Animal Microbiome

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Potential role of key rumen microbes in regulating host health and growth performance in Hu sheep

Ximei Xie[1], Huan Yang[1], Xingang Zhao[1], Li Teng[1], Yuze Yang[2*] and Hailing Luo[1*]

Abstract

Background Average daily gain (ADG) is an important component affecting the profitability of sheep. However, research on the relationship between rumen microbes and sheep growth phenotype is still very lacking. Therefore, in this study, 16 Hu sheep were selected from a cohort of 318 sheep assigned to the same feeding and management conditions, and divided into high growth rate (HADG, n = 8) group and low growth rate (LADG, n = 8) group according to the extreme ADG value. Then, the differences in rumen microbes, rumen fermentation and animal immune parameters were further compared between groups to explore the potential role of rumen key microbes in regulating the health and growth performance of Hu sheep hosts.

Results The results showed that specific pathogenic bacteria associated with ADG, including *Anaerotruncus*, *Sediminibacterium* and *Glaesserella*, exhibited significant correlations with interleukin-6 (IL-6) and immunoglobulin G (IgG). These interactions disrupt immune homeostasis in the host, leading to a metabolic prioritization of energy resources toward immune responses, thereby impairing growth and development. *Succinivibrio_dextrinosolvens* was enriched in HADG sheep and exhibited a significant positive correlation with propionate levels. This promoted propionate production in the rumen, enhancing the metabolic activity of carbohydrate, amino acid and energy metabolism, ultimately contributing to higher ADG in sheep. Importantly, random forest analysis results showed that *Succinivibrio_dextrinosolvens* could classify sheep into HADG and LADG with a prediction accuracy of 81.2%. Additionally, we identified 34 bacteria belonged to connectors in the HADG co-occurrence network, including *Alloprevotella*, *Phascolarctobacterium*, *Anaerovibrio*, *Butyricicoccus*, *Ruminococcaceae_noname*, and *Roseburia*, etc., which play an important role in the degradation of carbohydrates and convert them into short-chain fatty acids (SCFAs), maintaining rumen health, and modulating inflammation.

Conclusions In summary, key microbes in the rumen affect the overall healthy homeostasis and rumen fermentation of the host, leading to changes in energy utilization, which in turn affects the average daily gain of Hu sheep. *Succinivibrio_dextrinosolvens* is a promising biomarker for selecting high growth rate sheep in the future. This study provides a new method to manipulate rumen bacteria to improve growth performance in sheep.

Keywords Sheep, Average daily gain, Rumen bacteria, Rumen fermentation immune homeostasis, Metagenome sequencing, Random forest

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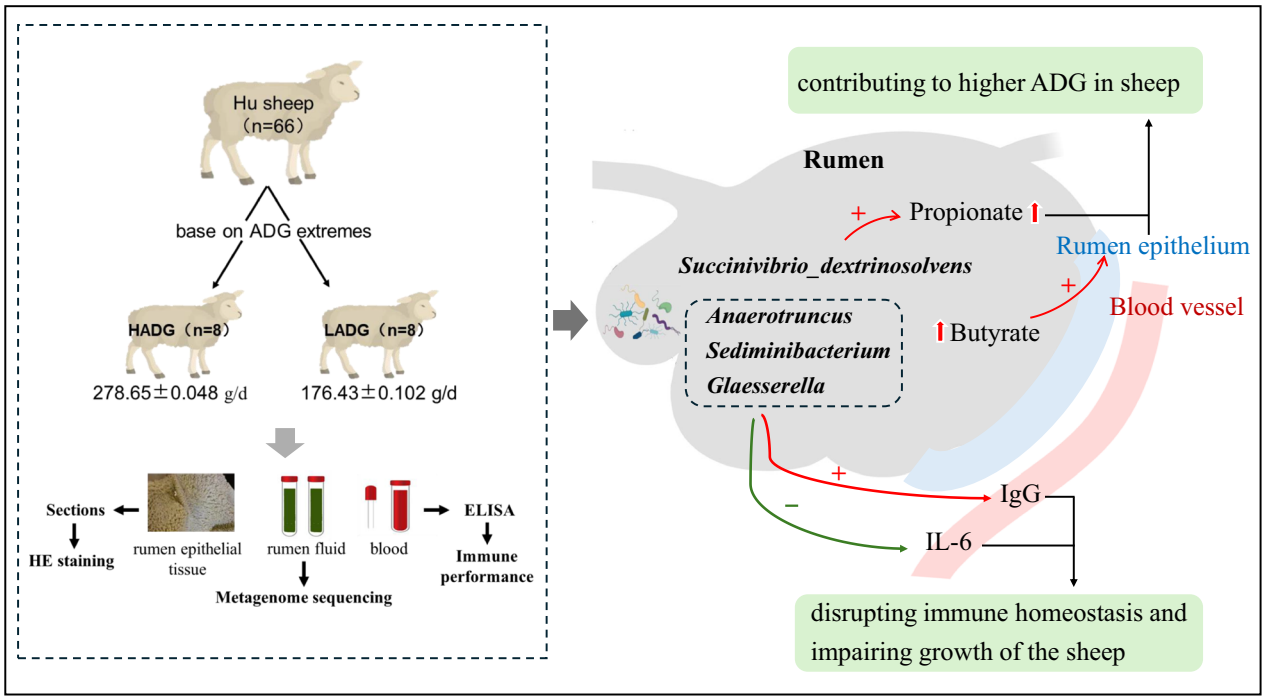
Graphic abstract



Introduction

Growth performance is a core driver of economic efficiency in the meat sheep industry, where average daily gain (ADG)—a critical indicator reflecting the growth potential of lambs from birth to weaning and post-weaning up to six months of age—largely determines the profitability of farming operations [5]. Phenotypic variation in ADG is not only influenced by genetic background and dietary nutrient levels but is also closely linked to the dynamic equilibrium of host-microbe interaction networks. The rumen microbiota, often termed the "second genome" of the host, plays a pivotal role in physiological processes such as nutrient metabolism, immune modulation, and energy allocation [58, 70, 71, 78], offering a breakthrough perspective for deciphering host growth heterogeneity. Nevertheless, further research is imperative to advance this field.

Growing evidence highlights the intricate connections between rumen microbiota and host health and productivity [20, 38]. Rumen microbiota transplantation has been shown to reshape endogenous microbial structures and enhance growth performance in calves and lambs [6, 92]. Previous studies have revealed that specific functional microbial taxa optimize host growth through metabolic network remodeling by comparing rumen microbiota between high- and low-efficiency ruminants. Specifically, members of the *Succinivibrionaceae* family competitively inhibit hydrogen utilization pathways in methanogens while stimulating the succinate synthesis pathway [56], thereby improving ADG and feed efficiency [12, 16, 96, 97]. *Ruminobacter amylophilus* and *Prevotella ruminicola* enhance feed digestion and protein degradation capabilities in the rumen of Holstein dairy cows, thereby improving feed efficiency [17]. The abundance of *Ruminococcaceae_NK4A214_* group is positively correlated with lipid-soluble vitamin absorption regulation. Through fatty acid metabolism pathways [94], this taxon enhances host immune function and demonstrates tight associations with improved feed efficiency in low residual feed intake (RFI) Angus cattle [50, 51]. Previous studies have predominantly focused on how gastrointestinal microbiota influence bovine metabolic activities, thereby contributing to variations in host feed efficiency, whereas significant microbial divergences exist between cattle and Hu sheep. Emerging evidence indicates that Hu sheep with divergent feed efficiency exhibit distinct rumen microbiota profiles [96, 97]. Specifically, *Prevotella* and *Mitsuokella*, as key players

in carbohydrate degradation and fermentation, critically contribute to acetate biosynthesis, thereby enhancing feed efficiency in Hu sheep [95].

Notably, microbial dysbiosis (e.g., pathogen proliferation) not only depletes feed energy but also triggers subclinical inflammation and compromises gastrointestinal barrier function, thereby suppressing growth potential. For example, *Bacteroides* can induce endogenous infections and exacerbate nutrient loss, with *Bacteroidales S247* being strongly associated with low feed efficiency (FE) in pigs [29]. Additionally, colonization of *Campylobacter avium* and *Helicobacter pullorum* in chicken ceca correlates with inflammatory responses and reduced FE [15]. However, our understanding of how rumen pathogenic bacteria in Hu sheep impact host health and thereby inhibit growth remains limited.

We concluded that current research on how rumen microbiota regulates host health and growth rate in Hu sheep through dual "metabolic-immune" pathways remains limited, and key functional microbial taxa and their mechanisms await elucidation. To address these gaps, this study employed an ADG-based grouping strategy in sheep under identical feeding and management conditions, macrogenomics was used to assess differences in rumen microbes in terms of composition and function between the two groups, to compare between-group differences in animal immune performance and rumen development and fermentation. Our objectives were to: (1) characterize differences in rumen microbial community structure and function between high- and low-ADG Hu sheep; (2) identify rumen microbial biomarkers capable of classifying Hu sheep with divergent growth rates; and (3) explore the mechanisms by which keystone microbial taxa influence host health and average daily gain (ADG). These findings may deepen our understanding of the "microbiota-host" coevolutionary mechanisms in Hu sheep and provide a theoretical foundation for synchronously improving host health and farming efficiency through microecological agents, probiotic interventions, or genetic breeding strategies.

Materials and methods

Animal

The Institutional Animal Care and Use Committee of the China Agricultural University (Permit number: AW22901202-1-3) granted approval for the animal studies used in this investigation. A total of 318 healthy, disease-free male lambs with detailed pedigree records and similar birth dates (to minimize age-related variability) were selected from Pangda Sheep Breeding Farm (Hangzhou, China) to construct a test sheep population. Throughout the entire experiment, a consistent standardized feed formulation (detailed in Supplementary Material Table S1) was used. To ensure compositional stability of feed ingredients, key components—Corn, Soybean meal, Bran, Soybean straw, Peanut seedlings, Garlic peel, Baking soda, Salt, and Premix—were purchased in a single batch based on estimated intake volumes and stored in a low-humidity warehouse. Tofu residue was procured weekly from the same supplier to maintain batch-to-batch consistency. Additionally, clean, fresh drinking water was provided ad libitum to all sheep. All experimental sheep had no history of clinical diseases during the trial. Prior to weaning, they received uniform vaccinations. Regular deworming protocols (oral ivermectin administration) and pen disinfection were systematically implemented, while antibiotic usage was strictly prohibited throughout the study period. Growth parameters were systematically tracked from birth, with birth weight and weaning weight (measured at 3 months of age) recorded. From this cohort, 66 Hu sheep male lambs were randomly selected for extended longitudinal monitoring until 6 months of age. Final body weight at 6 months (44.7 ± 0.75 kg, expressed as mean ± SEM) was determined to calculate postnatal average daily gain (ADG, g/d). The ADG of all 66 Hu sheep was 227.45 ± 3.72 g/d with a coefficient of variation of 13.3% (Table S2). All 66 Hu sheep were ranked according to their individual ADG, and then the top 8 were selected as the high growth rate group (HADG, ADG: 278.65 ± 0.048g/d) and the lowest 10 were selected as the low growth rate group (LADG, ADG: 176.43 ± 0.102g/d) (Table S2). A significant difference in ADG was observed between the two groups ($t = 12.896$, $P < 0.01$), with a Cohen's $d = 6.448$, indicating substantial between-group differences and a remarkably large effect size. In addition, the results of the covariate analysis (linear mixed models) showed that the confounders birth weight ($P = 0.538$), weaning age ($P = 0.223$), and genetic background ($P = 0.131$) did not significantly affect ADG. The power value for the number of biological replicates in the subgroups used in this experimental design ($n = 8$) has been verified using power analysis and is 1, indicating that the subgroups used in this experimental design are reasonable.

