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Sex-induced alterations in rumen microbial communities and metabolite profiles: implications for lamb body weight

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Abstract

Background Microbiota-metabolome interactions play a crucial role in host physiological regulation and metabolic homeostasis. The aim of this study was to investigate that sex induces alterations in rumen microbial community composition and metabolite profiles in lambs and the influence on body weight. This study aimed to demonstrate that sex- induced alterations in rumen microbial community and metabolite profiles and blood indices and their linkage to growth performance in lambs.

Results This study examined (growth indices, serum indices, rumen fermentation parameters, rumen fluid microbiota community and metabolome profiles) in 180 Hu lambs (90 males, and 90 females) with the same age and diet. At six months, male lambs showed significantly greater body weight, serum indices (glutamic pyruvic transaminase, glutamic oxalacetic transaminase, growth hormone, glucagon-like peptide 1, and ghrelin), and molar percentage of propionic acid, isobutyric acid, butyric acid, isovaleric acid and valeric acid compared to female. However, male had lower VFA molar concentrations (acetic acid, propionic acid, butyric acid, and TVFAs), acetic acid/propionic acid, and VFA molar percentage (acetic acid) than female. Significant sex-related differences were observed in rumen microbiota and metabolic enrichment between genders. Moreover, compared with the females lambs, the relative abundance of *Succiniclasticum*, *uncultured_rumen_bacterium*, *NK4 A214_group*, *Veillonellaceae_UCG_001* and *Butyrivibrio* in the male lambs has been significantly increased, while the relative abundance of *Prevotella* has been significantly decreased ($P < 0.05$). Notably, there were significant rumen microbiota-metabolite interactions, especially Firmicutes and Bacteroidota as dominant phyla in the sheep rumen with significant differences in correlation with rumen metabolic modules. Additionally, there are pronounced correlations among the microbiota, particularly within the Firmicutes phylum. Furthermore, the up-regulated metabolites in the rumen fluid of male lambs were predominantly enriched in the amino acid metabolite pathway, and these metabolites exhibited a significant positive correlation with body weight. However, the metabolites that were up-regulated in ewe lambs were predominantly enriched in the lipid metabolic pathway, and these metabolites exhibited a significant negative correlation with body weight. Moreover, lamb rumen microbial markers (*Lachnospiraceae_UCG_008*, *Saccharofermentans*, *unclassified_Clostridia*, *Christensenellaceae_R_7_group*, *Anaerovorax*, *Mogibacterium*, and *unclassified_Erysipelotrichaceae*) and metabolic markers (C75, 4-Coumarate, Flibanserin,3-Amino-5-mercapto-1,2,4-triazole, 1,3-Propane sultone, Fingolimod phosphate ester, S-,) were significantly positively correlated with body weight, but lamb rumen microbial markers

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(*Anaeroplasma*, *unclassified_Acholeplasmataceae*, *uncultured_rumen_bacterum_4c28 d_15*) and metabolic markers (Mozenavir, Reduced riboflavin, PG(18:2(9Z,12Z)/0:0), Cowanin) were significantly negatively correlated body weight.

Conclusions This study shows that sex-induced alterations in rumen microbial communities and metabolite profiles, adapting to the growth and development of lambs. The findings may help develop targeted strategies to optimize sheep rumen microbiota and improve productivity.

Keywords Hu sheep, Rumen microbiota, Rumen metabolome, Rumen fermentation, Serum indicators

Introduction

Sheep (*Ovis aries*) is a key livestock species that has been selectively bred through natural and artificial selection since the Neolithic era [1], while rely on the rumen microbes to convert plant feeds like straw, hay, silage, and grass into meat, wool, fur, and milk [2–4]. The host gut microbiota is known as the "second genome", they can interact with the host and influence various physiological functions, including metabolic homeostasis, immune regulation, digestion, and adaptation to environmental changes [5–7]. This interaction plays a crucial role in the overall health and physiological functioning of the host. The study showed that microbes, as important regulators of ruminant growth performance, exhibit significant heritability and form a regulatory network with host genes, impacting immune regulation, digestion, metabolism, animal productivity, meat quality, and host health [4, 8, 9]. However, the microbial composition varies among breeds and is influenced by host genetics, diet, sex, age, geographic range, and other factors [10–12]. Furthermore, a complex regulatory network affects body weight, host genetics, and rumen microbes in sheep. For example, the rs405307925 variant in the *TOX* gene influences body weight by affecting the *Lachnospiraceae ND3007 group*, which impacts body weight through volatile fatty acids (VFAs) production [10]. Moreover, body weight heritability is 39%, with rumen microbiota contributing 20% to the phenotypic variance in Hu sheep at 180 days [10].

Small molecule compounds metabolized by the host modulate the gut microbiota, offering new insights into host-microbe interactions [13]. The primary interactions between microbiota and the host involve the consumption and production of metabolites, establishing a bidirectional relationship in which each party influences the frequency and abundance of the other [14]. It's worth noting that rumen microbiota generate VFAs and nutrients through fermentation, providing up to 70% of the host's energy, with over 70% of VFAs absorbed through the rumen epithelium [10, 15, 16]. In particular, VFAs bridge microbiota and host interactions, influencing metabolism, immune responses, and host health [10, 17]. Research indicates that VFAs raise adenosine monophosphate (AMP) concentration and the AMP/ATP ratio in skeletal muscle, activate AMP-activated protein kinase (AMPK), promote peroxisome proliferator–activated receptor gamma coactivator 1α (PGC1α) phosphorylation, enhance fatty acid uptake and oxidation, increase glucose uptake and production, and inhibit lipogenesis and glycolysis [18]. Interestingly, increasing serum VFAs concentration can improve glucose tolerance in patients with type 2 diabetes [19], and butyrate has a significant effect on the blood glucose level of sheep, while this effect is strongly associated with the body initial blood glucose [20]. Research has demonstrated that VFAs are crucial in the process of lipid biosynthesis [21]. Concurrently, in vivo stable isotope labeling studies and dietary interventions have indicated that acetate serves as a precursor for the hepatic synthesis of C16 and C18 fatty acids, as well as their associated glycerolipids within the metabolic cycle [22]. The rumen metabolome is an important tool for studying rumen metabolism, and small molecular compounds in the rumen, such as VFAs, bile acids, and succinic acid, have a significant effect on host phenotype [16, 23, 24]. Studies suggest that gastrointestinal microbiota can affect body weight in sheep by regulating metabolism [10], with microbial effects mediated by changes in metabolite concentrations [14]. Furthermore, microbiota modifies the amino acid profile of the host by metabolizing amino acids derived from the feeds, which may play a role in regulating amino acid homeostasis in peripheral organs and tissues [25]. Ruminants depend on the microbiota in their rumen to break down feed proteins into ammonia and VFAs for their physiological needs [8, 17, 18]. Microbiota display metabolic differences in substance preferences and utilization methods during growth and reproduction, adapting to habitats and resisting disturbances through varied metabolic behaviors [26]. However, although less behaviorally active, metabolites resemble microbial communities by transforming, assembling, and performing ecological functions [27]. Microbiota-metabolome interactions are closely linked to host condition, while host nutritional homeostasis is regulated by both the microbiota and the host's metabolic system [14, 25].

We posited that the observed variations in growth and development between male and female lambs could be attributed to sex-specific differences in rumen microbial

communities and their associated metabolites. Therefore, this study investigates the effects of sex-induced rumen microbial community and metabolome variations on lamb body weight through comprehensive analyses of growth performance, serum parameters, rumen fermentation characteristics, rumen fluid microbiota, and metabolomic profiles under identical age and dietary conditions. The findings provide insights for health management and the design of precise microbial intervention strategies to modulate rumen microbiome-metabolome interactions, thereby enhancing ovine productivity.

Methods

Lamb and experimental design

One hundred and eighty Hu lambs (90 males and 90 females) of similar weight and age were selected from Science and Technology Service Workstation of Jiangxi Academy of Agricultural Sciences (Ganzhou Lvlinwan Agriculture and Animal Husbandry Co., Ltd., Ganzhou, China). The lambs were weaned at 45 days (HF weaning weigh 11.36 ± 0.23 kg, HM weaning weigh 11.52 ± 0.19 kg) and raised under identical conditions (adjacent fences, half open-front livestock house, natural lighting, unpowered ventilator) until 6 months of age. Throughout the study all lambs had consistent feeding conditions with ad libitum access to feed and water, receiving a total mixed diet at 08:30 and 17:30 daily. The diet consisted of digestible energy at 10.82 MJ/kg, crude protein at 16.95%, neutral detergent fiber at 27.68%, acid detergent fiber at 17.31%, calcium at 0.65%, and phosphorus at 0.35% for the total mixed ration. Detailed nutrient fractions are described in Wang et al. [28]. All animal experimental designs and feeding management were approved by the Institute of Animal Husbandry and Veterinary, Jiangxi Academy of Agricultural Sciences (2010 − JAAS − XM − 01).

Sampling and measurements

Body weights and body size indicators

At 180 days of age, the body weights (BW) of all lambs were measured with calibrated electronic scales, while body size indices such as body height (BH), hip height (HH), body length (BL), chest circumference (CHC), and circumference of cannon bone (CCB) were measured using a soft ruler.

Serum biochemistry and hormones

Blood samples of 5 ml were collected from the jugular vein of 180 experimental sheep using a vacuum tube (Jiangxi Jingzhi Technology Co., Ltd., Nanchang, China) before feeding. The blood was kept at room temperature for 2 h, and then serum samples were collected by centrifuging in a low − speed centrifuge (TDL − 80 − 2B, Anting Scientific Instrument Factory, Shanghai, China) at 3500 rpm for 10 min, serum was separated and transfer it to a clean, labeled Eppendorf tube by storing at − 80 °C pending further analysis serum biochemical and hormones. Subsequently, the glucose (GLU), alkaline phosphatase (AKP), pyruvate kinase (PK), creatine kinase (CK), total cholesterol (TC), triglyceride (TG), creatinine (CRE), low-density lipoprotein (LDL), high density lipoprotein (HDL), glutamic-pyruvic transaminase (GPT), glutamic oxalacetic transaminase (GOT), and urea nitrogen (UN) index was determined by using the relevant kit provided by Nanjing Jian Cheng Bioengineering Institute (Nanjing, China) according to the instructions. Moreover, serum hormones cholecystokinin (CCK), growth hormone (GH), glucagon-like peptide 1(GLP-1), insulin-like growth factor 1(IGF-1), and insulin (INS) measurements followed the kit instructions (Shanghai Ke Xing Biotechnology Co., Ltd., Shanghai, China).

