altered the microbial composition in the ileum and significantly increased the abundance of *Jeotgalicoccus, Turicibacter*, and *Bacillus*. *Jeotgalicoccus* is a Gram-positive coccus that has been repeatedly reported in poultry houses and is widely distributed (Bindari, et al., 2021; Martin, et al., 2011; Qu, et al., 2024). Additionally, *Jeotgalicoccus* is present in the chicken ileum, and its abundance negatively correlates with the ratio of Helper T and Cytotoxic T cells, potentially affecting intestinal immunity (Song, et al., 2022a). *Turicibacter*, an anaerobic bacterium found in the intestines of various species, is associated with sex hormones (Wu, et al., 2022). Furthermore, research on chickens show its complex response to bile and bile acids, involving multiple protein pathways (Maki, et al., 2022). In chickens with necrotic enteritis, the abundance of *Turicibacter* in the ileum was significantly increased (Song, et al., 2022b), and similar results were observed in the intestines of mice with DSS-induced colitis (Zhang, et al., 2021), suggesting that it could be an intestinal pathogen. In contrast, *Bacillus* may function as a probiotic(Gao, et al., 2017; Knap, et al., 2010; Nguyen, et al., 2015). Our study, for the first time, highlighted a strong association between *Romboutsia, Jeotgalicoccus, Toricibacter*, and *Bacillus* in regulating immune function in geese. However, the exact functional network is complex and requires further experimental validation.

Through transcriptome sequencing, a total of 17 and 21 KEGG pathways were enriched in the ileum and spleen, respectively. Notably, the “Intestinal immune network for IgA production” pathway was differentially expressed in the spleen. Subsequent STEM analysis of DEGs in the ileum and spleen revealed that clusters 27 and 29, which exhibited similar expression patterns, were both enriched in this pathway, highlighting a strong association between the genes in these clusters and our phenotype. Among them, *KEL, SERPING1, CALR*, and *OSTN* were further identified as key genes. *KEL*, the gene responsible for the Kell blood group, is primarily expressed in erythrocytes and tissues (Cartron, et al., 1998). It shows higher transcriptional activity in erythrocytes and basic transcriptional activity in non-erythrocytes (Camara-Clayette, et al., 2001). Recent studies have highlighted its involvement in immune responses related to erythrocytes and the complement system (Mener, et al., 2018; Patel, et al., 2018). *SERPING1*, which encodes the C1 inhibitor protein, plays a critical role in complement regulation by inhibiting the activation of C1s and C1r (Su, et al., 2024). Beyond its role in the complement system, *SERPING1* is also involved in regulating the coagulation, contact, and fibrinolytic systems (Cicardi, et al., 2005). In chickens, bioactive substances like probiotics have been shown to influence the expression and methylation of the *SERPING1* gene in the cecal tonsils (Dunislawska, et al., 2023). Furthermore, differential expression of the *SERPING1* gene was observed in the spleens of immunosuppressed chickens (Guo, et al., 2020). In geese, *SERPING1* is involved in the complement and coagulation cascade processes (Hu, et al., 2024). *CALR* encodes an endoplasmic reticulum-resident protein, calreticulin, which is involved in a variety of cellular processes (Fucikova, et al., 2021), including antigen processing and presentation (Gold, et al., 2010). *OSTN* encodes osteocrin, which is closely related to bone development (Moffatt, et al., 2007). Its role in the avian immune system has been rarely mentioned. In our study, *KEL, SERPING1, CALR*, and *OSTN* in the ileum and spleen appeared to mediate the effects of *R. lituseburensis* and act as key hub genes.

Additionally, the “Intestinal immune network for IgA production” pathway, which was downregulated in the spleen, included 3 key genes: *CCR9, TNFRSF13B*, and *AICDA*. *CCR9* encodes the chemokine (C-C motif) receptor 9, which induces the chemotaxis of IgA^{+} plasma cells, promoting their migration to the small intestine (Pabst, et al., 2004). *CCR9* is also expressed on T cells (Li, et al., 2022) and dendritic cells (Hadeiba, et al., 2008), thereby regulating the immune response. The *TNFRSF13B* gene encodes a lymphocyte-specific member of the tumor necrosis factor (TNF) receptor superfamily. In humans, variants of *TNFRSF13B* are significantly associated with serum levels of IgG and IgM (Liao, et al., 2012; Osman, et al., 2012). Moreover, studies have shown that *TNFRSF13B* is expressed differently in the spleens of goslings and adult geese, and is involved in the development of the goose immune system (Wang, et al., 2015). The *AICDA* gene, which encodes activation induced cytidine deaminase, has been repeatedly reported to be closely associated with hyper-IgM syndrome (Aghamohammadi, et al., 2009; Fazel, et al., 2017). In chickens, it initiates the conversion of immunoglobulin gene in B cells (Luo and Tian, 2010). In our study, we

speculated that their downregulation in the spleen, which subsequently affected serum immunoglobulin levels, resulted from changes in the expression of *KEL, SERPING1, CALR*, and *OSTN*.

In conclusion, *R. lituseburensis* significantly increased the abundance of *Jeotgalicoccus, Turicibacter*, and *Bacillus* in the ileum. Concurrently, it might downregulate the "Intestinal immune network for IgA production" pathway in the spleen (*CCR9, TNFRSF13B, AICDA*) via *KEL, SERPING1, CALR*, and *OSTN*, thereby contributing to the reduction of serum immunoglobulin levels.

Additional file Table S1. Basic information on sequencing data of all samples in this study

Additional file Table S2. The GO terms significantly enriched by the DEGs

Additional file Table S3. The KEGG pathways significantly enriched by the DEGs

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Declaration of competing interest

None.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psj.2025.105018.

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