ADG and KR value calculation and grouping

Experimental lambs' weights were obtained from the stock records maintained at the breeding farm. The daily weight gain of the sheep was calculated according to the following formula: $ADG = (W_i - W_j)/d$, KR values were calculated using the following formula): $KR\ (\%) = ADG / W_j^{0.75}$, where W_i is the body weight on day i, W_j is the

body weight on day j, d is the number of days between day i and day j.

Immune index analysis of serum

Blood was collected from the jugular vein of 16 test sheep the day before slaughter and centrifuged for 10 min at 3000 × g to separate serum. Serum samples were stored at − 20 ℃. Serum concentrations of tumor necrosis factor-α (TNF- α), interleukin (IL)-1β, IL-6, IL-10 and serum immunoglobulin (IgA, IgG, and IgM) were measured by using commercial ELISA kits (Shanghai Enzyme-linked Biotechnology Co., Ltd., China).

Measurement of morphological indices of rumen epithelium

Experimental lambs were slaughtered, a rumen tissue block of about 1.0 cm^2 in the ventral blind bursa of each test sheep was quickly clipped, rinsed with saline and fixed in 10% neutral-buffered formalin. To evaluate the morophological changes of the rumen epithelial tissue, 4 mm thick sections were embedded in paraffin and sliced in microtome. The trimmed sections were further stained with eosin and then subjected to light microscopic examination. The following parameters were examined and measured using Case Viewer software (version 2.3.0): the Papillae length and width, the Epithelium mucosae thickness, the Submucosa thickness, the Muscular thickness, and the Rumen wall thickness.

Ruminal fermentation parameters analysis

Water restriction was imposed before the 12 h of slaughtering, and after opening the abdominal cavity of the sheep, the viscera were immediately dissected, and the rumen was isolated. Rumen fluid samples were obtained by filtering the rumen contents through 4 layers of gauze cloth and stored in liquid nitrogen for subsequent analysis. Later on, the frozen samples were thawed at 4 °C and centrifuged at 3000 × g at 4 °C for 10 min. The VFAs were measured by using gas chromatography (Agilent 6890N, Santa Clara, CA, USA) with a chromatographic column (HP 19091N-213), according to the method of [79–82]. The NH_3-N concentration of rumen fluid was determined colorimetric technique (Spectrophotometer U-2900; Hitachi, Tokyo, Japan) as described by [83].

Metagenome sequencing

DNA was extracted using the HiPure Bacterial DNA Kit (Magen, Guangzhou, China) and DNA quality was assessed by Qubit (Thermo Fisher Scientific, Waltham, MA) and spectrophotometer (Thermo Fisher Scientific, Waltham, MA). Macrogenome sequencing was performed on an Illumina Novaseq 6000 sequencer using the PE 150 sequencing strategy. Qualified genomic DNA was firstly fragmented by sonication to a size of 350 bp, and then end-repaired, A-tailed, and adaptor ligated using NEBNext® ULtra™ DNA Library Prep Kit for Illumina (New England BioLabs, Ipswich, MA, USA) according to the preparation protocol. DNA fragments with a length of 300–400 bp were enriched by PCR. and the PCR products were purified using the AMPure XP system (Beckman Coulter, Brea, CA, USA), and then passed through, and detected in, the sequenced libraries using an Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA).

Genome sequencing was performed on the Illumina Novaseq 6000 sequencer using pair-end technology (PE 150). The raw data from the Illumina platform was filtered using FASTP (v0.18.0) [11]. After filtering, the resulting clean reads were used for genome assembly. Clean reads of each sample were assembled individually using MEGAHIT (v1.1.2) to generate sample-derived assembly. We used Meta Gene Mark (v3.38) to predict genes based on the final assembly contigs. All predicted genes of length > 300 bp were merged according to 95% identity and 90% coverage of reads using CD-HIT (v4.6) to reduce the number of redundant genes in downstream assembly steps. Using Bowtie (v2.2.5) to count reads numbers, the reads were realigned to the predicted genes [42]. The final gene catalog was obtained from non-redundant genes with gene reads count greater than 2.

Unigenes were annotated using DIAMOND (v0.9.24) aligned to deposited genes in the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. Additional annotations were performed based on carbohydrate activity (CAZymes).

Construction of random forest classifier

The Random Forest package in R was used for random forest analysis [8] to classify high or low ADG sheep and/or predict ADG in sheep by using rumen bacteria and ADG (HADG and LADG) of Hu sheep as inputs to the random forest model. The machine learning design references the description of Verhaar et al. [76]. To mitigate overfitting, a nested cross-validation design was implemented during model training. Hyperparameters of the model were optimized via five-fold cross-validation to minimize classification error. Model robustness was further validated using leave-one-out cross-validation (LOOCV) and permutation tests (1000 iterations) to assess the statistical significance of classification accuracy ($P < 0.05$). Receiver operating characteristic (ROC) curves were generated by plotting the true positive rate (TPR, sensitivity) against the false positive rate (FPR, 1 − specificity), with the area under the curve

(AUC) quantifying predictive performance. The UC-RF algorithm (AUC-optimized random forest) was applied to determine the prediction accuracy of each rumen bacterial feature based on the maximum AUC value.

Construction of microbial co-occurrence networks based on random matrix theory

The microbial co-occurrence network package in R was used to construct the microbial co-occurrence network between HADG and LADG, and Deng et al. [13] described the default parameters to identify microbial interactions. The network construction methodology involved twofold: (1) the ASVs detected in ≤ 50% of all samples were excluded [84]; (2) only filling with 0.01 in blanks with paired valid values. The fast-greedy modularity optimization procedure was used for module separation. The fast-greedy modularity optimization procedure was used for module separation. The within-module degree (Zi) and among-module connectivity (Pi) were calculated and plotted to generate a scatter plot for each network. In this study, we used the simplified classification as follows: (i) Peripheral nodes (Zi ≤ 2.5, Pi ≤ 0.62), which had only a few links and almost always to the nodes within their modules, (ii) Connectors (Zi ≤ 2.5, Pi > 0.62), which were highly linked to several modules, (iii) Module hubs (Zi > 2.5, Pi ≤ 0.62), which were highly connected to many nodes in their own modules, and (iv) Network hubs (Zi > 2.5, Pi > 0.62), which acted as both module hubs and connectors. These connectors, module hubs, and network hubs of the microbial network may play keystone roles in the microbial communities, which were called keystone species in this study.Statistical analysis.

The rumen fermentation characteristics, serum immune parameters were compared using t-test by IBM SPSS Statistics for Windows (version 26.0, IBM Corp, Armonk, NY, USA). Statistical significance was defined at $P < 0.05$. Correlation analyses were conducted by Spearman correlation analysis. Statistical significance was defined at $P < 0.05$.

Results

Production performance

In this study, 66 Hu sheep were randomly selected from 318 Hu sheep of close age to weigh their 6-month weight, and the ADG values were calculated based on birth weight and 6-month weight. We further screened 16 Hu sheep with extreme phenotypes using ADG

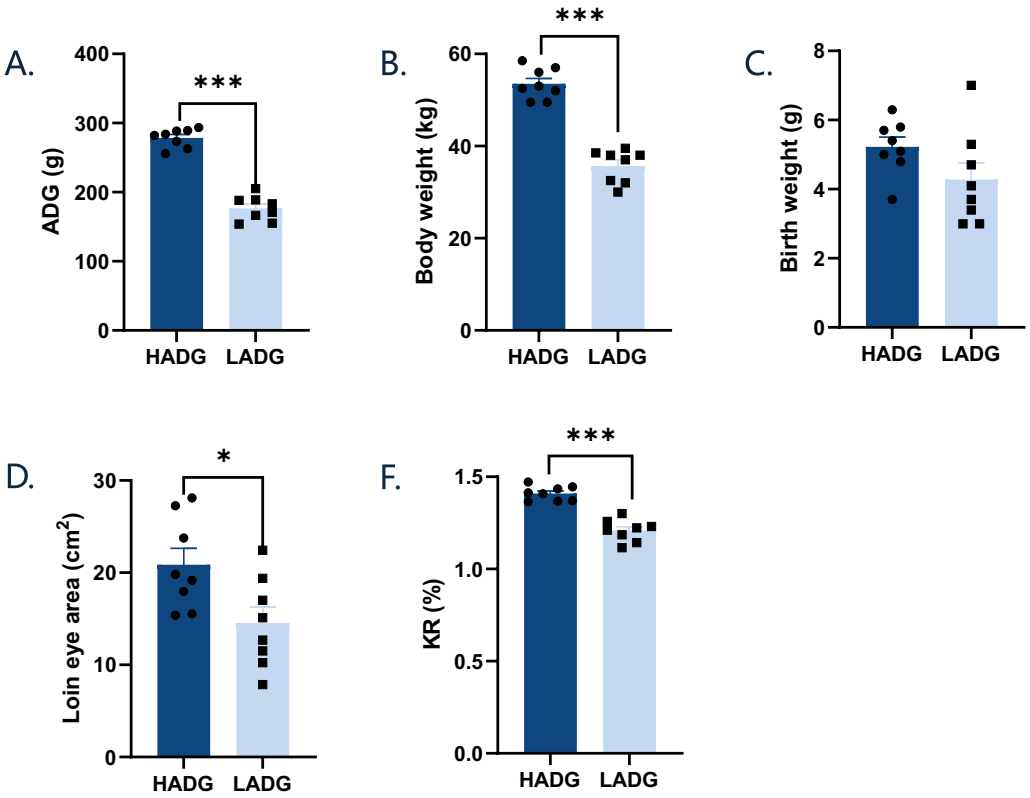


Fig. 1 Differences in growth performance between HADG and LADG sheep. **A**–**F** T-test for significance of differences in growth performance between the two groups. ADG, Body weight, Birth weight, Loin eye area and KR. $P < 0.05$: *, $P < 0.01$: **

as an index to divide them into HADG and LADG groups. No significant differences in birth weight were found between the two groups ($P=0.110$, Fig. 1C). In comparison with those in the LADG group, the Hu sheep in HADG group had an almost 49.9% higher 6-month weight, a 43.5% higher loin eye area, and a 16.6% higher KR values ($P<0.05$, Fig. 1A, B, D, F). In addition, correlation analysis showed a strong positive correlation between ADG and loin eye area, 6-month weight, and KR values ($r>0.6$, $P<0.05$, Table S3).