Rumen fluid pH, VFA and enzyme concentration

Rumen fluid was obtained from 180 lambs using a gastric tube sampler, with a volume of 50 mL collected per lamb, prior to feeding, following Wang et al. [28]. A portion of the samples was promptly analyzed for pH measurement (PHBJ-260 F, INESA Scientific Instruments Co., LTD, Shanghai, China), while the remaining samples were transferred into cryopreservation tubes, rapidly frozen in liquid nitrogen, and subsequently stored at −80 °C for future analysis. Moreover, the VFA molar content, including acetic acid (AA), propionic acid (PA), isobutyric acid (IBA), butyric acid (BA), isovaleric acid (IVA), valeric acid (VA), and total volatile fatty acids (TVFAs) was determined using a gas chromatograph (GC-7890B, Agilent Technologies) with reference to the method described in detail by Wang et. [28]. The VFA proportion (VFA molar content/TVFAs × 100%) and acetic acid/propionic acid (AA/PA) were calculated. Moreover, rumen fluid pepsase, β-glucosidase, lipase, xylanase, amylase, microcrystalline cellulase, and carboxymethyl cellulase measurements followed the kit instructions (Shanghai Ke Xing Biotechnology Co., Ltd., Shanghai, China).

DNA extraction and analysis of bacterial community in rumen

Bacterial DNA extraction was performed on 177 samples (Male lambs 89, female lambs 88) using the TGuide S96 Magnetic Stool DNA Kit (Tiangen Biotech (Beijing) Co., Ltd.) after removing 3 contaminated samples from 180 total rumen samples. The V3 − V4 hypervariable region of the bacterial 16S rRNA gene was amplified with primers 338 F (5′ − ACTCCTACGGGAGGCAGCA − 3′) and 806R (5′ − GGACTACHVGGGTWTCTAAT − 3′). The thermocycling profile included an initial denaturation at

95 °C for 5 min, followed by 20 cycles of denaturation at 95 °C for 30 s, 50 °C for 30 s, then 72 °C for 40 s, and a final extension at 72 °C for 7 min. The purified PCR products were collected and the paired ends was performed on the Illumina Novaseq 6000 platform (Beijing Biomarker Technologies Co., Ltd., Beijing, China).

Analysis of the metabolic profile of rumen fluid

Metabolomic measurements were performed on 177 (Male lambs 89, female lambs 88) of the 180 rumen samples after removing 3 contaminated samples. Metabolite assays are described in detail with reference to the previous article by Wang et al. [23]. Briefly, 100 μL of rumen fluid was mixed with 500 μL of an extraction solution containing an internal standard. Following the grinding and sonication of the rumen fluid in the presence of the extract and magnetic beads, the supernatant was obtained through centrifugation and subsequently subjected to vacuum drying. Thereafter, a suitable volume of extraction solution was introduced for re-dissolved, and the samples were analyzed utilizing the specified equipment. Finally, a liquid mass spectrometry system (Waters Acquity I-Class PLUS ultra-high per-formance liquid tandem Waters Xevo G2-XS QT high-resolution mass spectrometer, Waters, Milford, MA, USA) equipped with MassLynx V4.2 (Waters, Milford, MA, USA) data acquisition software was utilized, while Progenesis QI [29] software (version 4.0) facilitated peak extraction and alignment comparison to identify the metabolites for further analysis.

Bioinformatics analysis of rumen fluid sequencing

The rumen bacterial 16S rRNA gene High-throughput raw data were quality filtered with Trimmomatic [30] (version 0.33), followed by primer removal using Cutadapt [31] (version 1.9.1).The double-ended reads were spliced using USEARCH [32] (version 10) and the chimeras were removed and UCHIME [33] (version 8.1). The resulting amplicon sequence variants (ASVs) were denoised using DADA2 [34] in QIIME2 [35] (version 2020.6) with default parameters. Taxonomic annotation was performed using the Naive Bayes classifier in QIIME2 [35], referencing the SILVA [36] database (release 138.1) with a 70% confidence threshold. Beta diversity analysis uses binary Jaccard distance and sample sequence characteristics. Hierarchical clustering is performed with unweighted pair-group method with arithmetic mean (UPGMA), principal coordinates analysis (PCoA) and partialleast squares discriminant analysis (PLS-DA) tests for significant differences in beta diversity among sample groups. Furthermore, we used The ROC curve (receiver operating characteristic curve) is used to screen and evaluate biomarkers [37]. Sequence functional abundance was predicted using PICRUSt2 [38] (v2.2.0 − b). Meantime, the microbial genus level top80 correlation network was mapped based on correlation |R|> 0.3 using BMK Cloud (www.biocloud.net). Sequencing result datasets are available for download in the NCBI Sequence Read Archive (SRA) under search numbers PRJNA1180906 and PRJNA1180905.

The orthogonal projections to latent structures discriminate analysis (OPLS-DA) [39] model identified variable importance in projection (VIP) values for screening differential metabolites in each treatment group in the rumen. Finaly, identified metabolites were annotated using the KEGG database to construct rumen top5 differential metabolite enrichment network maps [40, 41]. Meanwhile, rumen metabolites were mapped to KEGG taxonomic entries using BMK Cloud (www.biocloud.net), and metabolic WGCNA module and microbial correlation heatmap and network mapping were performed.

Statistical analysis

Body size (BW, BH, HH, BL, CCB, and CHC), serum index (Glu, AKP, PK, CK, TG, CRE, LDL, HDL, GPT, GOT, BCA albumin, UN, CCK, GH, ghrelin, GLP-1, IGF-1, and INS) and rumen parameter (pH, AA, PA, BA, VA, IBA, IVA, TVFAs, A/P, AAR, PAR, BAR, VAR, IVAR, IBAR, pepsase, β-glucosidase, lipase, xylanase, amylase, microcrystalline cellulase, and carboxymethyl cellulase) of sheep were analyzed using nonparametric statistical methods (Mann–Whitney U test) in SPSS software (version 26.0) (SPSS Inc., Chicago, IL, USA). Redundancy analysis (RDA) and Pearson correlation heatmaps for microbial and body size indicators are performed through the BMK Cloud (www.biocloud.net). The analysis of serum indicators, rumen metabolic profiles, microorganisms, digestive enzymes, fermentation parameters, and their interrelationships was conducted utilizing Mantel's r analysis through R software. In addition, Pearson correlation heat map was drawn. The data were represented as the means ± Standard Error. $P < 0.05$ was considered significant.

Results

Analysis of body size indicators in lambs

Table 1 indicates that there were no statistically significant differences observed in the overall body weight and body size indices of lambs at the time of birth, and there were no significant differences in BW, BL and CHH when lambs were weaned at 45 days of age ($P > 0.05$). Moreover, HM lambs exhibited significantly greater BW, BH, HH, CCB, and CHC at 180 days compared to HF lambs. Additionally, their absolute growth in both BW, BH, HH, CCB, and CHC from 0 to 180 days was significantly higher than HF lambs ($P < 0.05$). In addition, correlation

Table 1 Influence of sex on body size index and correlation analysis between body size of lambs

Items		BW,kg	BH, cm	HH, cm	BL, cm	CCB, cm	CHC, cm
0 day	HF	2.95±0.07	36.25±0.25	36.34±0.30	30.91±0.28	5.69±0.48	33.58±0.27
	HM	3.10±0.07	36.49±0.23	36.49±0.27	31.42±0.29	5.89±0.06	34.12±0.30
	P-Value	0.126	0.589	0.890	0.273	0.029	0.140
45 day	HF	11.36±0.23	50.88±0.29	52.42±0.27	45.22±0.34	6.89±0.42	48.00±0.34
	HM	11.52±0.19	51.85±0.27	53.19±0.29	46.11±0.34	7.06±0.06	48.21±0.34
	P-Value	0.289	0.009	0.030	0.148	0.047	0.579
180 day	HF	28.12±0.45	65.64±0.35	66.66±0.35	61.67±0.64	7.57±0.06	68.86±0.43
	HM	31.94±0.42	67.94±0.34	69.60±0.33	61.45±0.52	8.18±0.04	72.24±0.58
	P-Value	< 0.001	< 0.001	< 0.001	0.518	< 0.001	< 0.001
0–180 days absolute growth	HF	139.84±2.40	0.16±0.00	0.17±0.00	0.17±0.00	0.01±0.00	0.20±0.00
	HM	160.19±2.29	0.17±0.00	0.18±0.00	0.17±0.00	0.01±0.00	0.21±0.00
	P-Value	< 0.001	< 0.001	< 0.001	0.299	< 0.001	< 0.001

Abbreviations: *HF* Hu female lamb, *HM* Hu male lamb, *BW* Body Weight, *BH* Body Height, *HH* Hip Height, *BL* Body Length, *CCB* Circumference of Cannon bone, *CHC* Chest Circumference

analysis between body weight and body size indicators revealed that BW showed significant positive correlation with BH, HH, BL, CCB, and CHC, and the overall positive correlation between body size indicators in the 180 d (Fig. 1A and B) ($P < 0.05$).

Analysis of serum index in lambs

From the Table 2, it was found that GPT, GOT, GH, ghrelin, and IGF-1 were significantly higher in HM serum than in HF, but TC, LDL and INS was significantly lower than in HF ($P < 0.05$). However, there was no significant difference in GLU, AKP, PK, CK, TG, CRE, HDL, BCA albumin, UN, CCK and GLP-1 between HM and HF serum ($P > 0.05$).

Analysis of rumen fermentation parameter in lambs

From the Table 3, it was found that VFA molar concentration (IBA and IVA,) pH, and VFA molar proportion (PAR, IBAR, BAR, IVAR, and VAR) were significantly higher in the rumen of HM than in HF, whereas the VFA molar concentration (AA, PA, BA, and TVFAs), AA\ PA, and VFA molar proportion (AAR) were significantly lower than those of HF ($P < 0.05$). However, rumen fluid VA molar concentration was not significantly different between HM and HF were not significantly different ($P > 0.05$). In addition, HM rumen pepsase, xylanase, amylase, and CMC was significantly higher than HF, while digestive enzyme β-glucosidase and lipase was significantly lower than HF ($P < 0.05$). However, rumen MCC had no significant effect between HF and HM ($P > 0.05$).

Analysis of the rumen microbiota of lambs

Analysis of rumen microbial diversity in HF and HM groups

A total of 13,660,700 base pairs of Reads were obtained from the sequencing of 177 samples. Following quality control and splicing of the paired-end reads, a total of 13,593,681 Clean Reads were generated. Each sample produced a minimum of 42,462 Clean Reads, with an average of 76,800 Clean Reads per sample. These mapped to 30 phyla, 72 classes, 197 orders, 396 families, 759 genera, and 972 species (Supplementary Table 1). Analysis of these sequences through Fig. 2A Venn diagram revealed

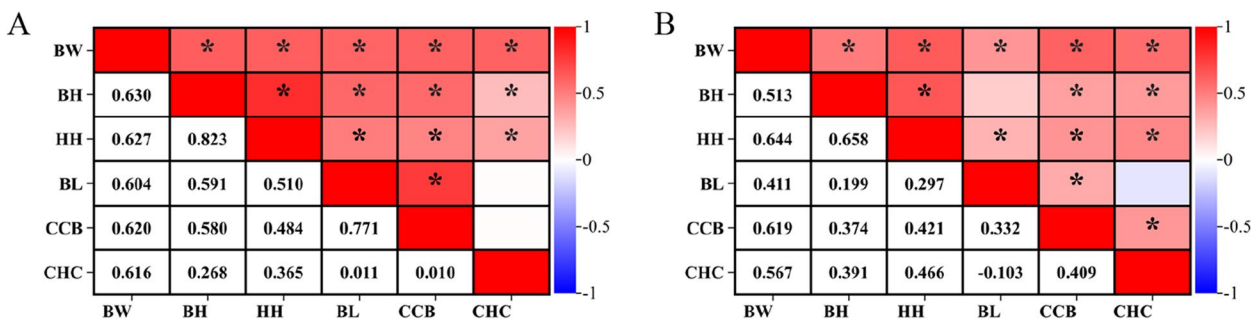

Fig. 1 Correlation analysis of body size indicators. Heat map of correlation between body size indices of HF (**A**) and (**B**). Note: HF: Hu female lamb, HM: Hu male lamb, * in the correlation heat map indicates $P < 0.05$. The values in the heat map represent correlation coefficients

Table 2 Effect of sex on serum biochemistry and hormones in lambs

Items		HF	HM	*P*-Value
Serum biochemistry	GLU, mmol/L	4.20±0.13	4.09±0.09	0.795
	AKP, King unit/100 mL	2.72±0.11	2.72±0.12	0.781
	PK, U/L	42.88±2.49	47.63±3.97	0.775
	CK, U/mL	0.66±0.03	0.64±0.03	0.211
	TC, mmol/L	3.34±0.09	3.14±0.09	0.027
	TG, mmol/L	0.20±0.01	0.22±0.01	0.131
	CRE, μmol/L	53.79±0.85	55.00±0.84	0.696
	LDL, mmol/L	2.25±0.09	2.05±0.17	0.003
	HDL, mmol/L	1.72±0.08	1.64±0.09	0.376
	GPT, U/L	29.22±0.90	31.72±0.97	0.044
	GOT, U/L	57.94±2.25	71.39±5.23	0.007
	BCA albumin, μg/μL	2.79±0.05	2.78±0.04	0.682
	UN, mmol/L	9.51±0.78	7.60±0.53	0.244
Serum hormones	CCK, pg/mL	310.19±7.57	320.18±7.01	0.145
	GH, μg/L	3.03±0.06	4.88±0.16	< 0.001
	ghrelin, ng/L	754.11±14.53	889.18±28.14	< 0.001
	GLP-1, pmol/L	10.77±0.23	11.21±0.24	0.239
	IGF-1, μg/L	281.82±6.93	352.86±4.79	< 0.001
	INS, mIU/L	82.12±2.12	78.56±3.22	0.032

Abbreviations: *HF* Hu female lamb, *HM* Hu male lamb, *GLU* Glucose, *AKP* Alkaline phosphatase, *PK* Pyruvate kinase, *CK* Creatine kinase, *TC* Total cholesterol, *TG* Triglyceride, *CRE* Creatinine, *LDL* Low-density lipoprotein, *HDL* High density lipoprotein, *GPT* Glutamic-pyruvic transaminase, *GOT* Glutamic oxalacetic transaminase, *UN* Urea nitrogen, *CCK* Cholecystokinin, *GH* Growth hormone, *GLP-1* Glucagon-like peptide 1, *IGF-1* Insulin-like growth factor 1, *INS* Insulin

that these sequences were assigned to 44,788 ASVs, of which 9,920 ASVs were shared among two groups (Fig. 2A). Next, the results of the analysis based on the binary Jaccard UPGMA (Supplementary Fig. 1), PCoA (Fig. 2B) and PLS-DA (Fig. 2C, R = 0.215, $P = 0.001$) analyses revealed significant differences in microbial presence between the HM and HF groups. Next, our analysis of rumen microbial α-diversity showed that in the HM group ACE (Fig. 2D), Chao1 (Fig. 2E), Shannon (Fig. 2F), and Simpson (Fig. 2G) were all significantly higher than in the HF group. Thus, sex significantly impacted rumen microbial diversity in lambs, with male lambs exhibited a heightened level of microbial diversity.

Taxonomic composition and characterization of the HF and HM Groups

Figure 3 illustrates the relative abundance of the top 10 abundant microbes at the phylum (A) and genus (B) levels in stacked histograms. At the phylum level, the 2 dominant phyla (Firmicutes and Bacteroidetes) accounted for more than 94.19% of the total bacterial count in the community (Fig. 3A). Among them, Firmicutes was the most prominent microbe, accounting for 51.99% in the HF group and 57.05% in the HM group (Fig. 3A). Bacteroidota and were the second most abundant phyla based on 16S rRNA sequencing (Fig. 3A). Meanwhile, compared with the HF group, the relative abundance of Firmicutes, Desulfobacterota, Verrucomicrobiota and Chloroflexi in the HM group has been significantly increased ($P < 0.05$), while the relative abundance of Bacteroidota and Synergistota has been significantly decreased ($P < 0.05$) (Fig. 3A). Furthermore, there were no significant differences observed in the abundance of Patescibacteria, Proteobacteria, unclassified Bacteria, and Actinobacteriota between HF and HM group ($P > 0.05$) (Fig. 3A).

At the genus level, the top 10 abundations accounted for more than 60.08% (Fig. 3B). In addition, 2 dominant genes *Prevotella* (HF 14.55%; HM 10.21%) and *Rikenellaceae_RC9_gut_group* (HF 10.37%; HM 9.80%) accounted for more than 20.00% of the total population (Fig. 3B). Meanwhile, compared with the HF group, the relative abundance of *Succiniclasticum*, *uncultured_rumen_bacterium*, *NK4 A214_group*, *Veillonellaceae_UCG_001* and *Butyrivibrio* in the HM group has been significantly increased ($P < 0.05$), while the relative abundance of *Prevotella* has been significantly decreased ($P < 0.05$) (Fig. 3B). However, HM and HF had no significant effect between *Rikenellaceae_RC9_gut_group*, *unclassified_Selenomonadaceae*, *unclassified_F082*, and *Selenomonas* ($P > 0.05$) (Fig. 3B). Moreover, it was found by constructing a network diagram of genus-level communities with top 80 abundance, which was dominated by the phylum Firmicutes in the both HM and HF group (Fig. 3C and D).

Table 3 Effect of sex on rumen fermentation parameter in lambs

Items		HF	HM	*P*-value
	pH	7.21±0.02	7.48±0.02	< 0.001
VFA molar concentration, mmol/L	AA	34.71±1.19	16.94±0.77	< 0.001
	PA	6.39±0.22	4.21±0.10	< 0.001
	IBA	0.81±0.02	1.00±0.10	0.016
	BA	5.12±0.26	2.83±0.10	< 0.001
	IVA	1.13±0.03	1.30±0.04	0.002
	VA	0.48±0.04	0.48±0.05	0.567
	TVFAs	48.61±1.65	26.63±0.89	< 0.001
	AA/PA	5.44±0.05	4.03±0.14	< 0.001
VFA molar proportion, %	AAR	71.31±0.19	61.13±1.25	< 0.001
	PAR	13.19±0.10	16.91±0.55	< 0.001
	IBAR	1.79±0.05	4.16±0.41	< 0.001
	BAR	10.20±0.22	11.32±0.49	< 0.001
	IVAR	2.52±0.08	5.25±0.20	< 0.001
	VAR	1.09±0.14	2.02±0.26	< 0.001
digestive enzyme	Pepsase, ug/L	12.40±0.19	13.45±0.21	< 0.001
	β- glucosidase, ng/L	1076.72±19.32	908.90±15.13	< 0.001
	Lipase, ng/mL	237.01±4.49	187.28±3.26	< 0.001
	Xylanase, pg/mL	251.04±3.65	322.62±4.37	< 0.001
	Amylase, umol/L	138.16±1.93	173.34±3.57	< 0.001
	MCC, pg/mL	105.39±2.55	102.59±1.45	0.544
	CMC, pg/mL	379.35±8.23	407.97±6.39	0.012

Abbreviations: *HF* Hu female lamb, *HM* Hu male lamb, *AA* Acetic acid, *PA* Propionic acid, *BA* Butyric acid, *IBA* Isobutyric acid, *IVA* Isovaleric acid, *TVFA* Total volatile fatty acid, *AAR* Acetic acid molar ratio, *PAR* Propionic acid molar ratio, *BAR* Butyric acid molar ratio, *AA/PA* Acetic acid ratio propionic acid, *IBAR* Isobutyric acid molar ratio, *IVAR* Isovaleric acid molar ratio, *MCC* Microcrystalline cellulase, *CMC* Carboxymethyl cellulase

Screening for biomarkers in the HM and HF groups

ROC curve analysis of rumen biomarkers in Hu sheep showed that species *Anaeroplasma*, *Lachnospiraceae_UCG_008*, *unclassified_Acholeplasmataceae*, *Saccharofermentans*, *unclassified_Clostridia*, *Christensenellaceae_R_7_group*, *uncultured_rumen_bacterium_4 C28 d_15*, *Anaerovorax*, *Mogibacterium*, and *unclassified_Erysipelotrichaceae* ranked in the top 10, with species *Anaeroplasma* having the highest contribution (Fig. 4A). Furthermore, the analysis of microbial markers in Fig. 4A, it was found that the HM group significantly increased *Lachnospiraceae_UCG_008* (AUC = 0.836, Fig. 4 A and B), *Saccharofermentans* (AUC = 0.827, Fig. 4 A and C), *Christensenellaceae_R_7_group* (AUC = 0.821, Fig. 4 C and D), *Mogibacterium* (AUC = 0.807, Fig. 4 A and E), *Anaerovorax* (AUC = 0.807, Fig. 4 A and F), *unclassified_Clostridia* (AUC = 0.826, Fig. 4 A and G), and *unclassified_Erysipelotrichaceae* (AUC = 0.807, Fig. 4 A and H), but significantly decreased *Anaeroplasma* (AUC = 0.913, Fig. 4 A and I), *unclassified_Acholeplasmataceae* (AUC = 0.833, Fig. 4 A and J), and *uncultured_rumen_bacterium_4 C28 d_15* (AUC = 0.820, Fig. 4 A and K) compared to the HF group.