Rumen fermentation parameters

Given the important role of rumen fermentation in ruminant growth and development, we addressed the differences in rumen fermentation parameters between the two groups. The concentrations of propionate and butyrate in the rumen of HADG sheep surpassed that in LADG sheep ($P<0.05$, Fig. 2B, C). This observation may suggest that different rumen microbes drive changes in VFAs in the rumen, underscoring the imperative the need to further explore the rumen microbiome. In addition, we further analyzed the correlation of VFAs with ADG and other phenotypes. Propionate was positively correlated with ADG ($r>0.6$, $P<0.05$, Fig. 2J), loin eye area ($r>0.8$, $P<0.01$, Fig. 2J), and KR ($r>0.5$, $P<0.05$, Fig. 2J).Butyrate was positively correlated with ADG ($r>0.5$, $P<0.01$, Fig. 2J), loin eye area ($r>0.5$, $P<0.05$, Fig. 2J), and KR ($r>0.6$, $P<0.05$, Fig. 2J).

Rumen epithelial morphology

More than 70% of the rumen VFAs were absorbed through the rumen epithelium and then supplied energy to the organism, so we processed the rumen epithelial tissues by HE staining and then observed the sections of the two groups. We found that rumen papilla width and muscular thickness were significantly increased in HADG sheep ($P<0.05$, Fig. 3B, C). Spearman's correlation analysis between rumen epithelial morphology and growth performance traits showed that rumen papilla width was positively correlated with ADG ($r>0.7$, $P<0.01$, Fig. 3G), BW ($r>0.6$, $P<0.05$, Fig. 3G), loin eye area ($r>0.5$, $P<0.05$, Fig. 3G) and KR ($r>0.8$, $P<0.01$, Fig. 3G), and muscular thickness with ADG ($r>0.6$, $P<0.01$ Fig. 3G), BW ($r>0.5$, $P<0.05$, Fig. 3G), loin eye area ($r>0.5$, $P<0.05$, Fig. 3G) and KR were positively correlated ($r>0.6$, $P<0.01$, Fig. 3G).

Given the close relationship between butyrate and rumen epithelium development, we conducted a correlation subanalysis between fermentation parameters and rumen epithelium morphology. The results showed a significant positive correlation between butyrate and both rumen papilla width ($r>0.8$, $P<0.01$, Fig. 3H) and rumen muscular thickness ($r>0.6$, $P<0.01$, Fig. 3H).

Profiling of the rumen metagenome

Metagenome sequencing generated a total of 1,093,090,812 raw reads, 68,318,175.75 ± 4,577,605.649 per sample (mean ± standard error of the mean [SEM]). After quality control and removal of host genes, 1,088,846,520 clean reads were retained, 68,052,907.5 ± 4,553,109.642 per sample. Since the samples were collected from the rumen, host sequence contamination is inevitable. Therefore, the filtered data clean reads need to be compared to the host reference

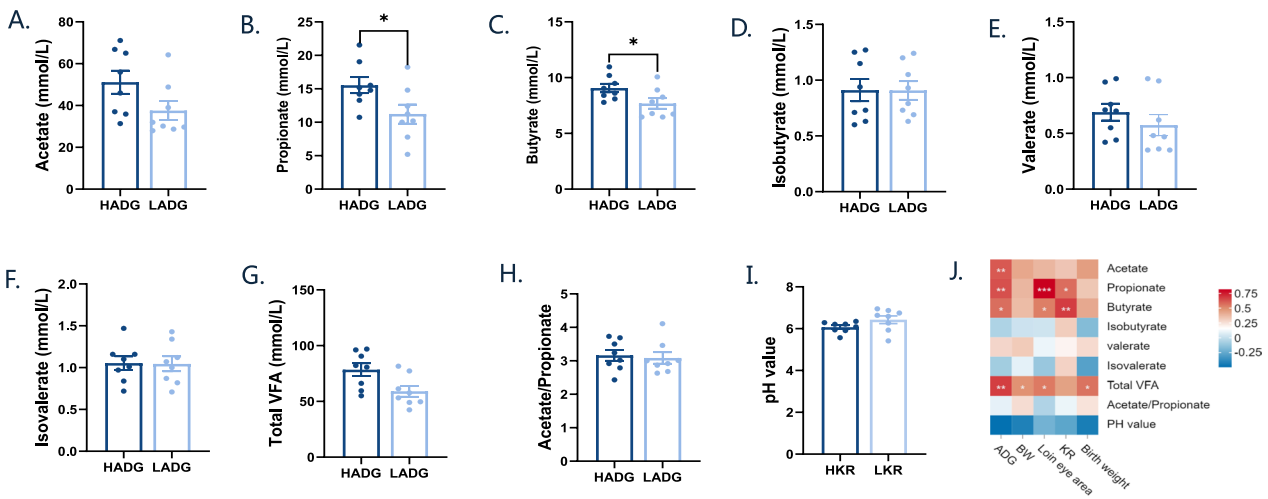


Fig. 2 Differences in rumen fermentation parameters between HADG and LADG sheep. **A**–**I** T-test for significance of differences in rumen fermentation parameters between the two groups. Acetate, Propionate, Butyrate, Isovalerate, Total VFA, Acetate/Propionate and pH value. $P<0.05$: *, $P<0.01$: **. **J** Relationship between rumen fermentation parameters and growth performance. Color shade and line width indicate the strength of correlation, red: positive, blue: negative

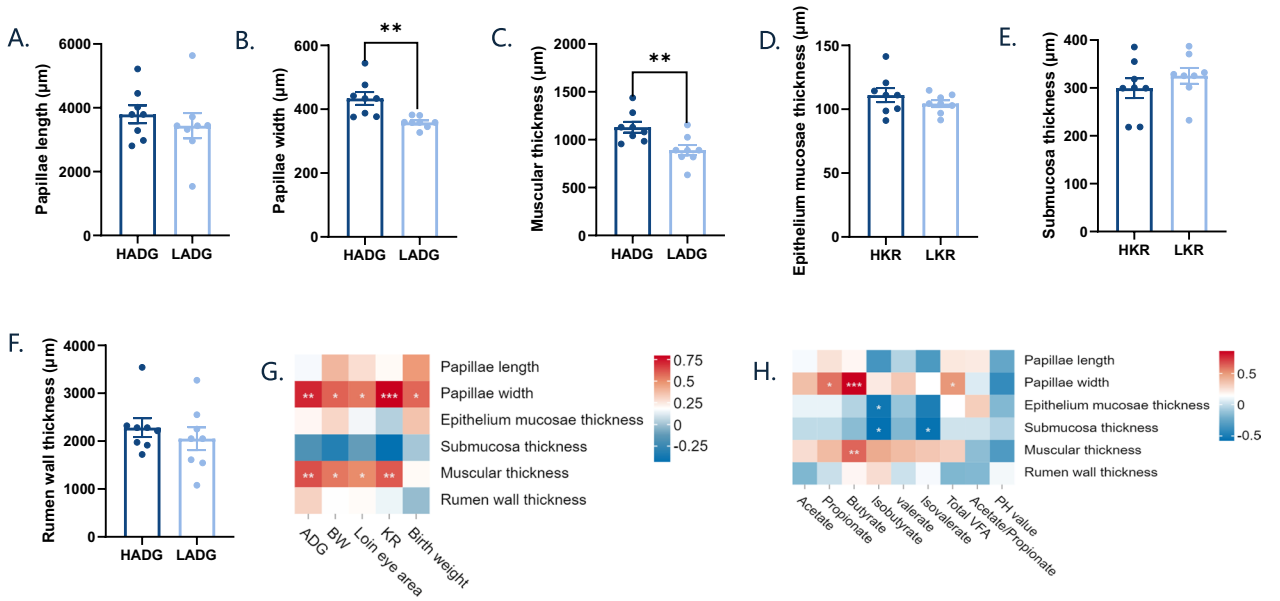


Fig. 3 Differences in rumen epithelial morphology between HADG and LADG sheep. **A**–**F** T-test for significance of differences in rumen epithelial morphology between the two groups. Papillae length, Papillae width, Muscular thickness, Epithelium mucosae thickness, Submucosa thickness and Rumen wall thickness. $P < 0.05$: *, $P < 0.01$: **. **G** Relationships between rumen epithelial morphology parameters and growth performance traits. Color shade and line width indicate the strength of correlation, red: positive, blue: negative. **H** Relationship between rumen epithelial morphology parameters and rumen fermentation parameters. Color shade and line width indicate the strength of correlation, red: positive, blue: negative

genome to filter the reads originating from the host, and 1,086,317,148 effective reads were obtained, 67,894,821.75 ± 4,516,166.627 for each sample. Finally, the MEGAHIT software was used to assemble effective reads, a total of 9,264,994 Contigs were obtained, 579,062.125 ± 35,961.4155 per sample. 4,271,553 and 4,993,441 Contigs were obtained in the HADG and LADG groups, respectively.

Compositional profiles of the rumen microbiome and taxonomic differences between the HADG and LADG sheep

Alpha-diversity calculations revealed no divergence of the Shannon and Chao indices ($P > 0.05$, Figure S1A), indicating unchanged rumen microbial richness and evenness between the two groups. Analysis of similarities (Anosim) based on Bray–Curtis distance showed differences between the two groups ($R = 0.164 > 0$, $P = 0.03 < 0.05$, Figure S1B), demonstrating a significant difference in rumen microbial structure between the HADG and LADG groups. The dominant phylum (Figure S1C) included *Bacteroidetes* (46.76 ± 2.13%), *Firmicutes* (9.15 ± 0.59%), *Proteobacteria* (1.15 ± 0.06%). The dominant genus (Figure S1C) included *Prevotella* (16.24 ± 1.67%), *Bacteroides* (1.71 ± 0.05%), *Succiniclasticum* (1.03 ± 0.11%). The dominant species (Figure S1C) were *Prevotella_sp.tf2-5* (1.25 ± 0.17%), *Prevotella_sp.tc2-28* (1.04 ± 0.16%), *Succiniclasticum_ruminis* (1.03 ± 0.11%).

The difference between groups of bacteria at the genus level was analyzed using the Wilcoxon rank-sum test (Fig. 4A), a total of 18 differential bacteria were identified, of which *Succinivibrio* and *Succinatimonas*, had a higher relative abundance in HADG sheep ($P < 0.05$); 16 bacteria such as *Selenomonas*, *Schwartzia, Blautia, Roseburia, Anaerotruncus and Sediminibacterium* had a higher relative abundance in LADG sheep ($P < 0.05$). Besides, linear discriminant analysis effect size (LEfSe) was used to screen the main specific bacteria between groups (Fig. 4B), and it was found that *Succinivibrio_dextrinosolvens* was enriched in the HADG group, whereas *Selenomonas_ruminantium* was enriched in the LADG group (LDA > 3).