Functional prediction of rumen microbiota between HF and HM

To predict the potential effects of rumen microbiota on host physiology, we performed PICRUSt2 using the KEGG database. It is worth noting that microbial class1 is mainly enriched in metabolism (HF 79.24%; HM 79.06%) (Fig. 5A), while there are significant differences in HF and HM ($P < 0.05$) (Fig. 5B). Next, we further analysis of class2 top10 metabolic pathways shows that carbohydrate metabolism (HF 9.28%; HM 9.22%), membrane transport (HF 3.06%; HM 3.15%), and signal transduction (HF2.11%; HM 2.17%) were significantly different between HF and HM ($P < 0.05$) (Fig. 5 C and D). Finally, based on an abundance threshold of greater than 0.50, we identified 19 significantly different metabolic pathways at the class 3 level (Table 4). As can be seen in Table 4, compared with the HF group, the relative abundance of pentose phosphate pathway, pyruvate, methane, biosynthesis of antibiotics, biosynthesis of amino acids, 2-oxocarboxylic acid, porphyrin and chlorophyll, and one carbon pool by folate in the HM group has been significantly increased, while the relative abundance of glyoxylate and dicarboxylate, TCA cycle, glycolysis/gluconeogenesis, fructose and mannose, galactose, oxidative phosphorylation, alanine, aspartate and glutamate, glycine, serine and threonine, metabolic pathways, pantothenate

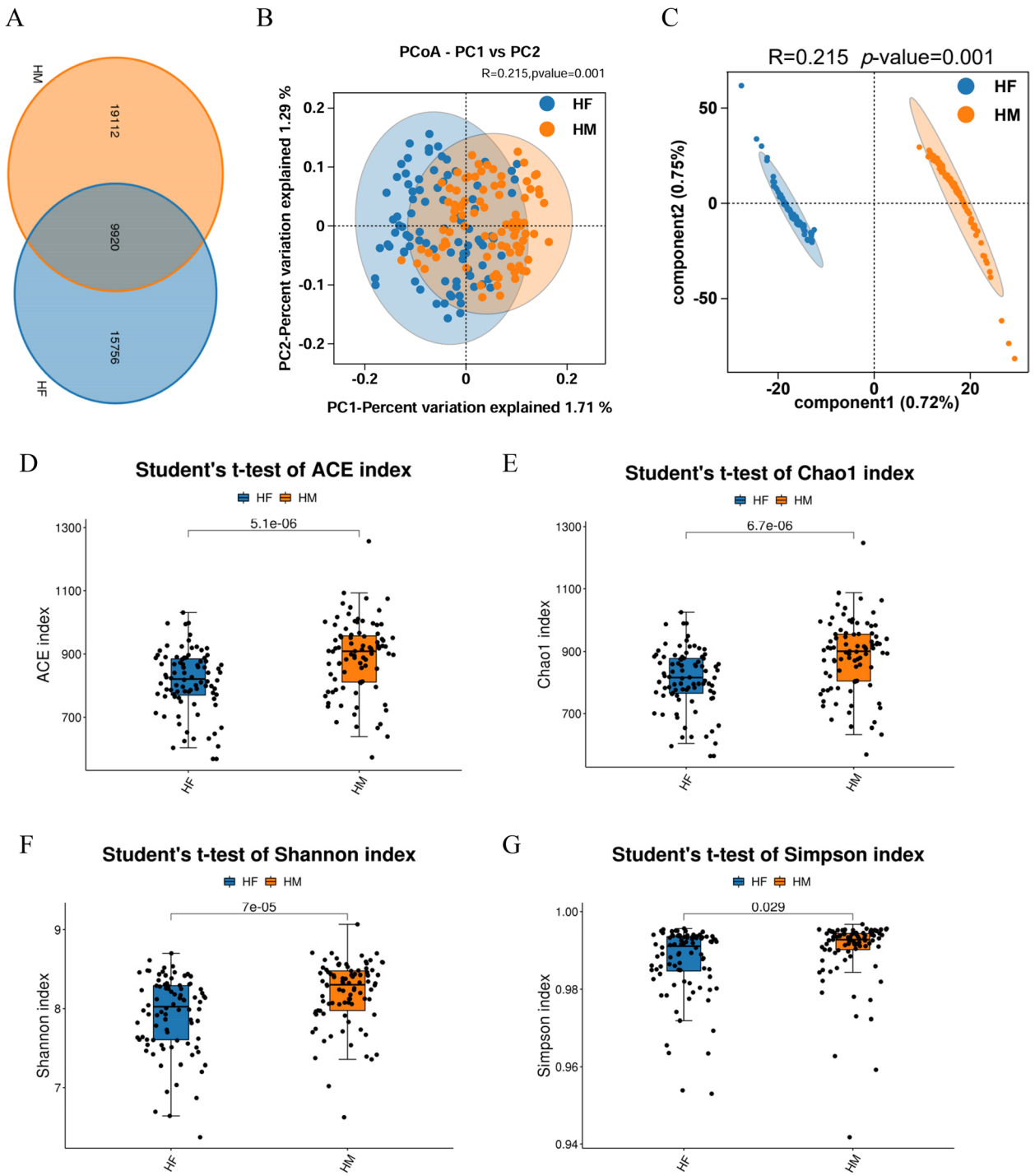

Fig. 2 Analysis of rumen microbial diversity. ASVs-Venn diagram analysis of HF and HM (**A**). Rumen microbial beta diversity PCoA (**B**) and PLS-DA (**C**) analysis of HF and HM. Rumen microbial alpha diversity indicators ACE (**D**), Chao1 (**E**), Shannon (**F**), and Simpson (**G**) analysis of HF and HM. Abbreviations: HF: Hu female lamb, HM: Hu male lamb

and CoA biosynthesis, pyrimidine has been significantly decreased ($P < 0.05$).

Analysis of rumen metabolism in lambs

Analysis of rumen differential metabolites across the HM and HF groups

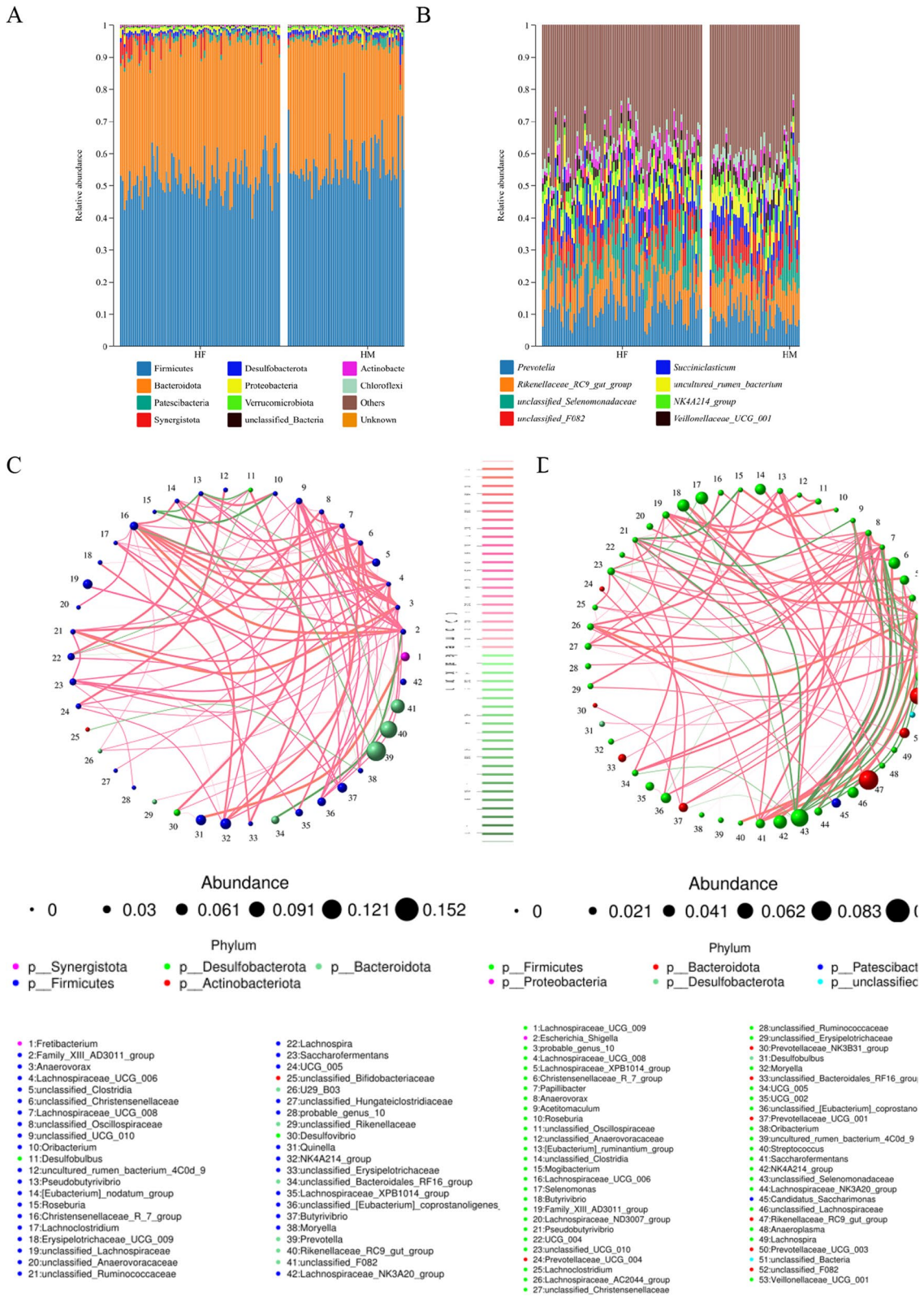

Fig. 3 Rumen microbial community composition in HF and HM. Rumen microbial annotation results at the top 10 phylum level (**A**) and genus level (**B**). Network diagrams of HF (**C**) and HM (**D**) microbial correlations at genus level top80 are constructed. Abbreviations: HF: Hu female lamb, HM: Hu male lamb

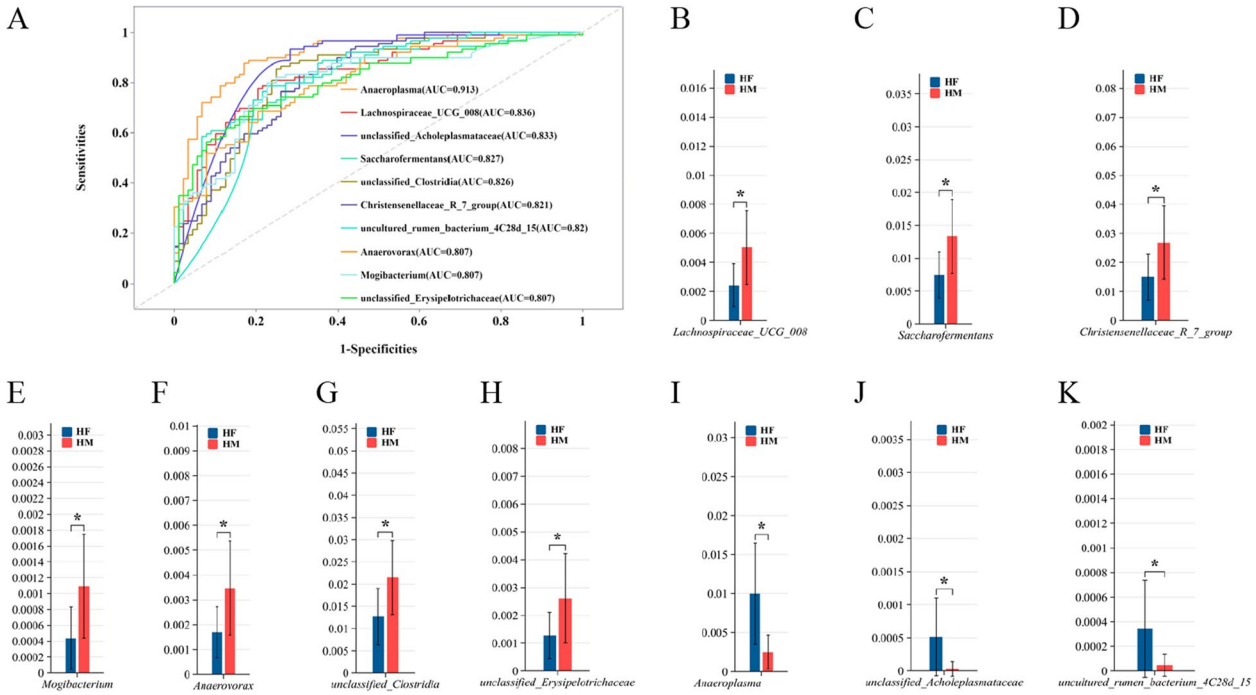

Fig. 4 Screening for biomarkers in the HM and HF groups. **A** receiver operating characteristic (ROC) curve to assess sheep rumen biomarkers. **B**-**K** shows the top 10 AUC microbial abundances. Note: HF: Hu female lamb, HM: Hu male lamb. * in the bar graph indicates $P < 0.05$

It can be seen from OPLS-DA that the samples of each group were distinguished. In this case, the model is reliable (Q2Y = 0.954 > 0.50) and can be used to screen for differential metabolites. Next, according to VIP > 1 and $P < 0.05$, in which 4982 metabolites were identified, containing 1833 (598 up-regulated and 1235 down-regulated) differential metabolites Fig. 6 A and B, Supplementary Table 2). Subsequently, we conducted a differential metabolite screening utilizing more rigorous screening criteria (VIP > 2, AUC > 0.8, $P < 0.05$) and performed correlation analyses with microbial genera and rumen parameters, as illustrated in Figs. 6C and D. The Mantel's r analysis of rumen microbial genus levels with rumen differential metabolites showed that microbiota was significantly correlated with the rumen differential metabolites Fig. 6 C) ($P < 0.05$). Additionally, the differential metabolites were significantly correlated with each other, with the up-regulated metabolites being significantly negatively correlated with the down-regulated metabolites ($P < 0.05$). However, a significant positive correlation existed between up-regulation and up-regulation metabolites, and between down-regulation and down-regulation metabolites ($P < 0.05$) (Fig. 6 C). Meanwhile, the differential metabolites and fermentation parameters of the rumen were analyzed. The results indicated significant differences in the correlations between the rumen fermentation parameters and both up-regulated and down-regulated metabolites. Among them, β-GLU, lipase, AA, PA, BA, TVFAs, AA/PA and AAR were significantly negatively correlated with up-regulated metabolites and significantly positively correlated with down-regulated metabolites ($P < 0.05$). However, xylanase, amylase, IVA, PAR, IBAR, IVAR, VAR and pH were significantly positively correlated with up-regulated metabolites and significantly negatively correlated with down-regulated metabolites ($P < 0.05$). However, pepsase, MCC, CMC, IBA, VA and BAR had essentially no significant correlation with the differential metabolites ($P > 0.05$) (Fig. 6 D).

Furthermore, we produced ROC curves for top 10 rumen metabolites analysis of metabolite markers by Fig. 6 E-O revealed that the HM group significantly increased 1,3-propane sultone (neg_15079, AUC = 1.000, Fig. 6 E and F), flibanserin (neg_15575, AUC = 1.000, Fig. 6 E and G), 3-Amino-5-mercapto-1,2,4-triazole (neg_15700, AUC = 1.000, Fig. 6 E and H), 4-Coumarate (neg_5752, AUC = 0.993, Fig. 6 E and I), fingolimod phosphate ester, S- (pos_14144, AUC = 0.987, Fig. 6 E and J), and C75 (neg_11832, AUC = 0.986, Fig. 6 E and K), and significantly decreased reduced riboflavin (pos_2999, AUC = 0.988, Fig. 6 E and L), cowanin (pos_2442, AUC = 0.985, Fig. 6

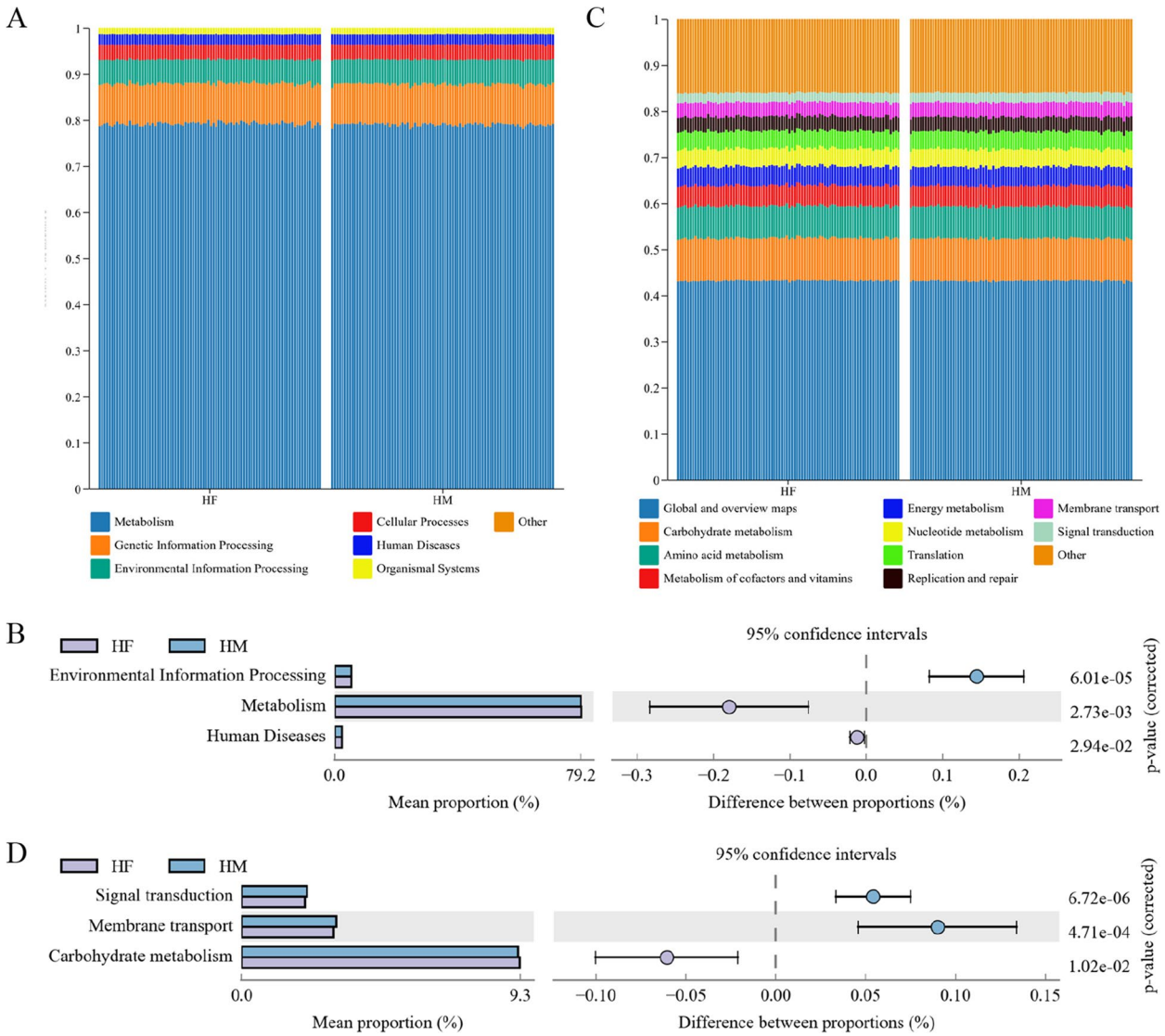

Fig. 5 PICRUSt2 was employed to predict the functional profiles of lamb rumen microbiota, showing (**A**) functional enrichment at Class 1 and (**B**) significantly altered pathways, followed by (**C**) Class 2 enrichment and (**D**) associated pathway differences. Abbreviations: HF: Hu female lamb, HM: Hu male lamb

E and M),PG (18:2(9Z,12Z)/0:0) (pos_3004, AUC =0.984, Fig. 6 E and N), and Mozenavir (pos_3005, AUC =0.984, Fig. 6 E and O) compared to the HF group ($P< 0.05$).

Additionally, the results of correlation analysis by body weight, rumen parameters, and rumen metabolism showed that pepsase, xylanase, amylase, PAR, VAR, BAR, pH, and BW were significantly negatively correlated with down-regulated metabolites markers (pos_2999, pos_2442, pos_3004, and pos_3005) of the HM group, but they were significantly positively correlated with up-regulated metabolites markers (neg_15079, neg_15575, neg_15700, neg_5752, pos_14144, and neg_11832) of the HM group ($P< 0.05$) Fig. 6 P). However, β-GLU, lipase, AA, PA, BA, TVFAs, AA/PA, and AAR were significantly positively correlated with down-regulated metabolites markers (pos_2999, pos_2442, pos_3004, and pos_3005) in HM group, but they were significantly negatively correlated with up-regulated metabolites markers (neg_15079, neg_15575, neg_15700, neg_5752, pos_14144, and neg_11832) in HM group ($P< 0.05$) Fig. 6 P).

Functional enrichment analysis of rumen metabolites in lambs

Rumen metabolites were annotated using the KEGG database, and the top 20 pathways with the highest

Table 4 Significant differences pathways between the HF and HM groups in metabolism

Class1	Class2	Pathways ID	HF	HM	*p*-value
Metabolism	Carbohydrate	Glyoxylate and dicarboxylate	0.725	0.716	0.000
		Pentose phosphate pathway	0.704	0.711	0.009
		Citrate cycle (TCA cycle)	0.667	0.655	0.006
		Glycolysis/Gluconeogenesis	1.022	1.016	0.029
		Pyruvate	0.941	0.957	0.000
		Fructose and mannose	0.747	0.723	0.001
		Galactose	0.615	0.592	0.003
	Energy	Oxidative phosphorylation	0.955	0.944	0.019
		Methane	0.605	0.613	0.000
	Amino acid	Alanine, aspartate and glutamate	0.920	0.911	0.032
		Glycine, serine and threonine	0.827	0.821	0.008
	Global and overview maps	Metabolic pathways	17.414	17.326	0.000
		Biosynthesis of antibiotics	5.826	5.837	0.016
		Biosynthesis of amino acids	4.086	4.113	0.016
		2-Oxocarboxylic acid	0.845	0.853	0.026
	cofactors and vitamins	Porphyrin and chlorophyll	0.841	0.868	0.002
		One carbon pool by folate	0.765	0.773	0.029
		Pantothenate and CoA biosynthesis	0.600	0.596	0.008
	Nucleotide	Pyrimidine	1.814	1.799	0.004

Abbreviations: *HF* Hu female lamb, *HM* Hu male lamb

number of selected metabolites are shown in Fig. 7A, including amino acid metabolism, biosynthesis of other secondary metabolites, digestive system, lipid metabolism, membrane transport, metabolism of cofactors and vitamins, nervous system, nucleotide metabolism, and xenobiotics biodegradation and metabolism. Meantime, Mantel's results showed that rumen metabolites was significantly correlated with rumen VFA molar content (AA, PA, BA, IVA, VA, and TVFAs) (Fig. 7 B), VFA molar ratios (AA/PA, AAR, PAR, IBAR, BAR, IVAR, and VAR) (Fig. 7 C), and rumen (pepsase, β-GLU, lipase, xylanase, amylase, MCC, and CMC) ($P < 0.05$) (Fig. 7 D).