Spearman rank correlation network further analyzed the correlation between the above rumen bacteria with intergroup differences at phylum, genus, and species levels and host growth-related phenotypes (Fig. 4C). A total of 26 bacteria were identified that were significantly associated with ADG, and these findings classified them as ADG-associated rumen microbes. At the species level, the relative abundance of *Succinivibrio_dextrinosolvens* enriched in HADG was significantly positively correlated with ADG ($r > 0.7$, $P < 0.05$), and *Selenomonas_ruminantium* enriched in LADG showed an opposite correlation with ADG

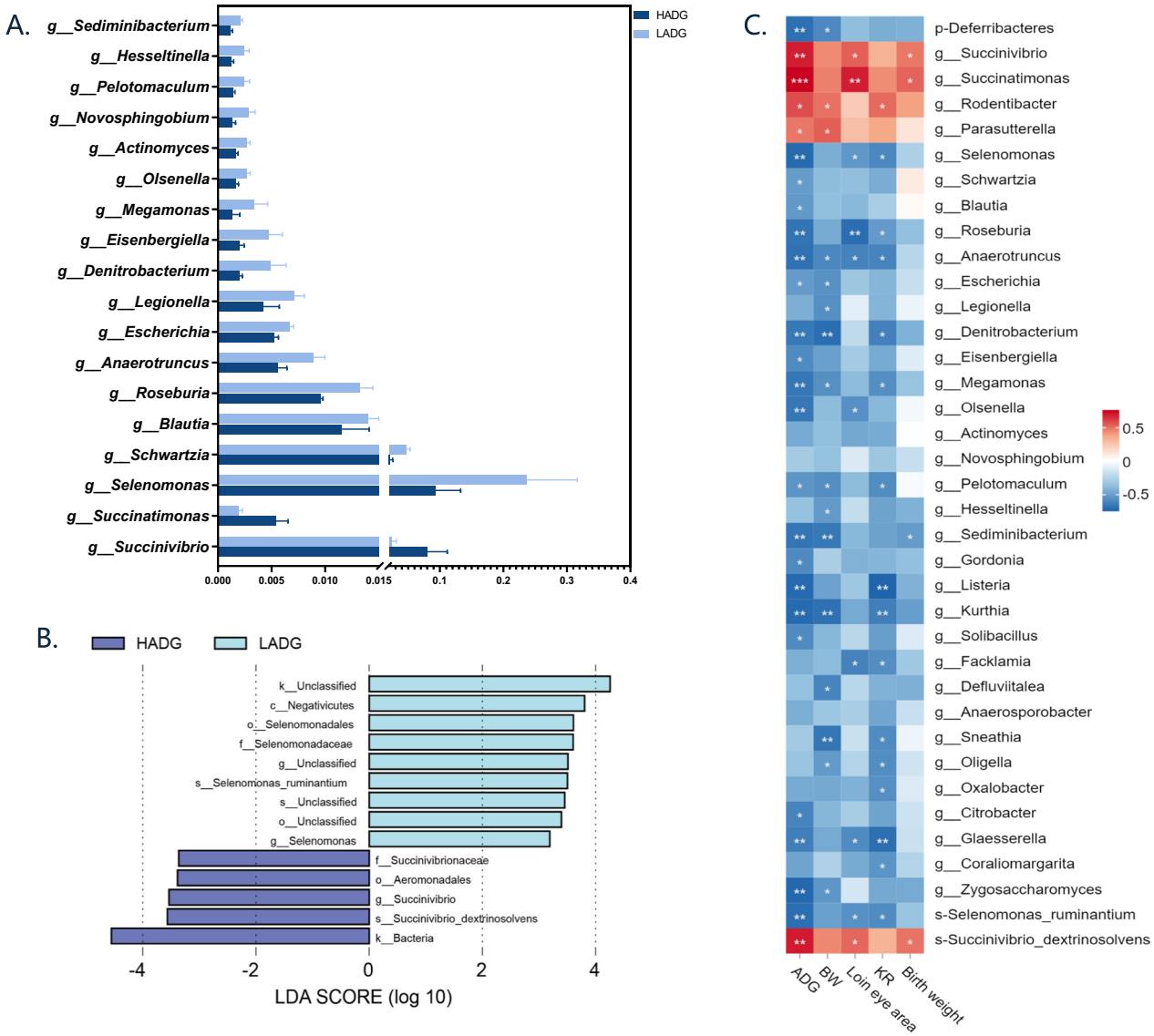


Fig. 4 Differences in the rumen bacteria of HADG and LADG sheep. **A** Analysis of differences in rumen microbial phylum and genus between HADG and LADG sheep, Wilcoxon rank-sum test for significant differences, with an adjusted *P*-value of < 0.05. **B** The significantly differential microorganisms based on LEfSe cladogram in metagenomic sequencing. **F** Heat map showing the correlation (Spearman's correlation, $P < 0.05$) between significantly different rumen bacteria and all sheep growth traits. Red indicates positive correlation and blue indicates negative correlation, the deeper the color, the higher the correlation

($r < -0.6$, $P < 0.05$). *Succinatimonas*, *Succinivibrio*, *Rodentibacter* and *Parasutterella* at the genus level were positively correlated with ADG ($r > 0.5$, $P < 0.05$), and were significantly increased in HADG. In addition, a total of 21 differential bacteria at the phylum and genus levels were negatively correlated with ADG ($r < -0.5$, $P < 0.05$), and all were significantly increased in LADG, including *Deferribacteres*, *Selenomonas*, *Schwartzia*, *Blautia*, *Roseburia*, *Anaerotruncus*, *Escherichia*, *Denitrobacterium*, *Eisenbergiella*, *Megamonas*, Olsenella, Pelotomaculum, Sediminibacterium, Gordonia, Listeria, Kurthia, *Solibacillus*, *Citrobacter*, *Glaesserella* and *Zygosaccharomyces*.

The rumen microbiota in relation to growth rate and rumen fermentation

Since carbohydrates are converted into VFAs and other substances to supply energy to the organism in the presence of rumen bacterial groups, a network of correlations between differential bacterial taxa and

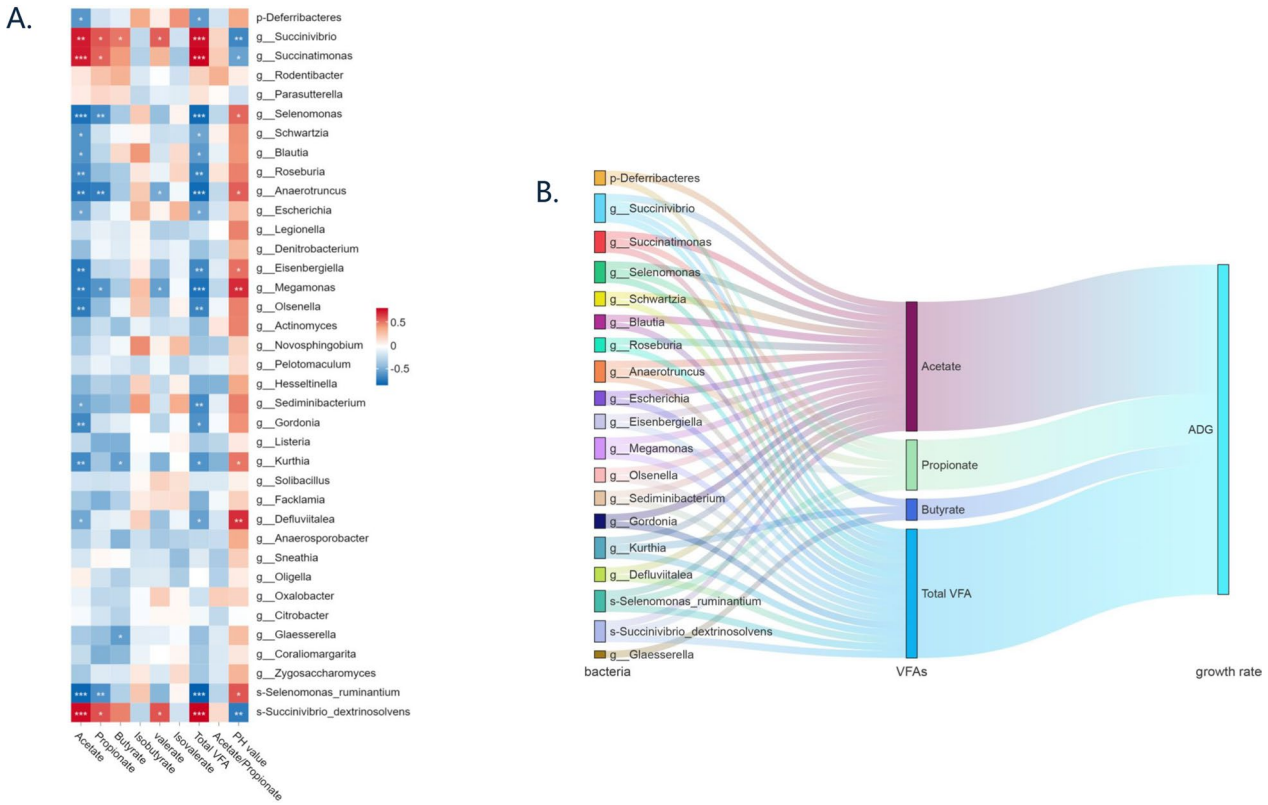


Fig. 5 The relationship of rumen microbes to rumen fermentation and production. **A** Heat map showing the correlation (Spearman's correlation, $P < 0.05$) ADG-related rumen bacteria and rumen fermentation parameters, all metrics were significantly different between the two groups of HADG and LADG. Red indicates positive correlation and blue indicates negative correlation, the deeper the color, the higher the correlation. **B** Sankey diagram showing the relationship between rumen bacteria, rumen fermentation parameters and growth rate

rumen VFA traits was further analyzed to investigate whether and how rumen bacterial variation is attributable to rumen VFA (Fig. 5A). The results showed that propionate was positively correlated with *Succinivibrio* and *Succinivibrio_dextrinosolvens* ($r > 0.5$, $P < 0.01$), negatively correlated with *Selenomonas_ruminantium* and 4 genera ($r < -0.5$, $P < 0.05$). Butyrate was positively correlated ($r > 0.5$, $P < 0.05$) with *Succinivibrio* ($r > 0.5$, $P < 0.05$). These observations suggest that differences in microbiome profiles may contribute to VFAs production and together play a role in the variation in growth rate of Hu sheep (Fig. 5B).

The rumen microbiota in relation to growth rate and organismal health homeostasis

Notably, 52% of the bacterial genera negatively correlated with ADG belong to pathogenic bacteria with pro-inflammatory effects or certain virulence, including *Blautia*, *Anaerotruncus*, *Escherichia*, *Eisenbergiella*, *Sediminibacterium*, *Gordonia*, *Listeria*, *Kurthia*, *Solibacillus*, *Citrobacter*, and *Glaesserella* (Fig. 4C). These findings suggest that the reason for the decrease in ADG may be related to the composition of rumen bacteria, and also emphasize the need for further exploration of the relationship between ADG and the overall health of the rumen and host.

Based on this, we compared the immune parameters of the HADG and LADG sheep sera to explore the role of changes in the overall health status of the host body in ADG variation. We had compared the immune parameters of sheep serum in HADG and LADG groups (Fig. 6A). The inflammatory factor IL-6 increased in the LADG compared to HADG ($P < 0.01$), while IgG was decreased at LADG ($P < 0.05$). In addition, correlations between immune parameters and growth performance traits were determined (Fig. 6A), in which IL-6 negatively correlate with ADG ($r < -0.6$, $P < 0.05$) and IgG positively correlating with ADG ($r > 0.7$, $P < 0.05$). These findings suggest that changes in ADG may be related to the imbalance of the overall health homeostasis of Hu sheep.