Subsequently, rumen KEGG top 20 enrichment point maps and top5 differential metabolite enrichment network maps were constructed as shown in Fig. 7 E and F, respectively. The top5 pathways enriched for HF vs HM differential metabolites were analyzed, which were mainly enriched in porphyrin metabolism, alpha-Linolenic acid metabolism, monobactam biosynthesis, TCA cycle, and taste transduction (Fig. 7 F). Next, the correlation analysis of rumen enzyme with KEGG top 5 enriched differential metabolites revealed that lipase, β-GLU, AAR, AA/PA, BA, PA, TVFAs, and AA were basically positively correlated with alpha-Linolenic acid metabolism, porphyrin metabolism and taste transduction, respectively, but negatively correlated with monobactam biosynthesis and TCA cycle pathway ($P < 0.05$) (Fig. 7 G). In addition, BW, pH, xylanase, amylase, PAR, and VAR were largely negatively correlated with alpha-Linolenic acid metabolism and taste transduction, respectively, but they were positively correlated with monobactam biosynthesis and TCA cycle pathway ($P < 0.05$) (Fig. 7 G). Moreover, CMC, MCC, and pepsase were not significantly correlated with the differential metabolites of the KEGG-enriched top 5 pathway ($P > 0.05$) (Fig. 7 G). Mantel's results indicated that the microbiota at the genus level in the rumen and the top 5 differential metabolites of the KEGG pathways were significantly correlated (Fig. 7 H) ($P < 0.05$). Additionally, there was a notable correlation among the differential metabolites themselves (Fig. 7 H)($P < 0.05$).

Finally, up-regulated differential metabolites were mainly enriched in amino acid metabolic pathways (glycine, serine and threonine metabolism, tyrosine metabolism, arginine and proline metabolism, cysteine and methionine metabolism, and phenylalanine metabolism) and down-regulated metabolites were mainly enriched in lipid metabolism (arachidonic acid metabolism, alpha-Linolenic acid metabolism, steroid hormone biosynthesis, linoleic acid metabolism, primary bile acid biosynthesis, biosynthesis of unsaturated fatty acids, and cutin, suberine and wax biosynthesis) ($P < 0.05$) (Fig. 7 I and J). Meantime, our overall results analysis the correlation of rumen most enriched pathway metabolites with rumen fermentation parameters and body weight in rams and ewes showed that metabolites (L-Arginine phosphate, O-Phospho-L-serine, L-Proline,

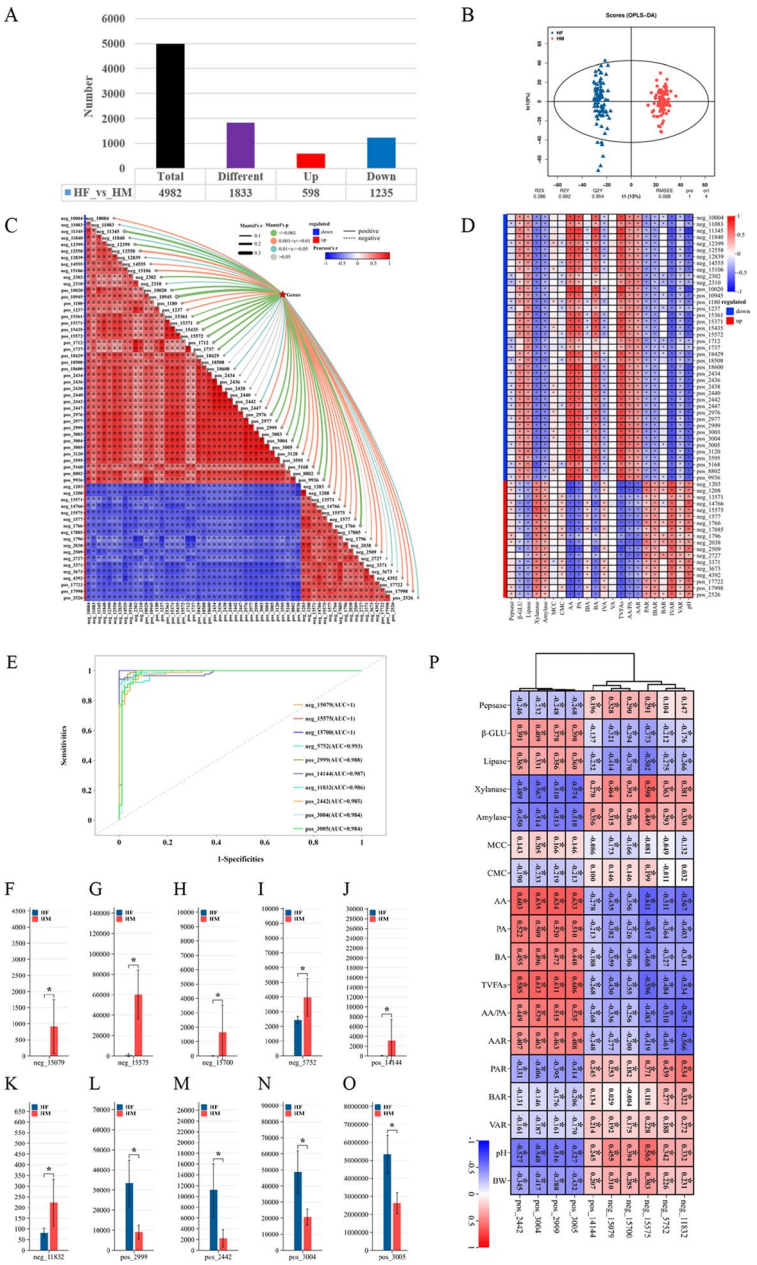

Fig. 6 Analysis of rumen differential metabolites in lambs. metabolite statistics histogram (**A**) and Plotting of OPLS-DA model scores (**B**) and for HF vs HM. **C** Mantel's r analysis of rumen microbial genus levels with rumen differential metabolites correlation. The width of the edges is representative of Mantel's r statistics associated with the respective distance correlations, while the color of the edges reflects the significance of Mantel's p statistic, where a gray line denotes indicating $P > 0.05$. **D** demonstrates the correlation between rumen fermentation parameters and rumen differential metabolites. Receiver operating characteristic (ROC) curve (**E**) to assess sheep rumen metabolites biomarkers. **F**-**O** shows the top 10 AUC metabolites biomarkers abundances. **P** Heat map analysis of metabolites biomarkers correlation with rumen fermentation parameters and BW. Note: HF: Hu female lamb, HM: Hu male lamb. * in the correlation heat map indicates $P < 0.05$. The values in the heat map represent correlation coefficients

Glyceric acid, L-Phenylalanine, trans-Cinnamic acid, L-Tyrosine, L-alpha-coumaric acid, L-Serine, Agmatine, 3-Amino-3-(4-hydroxyphenyl) propanoate, L-Threonine, L-Aspartic acid, L-Glutamate, 4-Coumarate, L-Glutamate, 4-Methylthio-2-oxobutanoic acid, L-Homoserine, et al.) were significantly positively correlated with body weight, VAR, PAR, amylase, and xylanase, but significantly negatively correlated with AA, TVFAs, PA, BA, AA/PA, and

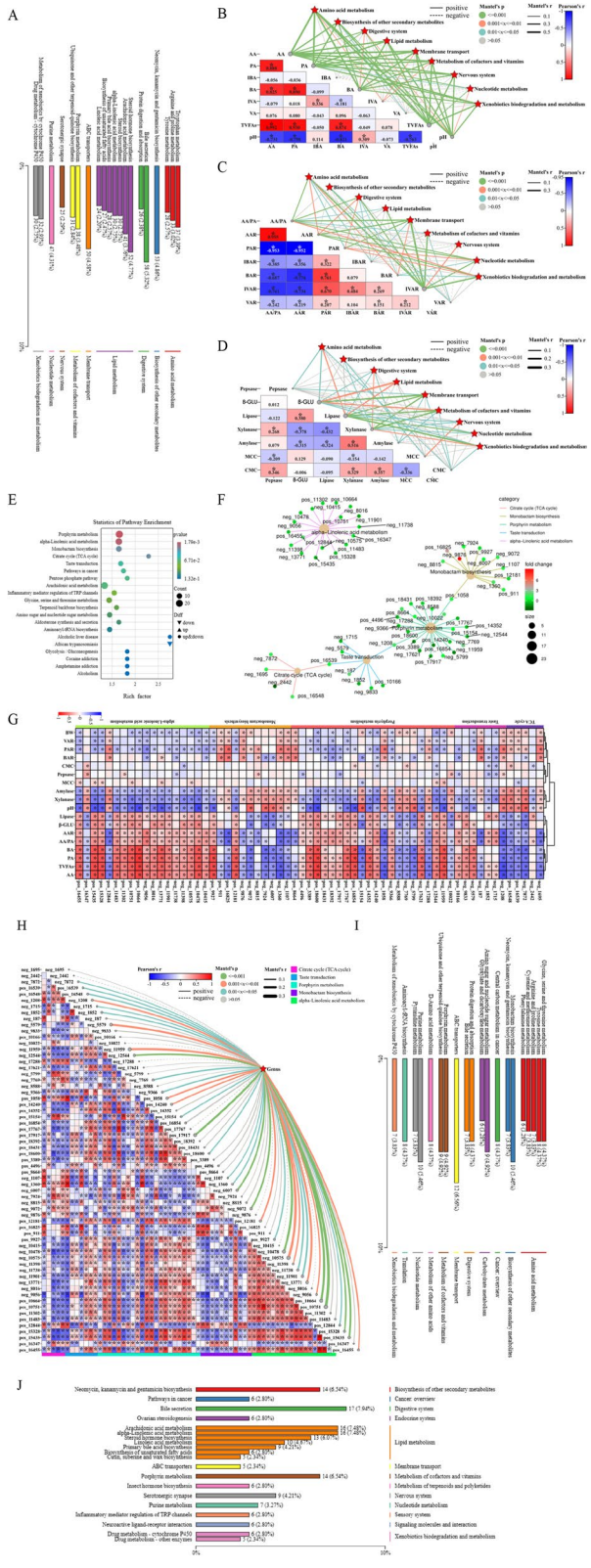

Fig. 7 Demonstrates KEGG enrichment of rumen metabolites among treatment groups, along with rumen microbes, fermentation parameters, and metabolite correlations. **A** KEGG pathway classification map of rumen metabolites. Mantel's r analysis rumen metabolites with rumen rumen VFA molar content (**B**), VFA molar ratios (**C**), and rumen enzymes (**D**) with correlation. The width of the edges is representative of Mantel's r statistics associated with the respective distance correlations, while the color of the edges reflects the significance of Mantel's p statistic, where a gray line denotes indicating $P > 0.05$. The figure shows the top 20 enrichment sites (**E**) and the top 5 networks enriched (**F**) for the rumen differential metabolite in KEGG. **G** Heat map analysis of rumen KEGG top 5 networks enriched differential metabolites correlation with rumen fermentation parameters and BW. **H** Mantel's r analysis of rumen microbial genus levels with rumen KEGG top 5 networks enriched differential metabolites correlation. The width of the edges is representative of Mantel's r statistics associated with the respective distance correlations, while the color of the edges reflects the significance of Mantel's p statistic, where a gray line denotes indicating $P > 0.05$. **I** Categorization map of KEGG pathways that upregulate rumen metabolites. **J** Categorization map of KEGG pathways that down-regulate rumen metabolites. Note: HF: Hu female lamb, HM: Hu male lamb, * in the correlation heat map indicates $P < 0.05$. The values in the heat map represent correlation coefficients