Next, we further explored the relationship between ADG-related rumen microbes and host health homeostasis. The results showed that *Anaerotruncus*,

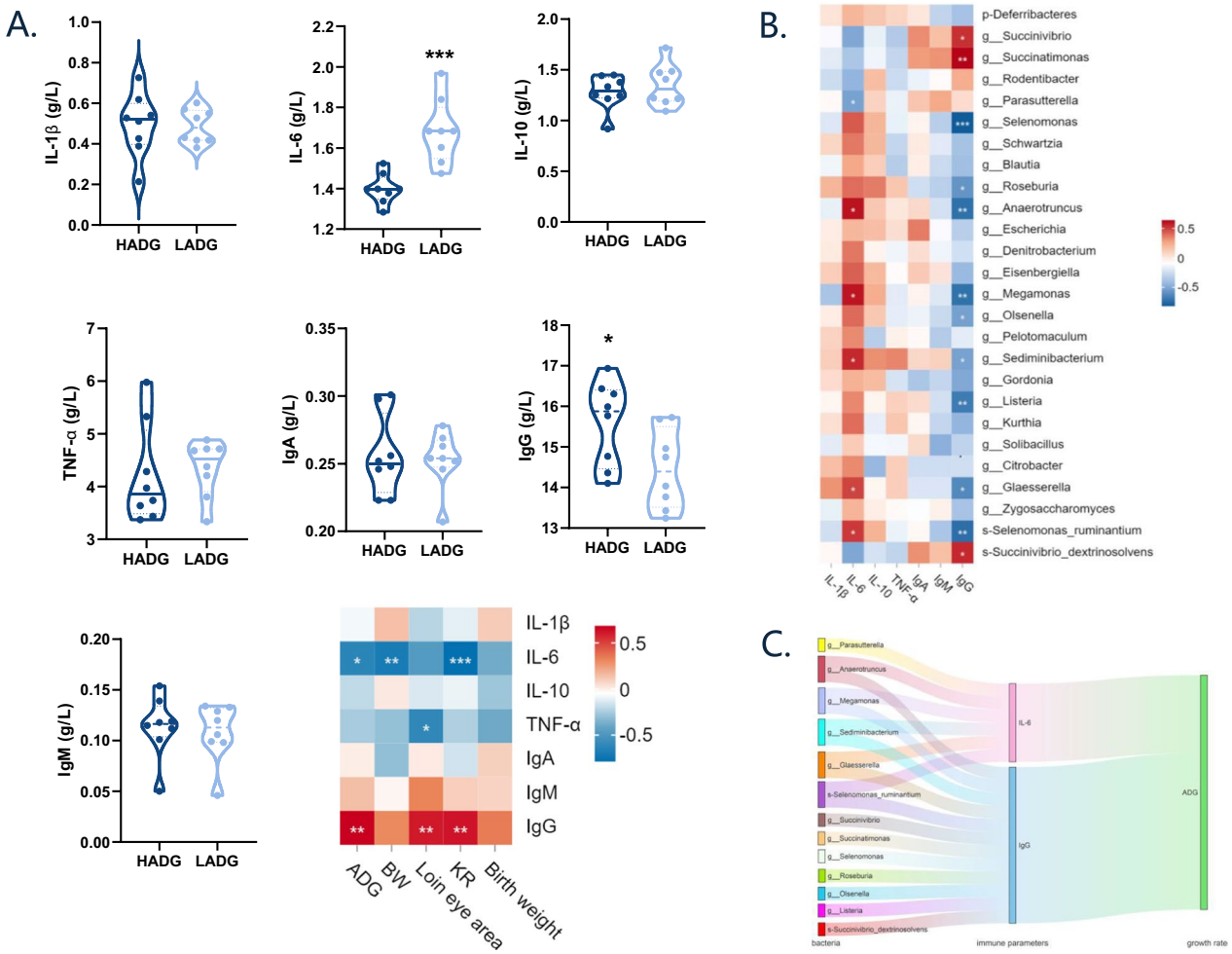


Fig. 6 The relationship of rumen microbes to host health and production. **A** Differences in serum immune parameters between HADG and LADG sheep. And relationships between serum immune parameters and growth performance traits, red: positive, blue: negative. **B** Heat map showing the correlation (Spearman's correlation, $P < 0.05$) ADG-related rumen bacteria and immune parameters, all metrics were significantly different between the two groups of HADG and LADG. Red indicates positive correlation and blue indicates negative correlation, the deeper the color, the higher the correlation. **C** Sankey diagram showing the relationship between rumen bacteria, animal immunity and growth rate

Sediminibacterium, and *Glaesserella* were positively correlated with IL-6 ($r > 0.5$, $P < 0.05$, Fig. 6B) and negatively correlated with IgG ($r < -0.5$, $P < 0.05$, Fig. 6B). These observations revealed that changes in rumen bacterial composition may have led to the destruction of the overall health homeostasis of Hu sheep, resulting in a decrease in ADG (Fig. 6C).

Rumen bacteria, VFAs and immune parameters classify host FE with high predictive accuracy

Using a random forest learning machine, we classified sheep with varying ADG using ruminal bacteria, VFAs, and immune parameters (Fig. 7). It is evident that IL-6 and IgG could classify HADG and LADG sheep with high accuracy (AUC > 0.844, Fig. 7A). Among VFAs, only propionate had the AUC values = 0.812 in classifying HADG and LADG sheep (Fig. 7B). At the species level, the rumen bacteria *Succinivibrio_dextrinosolvens* and *Selenomonas_ruminantium* were both able to classify different ADG sheep with a prediction accuracy of 81.2% and 89.1%, respectively (AUC = 0.812, AUC = 0.891, Fig. 7D).

Microbial interaction networks between high and low growth rate Hu sheep

Since bacteria-bacteria interactions are key regulators shaping the rumen microbiota, we further evaluated potential microbial interactions (Fig. 8A, B). We

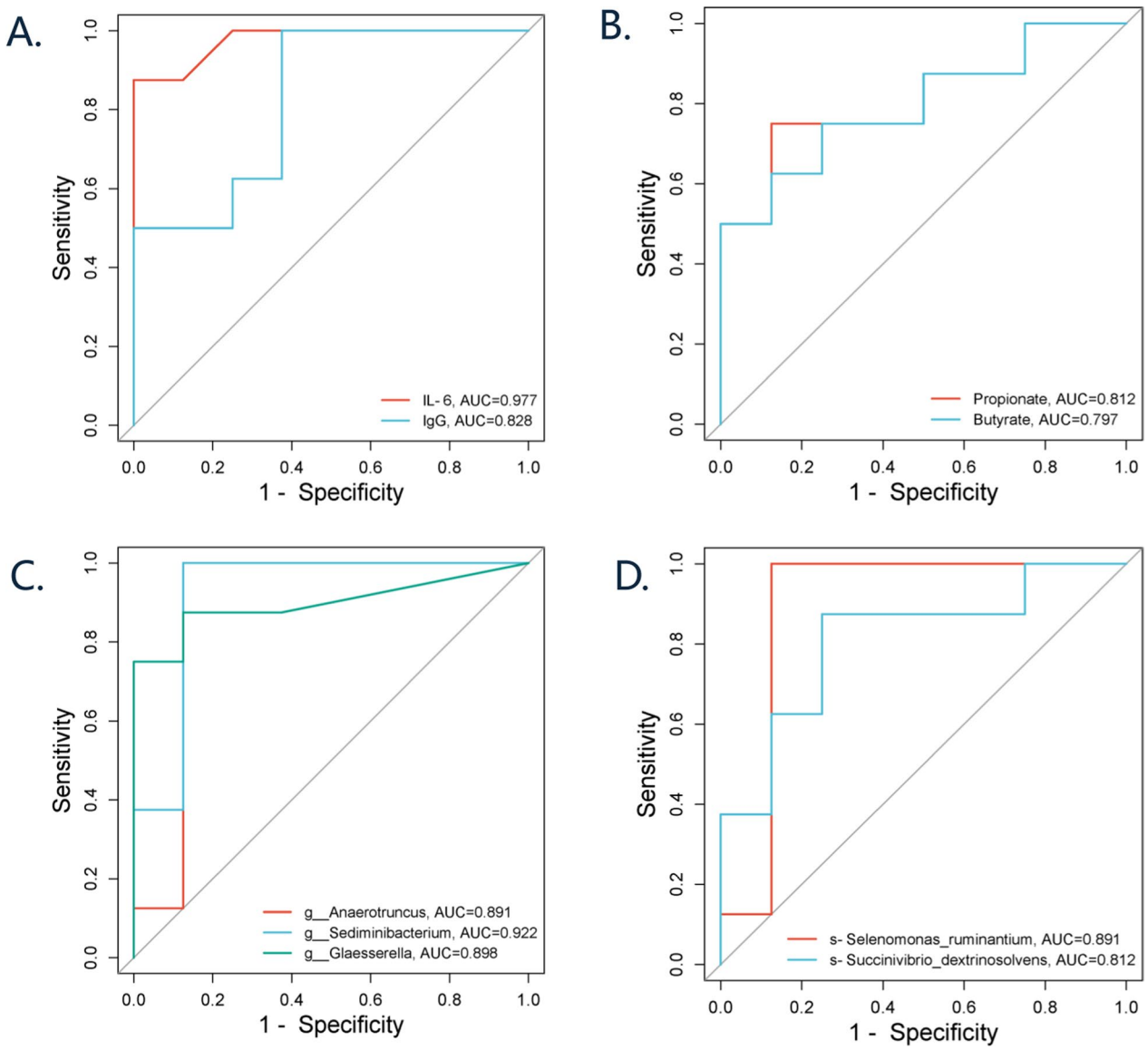


Fig. 7 Classification analysis based on random forest model. Classification of host growth rate (HADG vs LADG) using serum immune parameters (**A**), VFAs (**B**), and rumen bacteria (**C**, **D**)

identified 397 and 231 nodes, and 1311 and 405 edges in the molecular co-occurrence networks of HADG and LADG, respectively. In addition, we computed some topological properties commonly used in network analysis to characterize the complex patterns of interrelationships among microbes. Compared to LADG, the HADG molecular ecological network had higher average degree, average clustering coefficient, and network density, while the average network distance (average path length) between all pairs of nodes, and the network diameter were lower, suggesting a more complex HADG network and stronger interspecies connections (Table S4). Interestingly, we also identified the percentage of positively correlated connections in the HADG and LADG molecular ecological networks as 90.39% and 72.35%, respectively, indicating that the rumen microbes in both groups of Hu sheep tended to be in a cooperative rather than a competitive relationship, but more rumen microbes in the LADG sheep in were in a state of competing ecological niches a state.