AAR ($P < 0.05$) (Supplementary Fig. 2). Moreover, metabolites (5,6-Epoxytetraene,12-OPDA,9,10–12,13-Diepoxyoctadecanoate, 9(S)-HPETE, 3,6-Nonadienal, Crepenynate, 5(S)-HETE, 9,10-Epoxyoctadecanoic acid, 2,3-Dinor-8-iso prostaglandin F1alpha, Palmitic acid, Colnelenic acid, Arachidonate, Leukotriene D4, (9Z)-Octadecenoic acid, 15-Deoxy-Delta12,14-PGJ2, docosanedioate, 11,12-DHET, Hexadecanedioic acid, Erucic acid, 9,10-Dihydroxy-12,13-epoxyoctadecanoate, Glycocholic acid, 9-Oxononanoic acid, 7alpha-Hydroxy-3-oxo-4-cholestenoate, Glycochenodeoxycholate, Pregnanediol, 2(R)-HPOT, 9(S)-HOT, Rumenic acid, 3alpha,7alpha,12alpha,26-Tetrahydroxy-5beta-cholestane, 7alpha-Hydroxyandrost-4-ene-3,17-dione, Prostaglandin E2, Stearic acid, Prostaglandin F2alpha, Cortisol, Stearidonic acid, Dihomo-gamma-linolenate, 9(S)-HPOT, Prostaglandin G2, (-)-Jasmonic acid, 7alpha,27-Dihydroxycholesterol, et al.) of lipid metabolic pathways positively correlated with lipase, β-GLU, AAR, AA/PA, TVFAs, PA, BA, and AA, but significantly negatively correlated with body weight, VAR, PAR, amylase, and xylanase($P < 0.05$) (Supplementary Fig. 2).

Correlation analysis of lamb rumen microbiome and body size indicators

Overall, lamb body size indicators and microbial top 10 genus level correlation coefficients $|R| < 0.3$ (Fig. 8 A).

The *NK4 A214_group* were significantly positively correlated with BW, CHC, BH and CCB, while BH also had a significant positive correlation with *Veillonellaceae_UCG_001* ($P < 0.05$). However, *Prevotella* were significantly negatively correlated with CCB, BH and HH ($P < 0.05$). Furthermore, *unclassified_Selenomonadaceae* and *Selenomonas* were significantly positively correlated with CHC, but negatively with BL ($P < 0.05$). Additionally, *unclassified_Selenomonadaceae* were significantly negatively correlated with CCB ($P < 0.05$). *Succiniclasticum* were significantly positively correlated with BW, BL and CCB (Fig. 8 A and B).

Correlation analysis of lamb rumen function and serum index

Figure 9 shows the correlation analysis of the rumen microbiome, metabolites, fermentation traits, and serum profiles in lambs. Mantel's r analysis showed that rumen microbiota significantly correlated with serum IGF-1, GLU, GPT, and UN; rumen fermentation parameters significantly correlated with serum GH, PK, and TC; rumen enzyme activities significantly correlated with serum IGF-1, INS, and HDL; and rumen differential metabolites significantly correlated with GH, IGF-1, GLU, GPT, GOT, and UN ($P < 0.05$), especially the most significant effect on GH (Fig. 9 A). Moreover, the serum indicators exhibited overall weak intercorrelations ($|R| < 0.3$). Simultaneously, serum hormones (CCK, GH, ghrelin, GLP-1, IGF-1, and INS) showed an overall significant positive correlation ($P < 0.05$) (Fig. 9 A). Notably, serum biochemicals and hormones overall exhibited weak correlations ($|R| < 0.3$) with the top 10 microbial genus, only the *unclassified_Selenomonadaceae* exhibited a notable positive correlation with GLU and HDL, with a correlation coefficient |R| greater than 0.3. (Fig. 9 B).

Correlation analysis of rumen microbiome and rumen phenotype

Rumen microbiota genus level (xylanase) and differential metabolites (amylase, CMC, β-GLU, lipase and xylanase) were found to significantly affect rumen digestion enzymes by Mantel's r analysis ($P < 0.05$), especially the most significant effect on xylanase (Fig. 10 A). Subsequently, rumen microbiota genus level (AA, PA, BA, TVFAs and pH) and differential metabolites (AA, PA, BA, IVA, TVFAs and pH) were found to significantly affect rumen VFA molar concentration by Mantel's r analysis ($P < 0.05$), especially the most significant effect on TVFAs (Fig. 10 B). Finally, rumen microbiota genus level (IVAR) and differential metabolites (AA/PA, AAR, PAR, IBAR, IVAR and VAR) were found to significantly affect rumen VFA molar proportion by Mantel's r analysis ($P < 0.05$), especially the most significant effect on IVAR (Fig. 10 C). However, there was no significant correlation between serum indices and rumen digestion enzymes (pepsase, β-GLU, lipase, xylanase, amylase, MCC and CMC), VFA molar concentration (pH, AA, PA, IBA, BA, IVA, VA and TVFAs), and VFA molar proportion (A/P, AAR, PAR, IBAR, BAR, IVAR and VAR) ($P > 0.05$) (Fig. 10 A, B and C).

Overall, rumen enzyme activity and microbial top 10 genus level correlation coefficients $|R| < 0.3$, while $|R| > 0.3$ relationships were noted for *unclassified_Selenomonadaceae* and β-GLU (Fig. 10 D). Meantime, correlation coefficients $|R| < 0.3$ were found between rumen VFA molar concentration and the top 10 microbial genu, while $|R| > 0.3$ relationships were noted for *Prevotella* and the VFA molar concentrations (AA, PA, BA, and TVFAs), as well as *unclassified_Selenomonadaceae* and IVA molar concentrations (Fig. 10 D). Furthermore, correlation coefficients $|R| < 0.3$ were found between rumen VFA molar proportion and the top 10 microbial genu, while

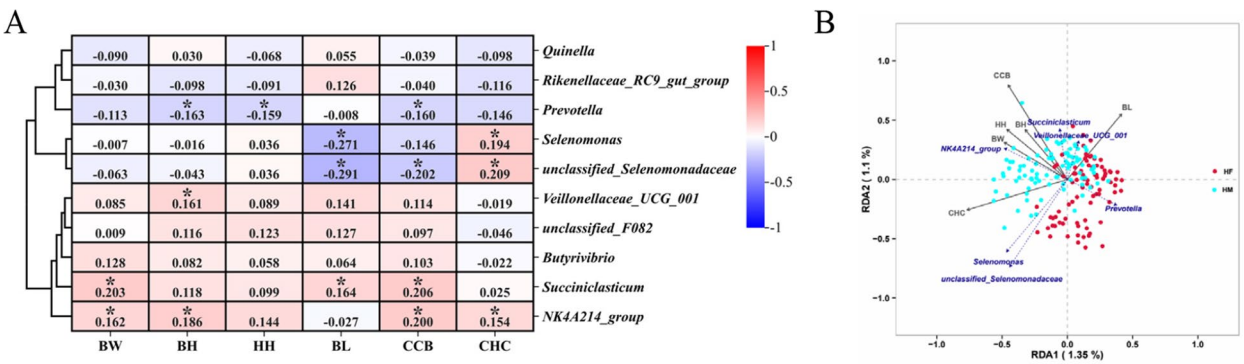

Fig. 8 **A** Heat map analysis of microbial correlation with body scale indicators. **B** RDA analysis of body size indices and microbes in lambs. Abbreviations: HF: Hu female lamb, HM: Hu male lamb, BW: Body Weight, BH: Body Height, HH: Hip Height, BL: Body Length, CCB: Circumference of Cannon bone, CHC: Chest Circumference, * in the correlation heat map indicates $P < 0.05$. The values in the heat map represent correlation coefficients

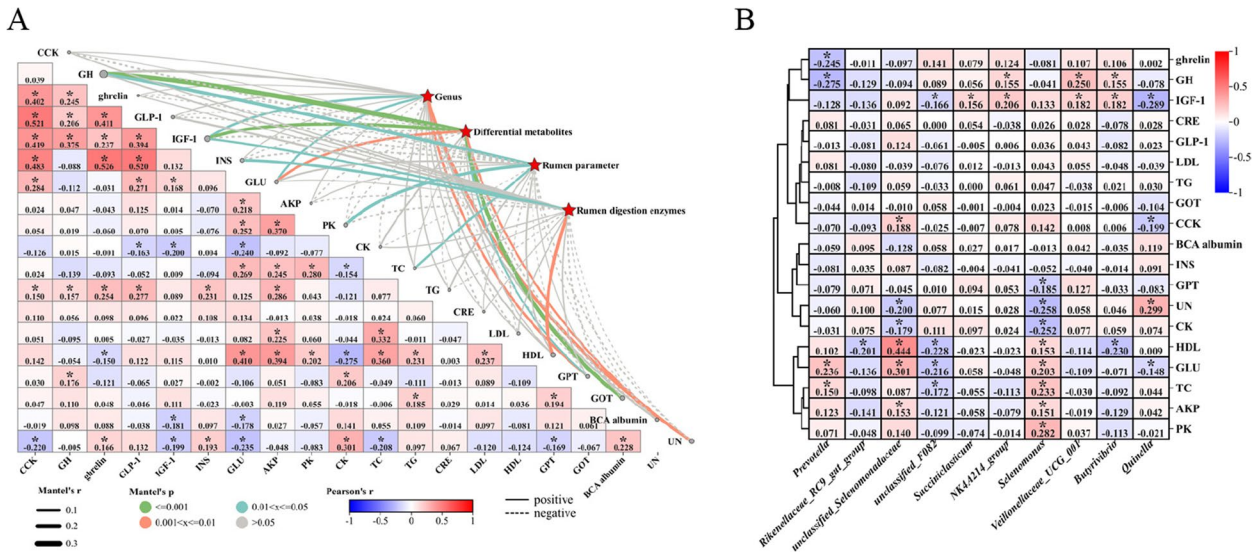

Fig. 9 Analysis of rumen microbes, differential metabolites, and rumen parameters correlated with serum indicators. **A** Mantel's r analyzed serum indicators (CCK, GH, ghrelin, GLP-1, IGF-1, INS, GLU, AKP, PK, CK, TC, TG, CRE, LDL, HDL, GPT, GOT, BCA albumin, and UN) and rumen microbes, differential metabolites, and rumen parameters. The width of the edges is representative of Mantel's r statistics associated with the respective distance correlations, while the color of the edges reflects the significance of Mantel's p statistic, where a gray line denotes indicating $P > 0.05$. **B** Heat map analysis of serum indicators (CCK, GH, ghrelin, GLP-1, IGF-1, INS, GLU, AKP, PK, CK, TC, TG, CRE, LDL, HDL, GPT, GOT, BCA albumin, and UN) and rumen microbes. Note: HF: Hu female lamb, HM: Hu male lamb, * in the correlation heat map indicates $P < 0.05$. The values in the heat map represent correlation coefficients