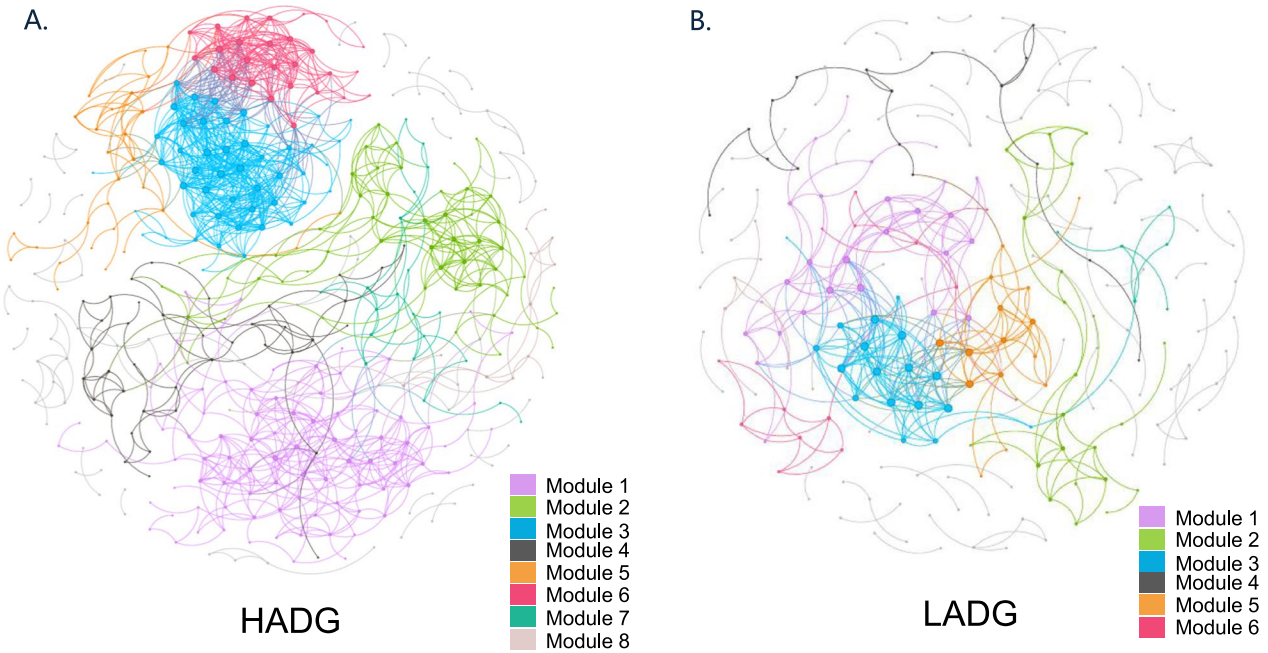


Fig. 8 Co-occurrence network of rumen bacteria based on correlation analysis in the HADG and LADG sheep

We then determined the microorganisms assigned to peripherals, connectors and module hubs in the HADG and LADG networks based on the RMT network analysis (Figure S2, Table S5, S6) 88.67% and 93.67% of the microbes in the HADG and LADG networks, respectively, belonged to peripherals, and the number of bacteria that were connected to those nodes were fewer. In the HADG network, 34 bacteria belonged to connectors, including *Alloprevotella*, *Phascolarctobacterium*, *Anaerovibrio*, *Butyricicoccus*, and *Ruminococcaceae_noname*, etc. Additionally, there are 12 bacteria in the LADG network that belong to connectors, including *Phascolarctobacterium*, and 7 bacteria, including *Butyricimonas*, are identified as module hubs. Furthermore, correlation analysis revealed significant negative associations between ADG and the genera *Nothophytophthora* ($r = -0.54$, $P < 0.05$), *Thraustotheca* ($r = -0.57$, $P < 0.05$), *Batrachochytrium* ($r = -0.58$, $P < 0.05$), *Gigaspora* ($r = -0.55$, $P < 0.05$), and *Candidatus Microgenomates* ($r = -0.57$, $P < 0.05$) (Figure S3).

Functional profiles of the rumen microbiome and differential functions between the HADG and LADG sheep

The function of the rumen microbiome was determined by the Kyoto Encyclopedia of Genes and Genomes (KEGG) atlas and the genes encoding CAZymes. Major classes, such as metabolism, genetic information processing, environmental information processing, and cellular processes, were found in both groups (Fig. 9). In addition, the subclass of each level 1 class is shown in Fig. 9B. Next, we used LEfse analysis was used to excavate the differences in KEGG functions of rumen bacteria from sheep with different growth rates (Fig. 9C), and the analysis revealed that a total of 34 metabolic pathways were enriched in the HADG group, among which 5 metabolic pathways related to carbohydrate metabolism, namely: Carbohydrate metabolism, Galactose metabolism, Pentose and glucuronate interconversions, Fructose and mannose metabolism, Glyoxylate and dicarboxylate metabolism. 4 metabolic pathways related to amino acid metabolism, namely: Alanine aspartate

(See figure on next page.)

Fig. 9 The functional microbiome profiles rumen microbiome between high and low growth rate sheep through functional annotation of metagenome with the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. **A** KEGG function at Class levels. **B** KEGG function at Subclass levels. **C** The differentially represented metabolic pathways at the Kyoto Encyclopedia of Genes and Genomes (KEGG) level 3 through linear discriminant analysis (LDA) effect size determination with LDA value > 3. D. Heat map showing the correlation (Spearman's correlation, $P < 0.05$) between those pathways (Fig. 9D) and related host phenotypes and VFAs. Red indicates positive correlation and blue indicates negative correlation, the deeper the color, the higher the correlation

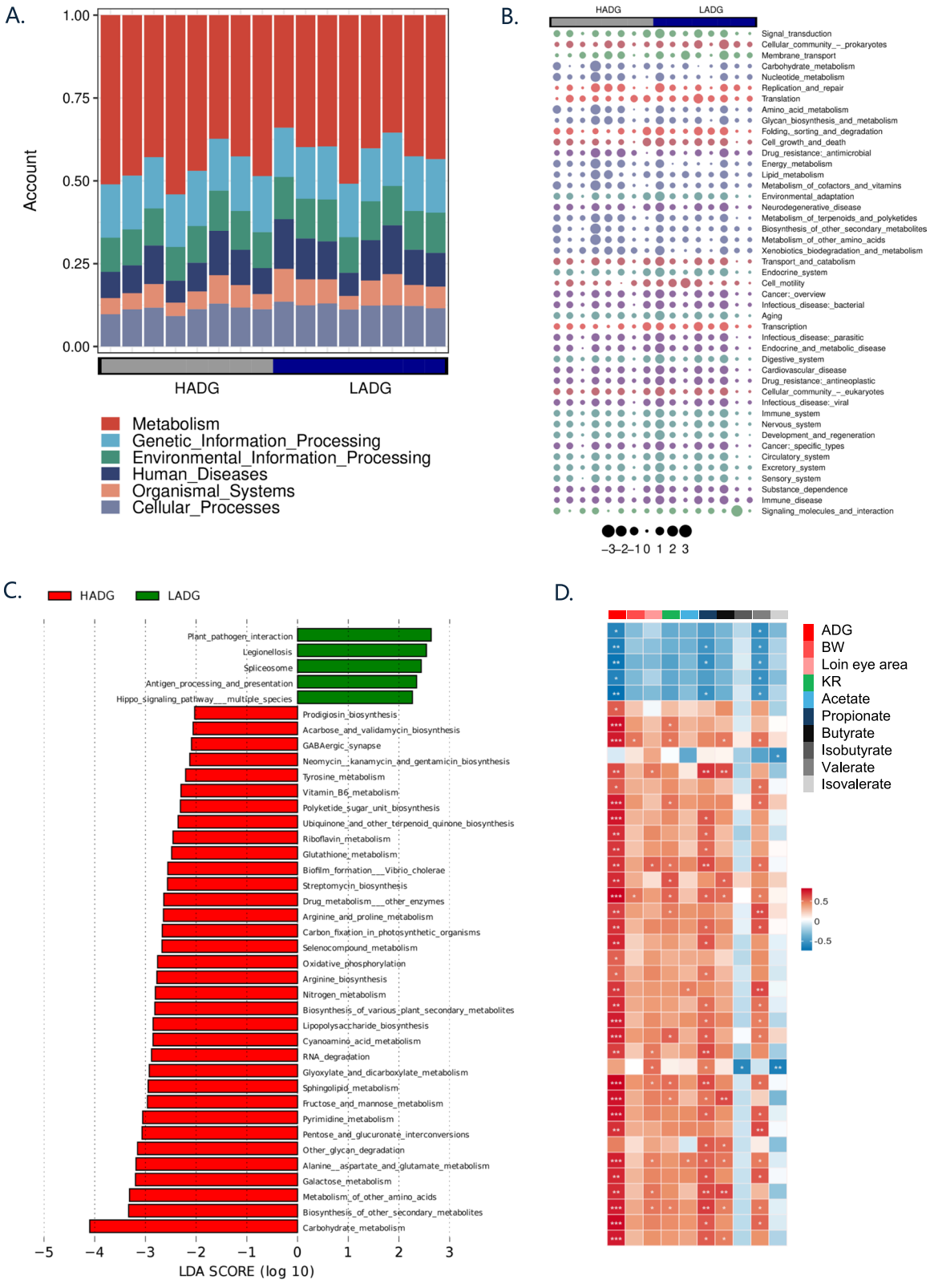


Fig. 9 (See legend on previous page.)

and glutamate metabolism, Arginine biosynthesis, Arginine and proline metabolism, and tyrosine metabolism. And 3 energy metabolism pathways, 2 of which are noteworthy: Nitrogen metabolism, Oxidative phosphorylation; and including Glutathione metabolism, Sphingolipid metabolism, Vitamin B6 metabolism. Besides, Plant pathogen interaction, Legionellosis, Spliceosome, Antigen processing and presentation, and Hippo signaling pathway_multiple species were found in the LADG group. In addition, the integrated key pathways and module metabolic maps helped us to fully understand the changes in rumen microbial functions between the two groups (Figure S4). We can clearly see that HADG sheep have higher metabolic activities in carbohydrate degradation and VFAs production as well as amino acid and nitrogen metabolism.

In addition, correlation analysis was further performed to estimate the association between the changes in microbiome functions and the growth phenotypes and VFAs of Hu sheep. Interestingly, these 3 carbohydrate pathways enriched in HADG ($r > 0.7$, $P < 0.01$), 3 amino acid metabolism pathways ($r > 0.6$, $P < 0.01$), and 3 energy metabolism pathways ($r > 0.5$, $P < 0.05$) were positively correlated with ADG, 2 carbohydrate metabolism pathways were positively correlated with loin eye area ($r > 0.5$, $P < 0.05$), and 4 carbohydrate pathways, 1 amino acid metabolism pathway, and 1 energy metabolism pathway were positively correlated with propionate ($r > 0.5$, $P < 0.05$), and 2 carbohydrate pathways were positively correlated with butyrate ($r > 0.5$, $P < 0.05$).

The CAZymes functional genes encoding carbohydrate-active enzymes in the rumen microbes of sheep with different growth rates were analyzed by

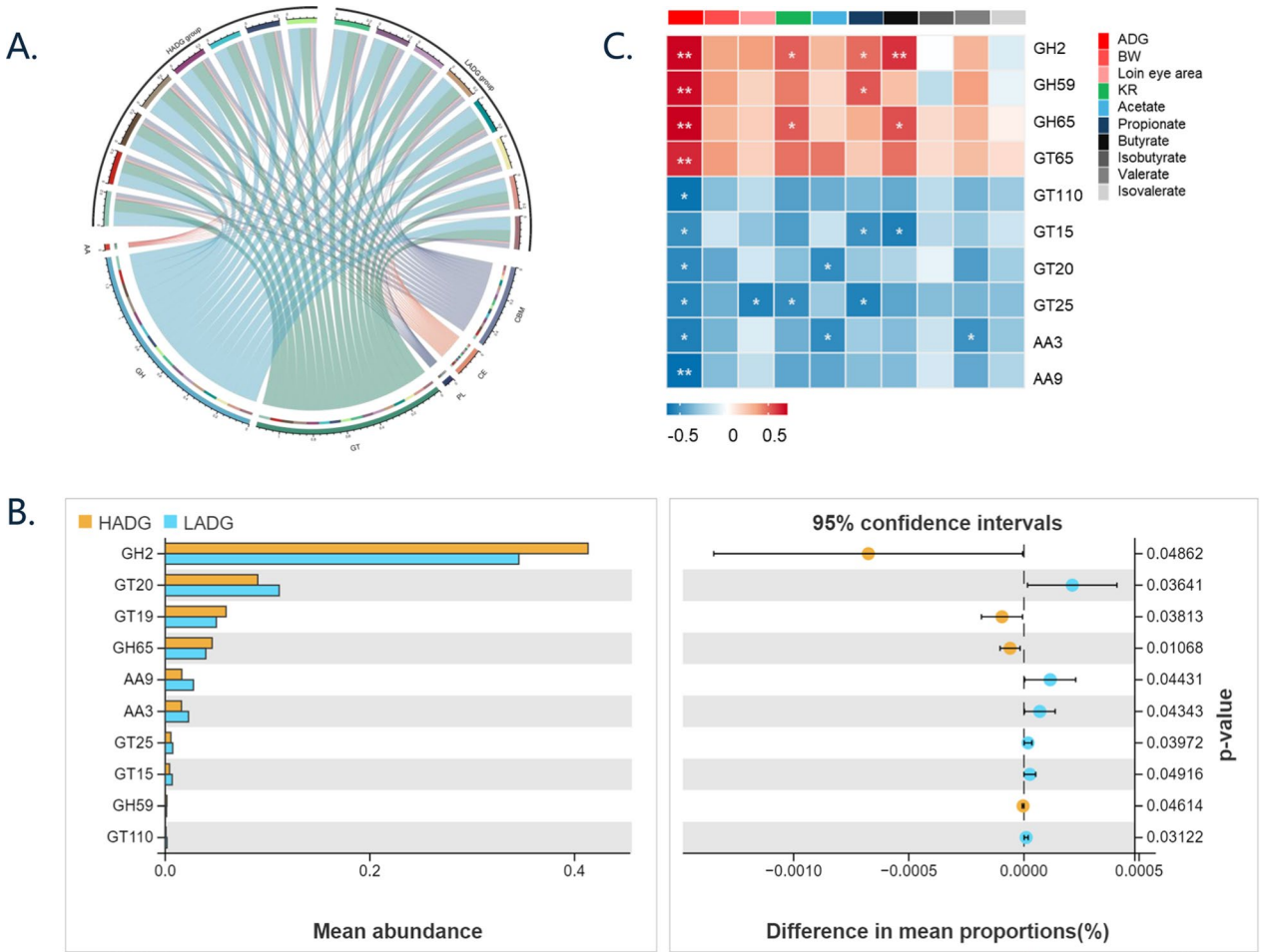


Fig. 10 Metagenomic analysis of the differentially represented genes encoding CAZymes between the HADG and low LADG groups. **A** The circle circos plot shows the different CAZymes patterns among the HADG and LADG groups. **B** Comparisons of the abundance of CAZymes genes of rumen microbiomes in the HADG and LADG groups by the Welch's t-test in metagenomic sequencing. **C** Heat map showing the correlation (Spearman's correlation, $P < 0.05$) between significantly different CAZymes genes and all sheep growth traits and VFAs. Red indicates positive correlation and blue indicates negative correlation, the deeper the color, the higher the correlation

differential analysis (Fig. 10A, B). The results showed that a total of 10 intergroup differential CAZymes genes were identified, of which 3 Glycoside Hydrolases (GH59, GH2, GH65) and 1 Glycosyl Transferase (GT19) were significantly elevated in HADG. 2 Auxiliary Activities (AA3, AA9), and 4 Glycosyl Transferases (GT15, GT20, GT25, GT110) were significantly elevated in LADG group. In addition, we further evaluated the relative abundance of CAZymes in relation to the growth phenotype with volatile acids (Fig. 12C). all CAZymes enzymes elevated in HADG were positively correlated with ADG ($r > 0.6$, $P < 0.01$), while all CAZymes enzymes elevated in LADG were negatively correlated with ADG ($r < -0.5$, $P < 0.05$).

Discussion

The rumen microbiome plays a pivotal role in the productivity and health of the host [34, 58]. Increasingly, research has focused on addressing the contribution of the composition and function of ruminant gastrointestinal microorganisms to changes in host feed efficiency. However, the relationship between rumen microorganisms and sheep growth performance remains unclear. In this study, 16 Hu sheep assigned to the same feeding and management conditions were used to elucidate the relationship between the rumen microbiome and host growth rate, revealing that rumen microorganisms affect rumen fermentation and alter host productivity and health homeostasis.

VFAs play a pivotal role in enhancing the growth and development of ruminants [19]. In this study, HADG sheep exhibited elevated ruminal butyrate concentrations, which were positively correlated with ADG. Butyrate improves the efficiency of short-chain fatty acid (SCFA) absorption in the rumen [55] and enhances total nutrient digestibility [24], thereby exerting profound impacts on host productivity [26],S. K. B. [70, 71]. On the other hand, butyrate additionally serves as a critical stimulant and regulator of rumen epithelial growth and functionality [63]. It enhances rumen epithelial development by promoting mitotic activity in epithelial cells [24, 68]. In this study, increased papillae width and muscular layer thickness were observed in the rumen epithelium, both showing positive correlations with butyrate concentration. Butyrate modulates rumen epithelial metabolic activity [85].The increase in rumen papilla width implies a greater surface area of the rumen in contact and a better capacity for nutrient uptake (e.g., propionate) and ion transport, which meets the host's energy needs [25]. Besides, the increased thickness of the rumen muscular layer promotes adequate contact between the chow and the microorganisms in the rumen and increases the absorption of nutrients. Studies have shown that feed efficiency responds to the absorption of VFA by rumen epithelial cells to a certain extent [59, 93]. These previous studies support our findings that butyrate, rumen epithelial development and ADG form a closely related interaction network that is critical to changes in host ADG [1, 37, 57].

In this study, the ruminal propionate concentration was found to be significantly higher in HADG Hu sheep compared to the LADG group, with a positive correlation observed between propionate levels and ADG. Propionate serves as a critical substrate for hepatic gluconeogenesis, contributing 45–60% of glucose supply to the host circulatory system and markedly enhancing energy utilization efficiency [46]. Succinctly, the above results indicate that propionate plays a pivotal role in enhancing feed efficiency, and the variations in growth performance of Hu sheep may be attributed to the rumen microbiota-driven restructuring of fermentation patterns [46, 86, 87]. Specifically, the enrichment of *Succinivibrio dextrinosolvens* in the HADG group was positively correlated with both propionate and ADG, suggesting that it may directly promote host growth by regulating VFA biosynthesis. This aligns with prior findings demonstrating significant enrichment of *Succinivibrio* taxa in Hu sheep and beef cattle with high efficiency [3, 96, 97]. As a key species within the genus *Succinivibrio*, *Succinivibrio dextrinosolvens* efficiently degrades starch to produce succinate, which is subsequently converted to propionate via the acrylate pathway [53, 77]. Notably, we also found a positive correlation between *Succinivibrio* and ADG in a study of young goats, with a strong ability to ferment carbohydrates and improve host energy supply [81]. Moreover, its metabolic activity consumes H_2 and CO_2, significantly reducing methane emissions and redirecting metabolic energy toward host tissue deposition for growth and development [65]. These results collectively demonstrate that *Succinivibrio dextrinosolvens* likely functions as a central hub in Hu sheep growth regulation through a coordinated "substrate degradation–propionate synthesis–energy conservation" mechanism. Future studies employ microbial consortium transplantation or targeted supplementation strategies in larger-scale cohorts with continuous phenotypic samples to establish causal relationships between *Succinivibrio dextrinosolvens* enrichment, propionate synthesis, and ADG enhancement, potentially utilizing it as a microbial agent to improve the growth performance of Hu sheep.

Numerous previous studies have demonstrated that *Succinivibrio dextrinosolvens* occupies a significant niche in the gastrointestinal tracts of animals with high feed efficiency [33, 48, 62]. Concurrently, our findings revealed a positive correlation between KR and *Succinivibrio dextrinosolvens*. KR is commonly used as

an important selection indicator for feed efficiency in the absence of or difficulty in obtaining individual animal feeding records [10, 27, 36]. The application of KR aids in improving animal feed efficiency without adversely affecting carcass traits [18, 23]. Future research efforts should focus on comprehensively elucidating its role in rumen microecology and developing effective microbial manipulation techniques to enhance feed efficiency and animal growth.

Random forest algorithms have demonstrated utility in predicting host growth performance through microbial community profiling [81, 89] and assessing disease risks [54, 90]. In this study, random forest analysis identified *Succinivibrio dextrinosolvens* as a potential biomarker for discriminating between HADG and LADG sheep cohorts with high predictive accuracy. These findings highlight the microbial-metabolite interplay in growth phenotype stratification. Future studies are required to improve the predictive accuracy and robustness, while the findings of this study provide foundational insights for developing microbial consortia to enhance ovine growth performance and may aid in breeding or selecting high-growth-performance sheep in future research.

A healthy microbiome is recognized as a hallmark of superior growth performance in ruminants [64]. However, in this study, the majority of significantly enriched bacterial genera in LADG Hu sheep were classified as opportunistic pathogens (e.g., *Anaerotruncus*, *Sediminibacterium*, *Glaesserella*), which exhibited negative correlations with ADG. Dysbiosis of the gastrointestinal microbiota may trigger pathogen proliferation in response to low-grade systemic inflammation [40]. Notably, *Anaerotruncus*, *Sediminibacterium*, and *Glaesserella* were concurrently positively correlated with IL-6 and negatively correlated with IgG. Pathogen-induced inflammatory responses necessitate metabolic reprogramming, redirecting nutrients (e.g., glucose, amino acids, fatty acids) toward immune activation [21, 22, 61, 75], thereby depleting energy reserves and impairing host growth efficiency [72]. *Anaerotruncus* is classified as an opportunistic pathogen or a pro-inflammatory bacterium with detrimental potential, with its pathogenic effects mediated through NF-κB signaling pathway activation and Nrf2 antioxidant pathway suppression [91]. Previous studies have confirmed that *Anaerotruncus* is negatively correlated with weaning weight in rabbits (Fang et al., 2019), which may impair muscle development by depleting total fat and saturated fatty acids [4, 40, 43, 96, 97]. *Sediminibacterium* may potentially induce or exacerbate intestinal inflammation [50, 51], inhibit microbial protein (MCP) synthesis [2], or directly or indirectly suppress lipid deposition [40], thereby contributing to impaired growth performance. *Glaesserella parasuis*, a key species within the genus *Glaesserella*, is a major pathogen in swine production [69]. During infection, it stimulates the release of multiple cytokines and chemokines, thereby inducing robust inflammatory responses [30, 31]. Furthermore, protein metabolism and amino acid metabolism pathways are critically involved in the proliferation and virulence of *Glaesserella* (e.g., proteolysis, amino acid uptake, and biosynthesis) [28]. Concurrently, its metabolic activity downregulates genes associated with cellular carbohydrate metabolism, the citric acid cycle, and the electron transport chain [7], ultimately impairing host growth performance. In conclusion, the enrichment of these pathogenic bacteria in the rumen may disrupt the balance of the host's immune status and impair the efficient conversion of nutrients, leading to suboptimal growth and production performance in livestock [57], Sordillo, 2016). However, these findings require further validation to comprehensively elucidate whether and how these pathogenic bacteria affect host health, with a focus on developing probiotic strategies to mitigate the negative impacts of these pathogenic microbial communities.

The previous analysis of microbes was based on a single bacterial genus or species. However, rumen microbes are a large microbial ecology, and there are interactions between microbes. Based on this, we further utilized molecular co-occurrence network analysis to assess different microbial interaction patterns among animals with different growth performance. In this study, we identified *Alloprevotella*, *Phascolarctobacterium*, *Anaerovibrio*, *Butyricoccus*, *Ruminococcaceae_noname*, and *Roseburia* cornerstone microorganisms in the microbial co-occurrence network of the HADG, which play an important role in the degradation of carbohydrates and converting them into short-chain fatty acids (SCFAs), maintaining rumen health, and modulating inflammation. The roles of the remaining bacterial genera in regulating host growth through microbiota-dependent mechanisms warrant further investigation. Notably, no significant positive correlations were observed between these genera and ADG or VFA concentrations in the present study. Therefore, future research should employ in vitro culturing systems or microbial transplantation approaches to elucidate their potential roles in maintaining gastrointestinal health and modulating SCFA production, thereby informing strategies to optimize sheep growth and health.

Alloprevotell, *Ruminococcaceae_noname* are probiotic bacteria and generate SCFAs to supply the body with energy and regulate gastrointestinal health [39, 99]. *Phascolarctobacterium* hardly uses carbohydrates for

growth but uses succinate as a substrate accompanied by propionate production [35]. *Anaerovibrio*, which plays an important role in the lipolytic activity of rumen contents in sheep [66], producing succinate and VFAs [32, 49]. *Anaerovibrio* may collaborate with *Phascolarctobacterium* to collaboratively convert carbohydrates into volatile fatty acids (VFAs), thereby providing energy to the host and enhancing growth. *Butyricoccus* is an important genus of butyrate-producing organisms in the gut, and it is also a key marker capable of distinguishing between healthy and abnormal gut microorganisms [30, 31, 44]. *Roseburia* contributes to anti-inflammatory signaling and barrier repair through butyrate production [98], while also maintaining energy homeostasis via its metabolic activity [60]. Studies have shown that microbial network analysis has become an essential tool for deciphering microbial interactions and identifying key taxa that can drive community composition and function regardless of abundance [14]. However, direct evidence elucidating how their collaborative mechanisms modulate host metabolism to enhance growth remains limited. Future studies are warranted to experimentally validate these interactions and translate our findings into practical applications.

Deepening the understanding of the functions of rumen microbes will help us better understand their contribution to phenotypes [41]. In this study, the carbohydrate metabolism, amino acid metabolism, and energy metabolism pathways showed higher activity in HADG sheep. Specifically, the KEGG function related to carbohydrate degradation was significantly enriched in HADG sheep, and was positively correlated with ADG and VFAs such as propionate and butyrate. These pathways include Galactose metabolism, Pentose and glucuronate interconversions, Fructose and mannose metabolism, Glyoxylate and dicarboxylate metabolism, indicating that the rumen microbiome of HADG sheep exhibits an increased carbohydrate-degrading capacity, resulting in hydrolyzate and pyruvate production, promoting host growth [86]. Additionally, we used the CAZyme database for comparison and found that it was mostly the GTs family that was elevated in the LADG group, whereas in the HADG group there was a preference for elevated abundance of the GHs family, with similar results observed in a previous study [74]. GTs catalyze the attachment of activated sugars to different receptor molecules such as proteins, nucleic acids, oligosaccharides, lipids, and small molecules in organisms, and it has been reported that they are enriched in feed inefficient cattle [45]. GHs are the most common enzymes in the rumen and include a large class of enzymes involved in the metabolism of polysaccharides such as starch, cellulose, lignin, and chitin, responsible for catalyzing the hydrolysis of glycosidic bonds in complex carbohydrates [9, 73], and promoting the production of VFAs in the rumen. The significant enrichment of GHs in the HADG group indicated that the HADG sheep rumen microbiome has a strong carbohydrate degradation capacity, which is consistent with the results of KEGG. Some genes from carbohydrate metabolism pathways are thought to predict FE in cattle [47], suggesting that the HADG sheep rumen microbiome is more metabolically active, especially in carbohydrate degradation, to support the energy requirements of the host.

In addition, our study identified enrichment of Amino acid metabolism and Nitrogen metabolism in the HADG group, and this enrichment may contribute to the organism's productive performance [88]. Specifically, 3 pathways related to amino acid synthesis, including Alanine aspartate and glutamate metabolism, Arginine biosynthesis, Arginine and proline metabolism, were positively correlated with ADG. Protein degradation results in the breakdown of true protein into amino acids and ammonia, which are used by rumen microorganisms to synthesize microbial protein (MCP) [52]. The enrichment of these amino acid biosynthetic pathways in the rumen of HADG sheep suggests a possible increase in microbial protein synthesis, and these microbial proteins synthesized in the rumen contribute 60–90% of the absorbed protein in the small intestine, better meeting the amino acid composition requirements of Hu sheep [67, 81, 86]. Conclusively, the carbohydrate metabolism, amino acid metabolism and energy metabolism of high-growth-rate were enhanced, and their rumen microbiome had a high feed fermentation capacity and energy utilization efficiency, thus meeting the energy needs of sheep.

Overall, the taxonomic and functional characteristics of the rumen microbiota differ between Hu sheep with divergent growth rates. The rumen microbe *Succinivibrio dextrinosolvens* plays a critical role in propionate biosynthesis, not only optimizing carbohydrate metabolism but also reducing energy loss during fermentation, thereby directing feed energy toward growth performance. *Anaerotruncus*, *Sediminibacterium*, and *Glaesserella* exhibit significant correlations with IL-6 and IgG, disrupting the balance of host health homeostasis and impairing host growth. Future studies should employ targeted interventions such as rumen microbiota transplantation (RMT) toto investigate the functional roles and mechanistic impacts of these bacteria in sheep growth, alongside large-scale trials to confirm the generalizability of these microbial associations and the robustness of the results.

Conclusions

In summary, key microbes in the rumen affect overall host health homeostasis and rumen fermentation, leading to changes in energy utilization, and thus affecting the average daily gain (ADG) of Hu sheep. Some pathogenic bacteria, such as *Anaerotruncus*, *Sediminibacterium* and *Glaesserella*, may disrupt the balance of the host's immune status, leading to changes in nutrient conversion and energy supply, which will impair the host's growth performance. Sheep with higher ADG are enriched in *Succinivibrio_dextrinosolvens*, which contributes to the production of propionate, as well as carbohydrate, amino acid and energy metabolism, thereby meeting the nutritional and energy needs of sheep for rapid growth and promoting host growth. Random forest machine learning analysis showed that it can be used as an effective biomarker to predict animal growth rate in the future, with a prediction accuracy of 81.2%. Therefore, our findings provide fundamental insights into how microbiota-dependent mechanisms shape health and growth performance in Hu sheep, while also laying the groundwork for future investigations targeting microbial interventions that leverage dynamic microbial-immune crosstalk to optimize animal health and production efficiency. Notably, given the current limitations in sample size and validation of results in this study, future studies should validate the functional roles of these key rumen microbes in sheep growth through larger and more diverse cohorts and in vivo experiments (e.g., microbial transplantation).

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42523-025-00412-0.

Figure S1 Differences in α-diversity and β-diversity of rumen microbes in the two groups and distribution of Top 10 rumen bacteria at phylum and genus level. **A** Differences in rumen microbial α-diversity between the two groups. **B** Analysis of Similarity (ANOSIM) is a non-parametric statistical method used to assess microbial5community structure by testing whether inter-group differences (e.g., between experimental conditions) are significantly greater than intra-group differences. This determines the biological relevance of predefined groupings. The test generates an R statistic ranging from −1 to 1: 1 > R > 0: Inter-group dissimilarity exceeds intra-group variation (higher R values indicate stronger separation); −1 < R < 0: Intra-group variation dominates over inter-group differences (grouping lacks meaningful distinction). Statistical significance is defined as $P < 0.05$. **C** Bar charts illustrating the distribution of dominant bacterial phyla, genera, and species.

Figure S2 Analysis of zipi values of edges of HADG and LADG rumen microbial co-occurrence networks

Figure S3 Correlations Between Keystone Microbiota and Host Phenotypes, Volatile Fatty Acids (VFAs), and Immune Parameters

Figure S4 Microbial functions involved carbohydrate metabolism, amino acid metabolism, and nitrogen metabolism in the rumen of HADG and LADG sheep. **A** Carbohydrate metabolism pathways. **B** Amino acid metabolism pathways. The red text represents KEGG pathways, KEGG modules, KEGG enzymes, or metabolites enriched in the rumen microbiome of HADG sheep (LDA score > 2, $P < 0.05$). **C** Nitrogen metabolism pathways. The red text represents KEGG pathways, KEGG modules, KEGG enzymes, or metabolites enriched in the rumen microbiome of HADG sheep (LDA score > 2, $P < 0.05$).

Table S1 Experimental diet composition and nutrient levels (%, dry matter basis)

Table S2 ADG values of 66 Hu sheep and details of 16 Hu sheep

Table S3 Correlation between phenotypes in Hu sheep

Table S4 Network topology parameters for HADG and LADG sheep

Table S5 Analysis of zipi values of edges of HADG rumen microbial co-occurrence networks

Table S6 Analysis of zipi values of edges of LADG rumen microbial co-occurrence networks

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Author contributions

Ximei Xie, Hailing Luo and Yuze Yang designed the study, Ximei Xie performed molecular work, and bioinformatics, statistical analyses and drafted the manuscript, and Hailing Luo, Huan Yang, Xingang Zhao, Li Teng, and Yuze Yang contributed to the revision of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

Illumina data are available at NCBI (BioProject ID: PRJNA1125523)

Declarations

Competing interests

The authors declare no competing interests.

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