|R|> 0.3 relationships were noted for *unclassified_Selenomonadaceae* and IVAR, as well as *Selenomonas* and BAR (Fig. 10 D).

Rumen metabolite-microbe correlation analysis

Metabolite modules and microbiota were screened for the top 30 based on a CCP < 0.05. Data with at least one correlation coefficient in this range were used for metabolite module-microbes correlation plots and network diagrams, which containing 11 metabolic modules and 17 phyla (Supplementary Table 2 and 3). Overall, the correlation between microbiota and metabolic modules was |CC|< 0.3 (Supplementary Table 3). Next, we analyzed the correlation between top10 differential microbes and metabolic modules. The Bacteroidota was significantly correlated with MEturquoise (CC =0.204, P= 0.006), MEyellow (CC =0.182, P= 0.015), MEpink (CC =0.184, P= 0.014), MEblue (CC =−0.310, P= 0.000), MEgreen (CC =−0.192, P= 0.011), and MEgrey (CC =0.178, P= 0.018) (Fig. 11 A and B). Meantime, Firmicutes was significantly correlated with MEturquoise (CC =−0.224, P= 0.003), MEyellow (CC =−0.224, P= 0.003), MEblue (CC =0.249, P= 0.000), MEgreen (CC =0.234, P= 0.002), and MEgrey (CC =−0.154, P= 0.041) (Fig. 11 A and B). In addition, Synergistota was significantly correlated with MEpurple (CC =0.262, P= 0.000), MEyellow (CC =0.309, P= 0.000), and MEmagenta (CC =0.171, P= 0.023) (Fig. 11 A and B). Furthermore, Desulfobacterota was significantly correlated with MEyellow (CC =−0.177, P= 0.018), MEpink (CC =−0.216, P= 0.004),

(See figure on next page.)

Fig. 10 Analysis of rumen microbes, differential metabolites, and serum indicators correlated with rumen parameters. **A** Mantel's r analyzed rumen digestion enzymes and rumen microbes, differential metabolites, and serum indicators. **B** Mantel's r analyzed rumen VFA molar concentration, pH, and rumen microbes, differential metabolites, and serum indicators. **C** Mantel's r analyzed rumen VFA molar proportion and rumen microbes, differential metabolites, and serum indicators. The width of the edges is representative of Mantel's r statistics associated with the respective distance correlations, while the color of the edges reflects the significance of Mantel's p statistic, where a gray line denotes indicating $P > 0.05$. **D** Heat map analysis of microbial correlation with rumen digestion enzymes, rumen VFA molar concentration and proportion. Note: HF: Hu female lamb, HM: Hu male lamb, * in the correlation heat map indicates $P < 0.05$. The values in the heat map represent correlation coefficients. AA: Acetic acid, PA: Propionic acid, BA: Butyric acid, IBA: isobutyric acid, IVA: isovaleric acid, TVFA: Total volatile fatty acid, AAR: Acetic acid molar ratio, PAR: Propionic acid molar ratio, BAR: Butyric acid molar ratio, AA/PA: Acetic acid ratio propionic acid, IBAR: isobutyric acid molar ratio, IVAR: isovaleric acid molar ratio, MCC: Microcrystalline cellulase, CMC: Carboxymethyl cellulase

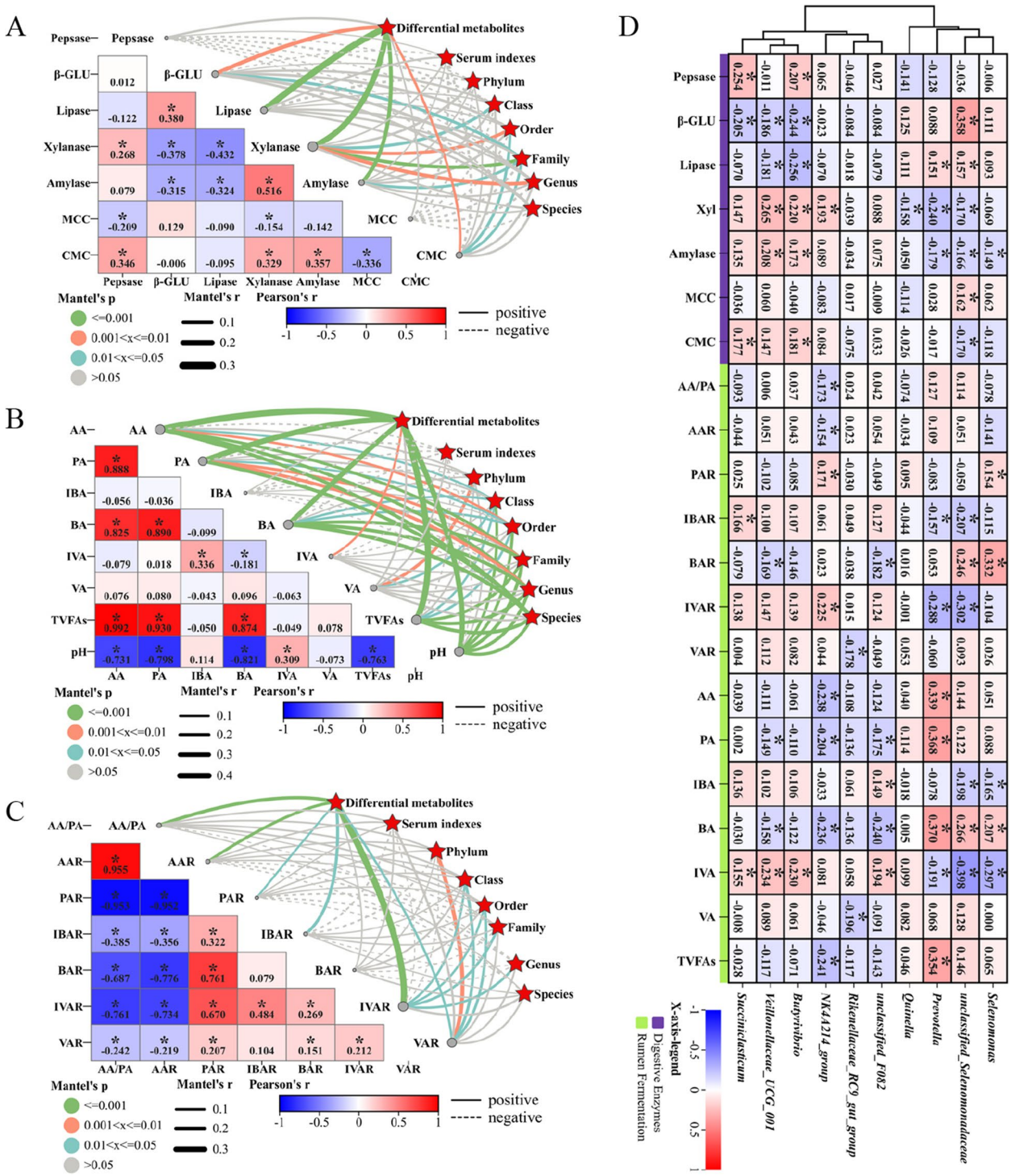

Fig. 10 (See legend on previous page.)

MEblue (CC = 0.252, P = 0.000), MEgreen (CC = 0.170, P = 0.023), and MEgrey (CC = −0.222, P = 0.003) (Fig. 11 A and B). Additionally, Verrucomicrobiota was significantly correlated with MEturquoise (CC = −0.246, P = 0.000), MEpurple (CC = −0.175, P = 0.020), MEyellow (CC = −0.354, P = 0.000), MEmagenta (CC = −0.242, P = 0.001), MEpink (CC = −0.228, P = 0.002), MEblue (CC = 0.391, P = 0.000), MEgreen (CC = 0.196, P = 0.009), and MEgrey (CC = −0.239, P = 0.001), while Chloroflexi was significantly correlated with MEturquoise (CC = −0.232, P = 0.002), MEpurple (CC = −0.176, P = 0.019), MEyellow (CC = −0.295, P = 0.000), MEpink (CC = −0.196, P = 0.009), MEblue (CC = 0.297, P = 0.000), MEgreen (CC = 0.243, P = 0.001), and MEgrey (CC = −0.173, P = 0.021) (Fig. 11 A and B).

Further by analyzing the correlation of microbial-metabolite markers in rumen fluid, it was found that significantly higher microbial markers (*Lachnospiraceae_UCG_008*, *Saccharofermentans*, *unclassified_Clostridia*, *Christensenellaceae_R_7_group*, *Anaerovorax*, *Mogibacterium*, and *unclassified_Erysipelotrichaceae*) were significantly positively correlated with significantly higher metabolites (neg_15079, neg_15575, neg_5752, neg_15700, pos_14144, and neg_11832) in the HM group, but significantly negatively correlated with significantly lower metabolites (pos_2999, pos_2442,pos_3004, and pos_3005) in the HM (P < 0.05). In addition, significantly lower microbial markers (*Anaeroplasma*, *unclassified_Acholeplasmataceae*, and *uncultured_rumen_bacterium_4 C28 d_15*) in the HM group were significantly negatively correlated with significantly higher metabolites (neg_15079, neg_15575, neg_5752, neg_15700, pos_14144, and neg_11832) in the HM group, but significantly positively correlated with significantly lower metabolites (pos_2999, pos_2442,pos_3004, and pos_3005) (P < 0.05) (Fig. 11 C). Meantime, the results of correlation analysis by rumen microbial markers correlating with metabolic markers and host phenotypes

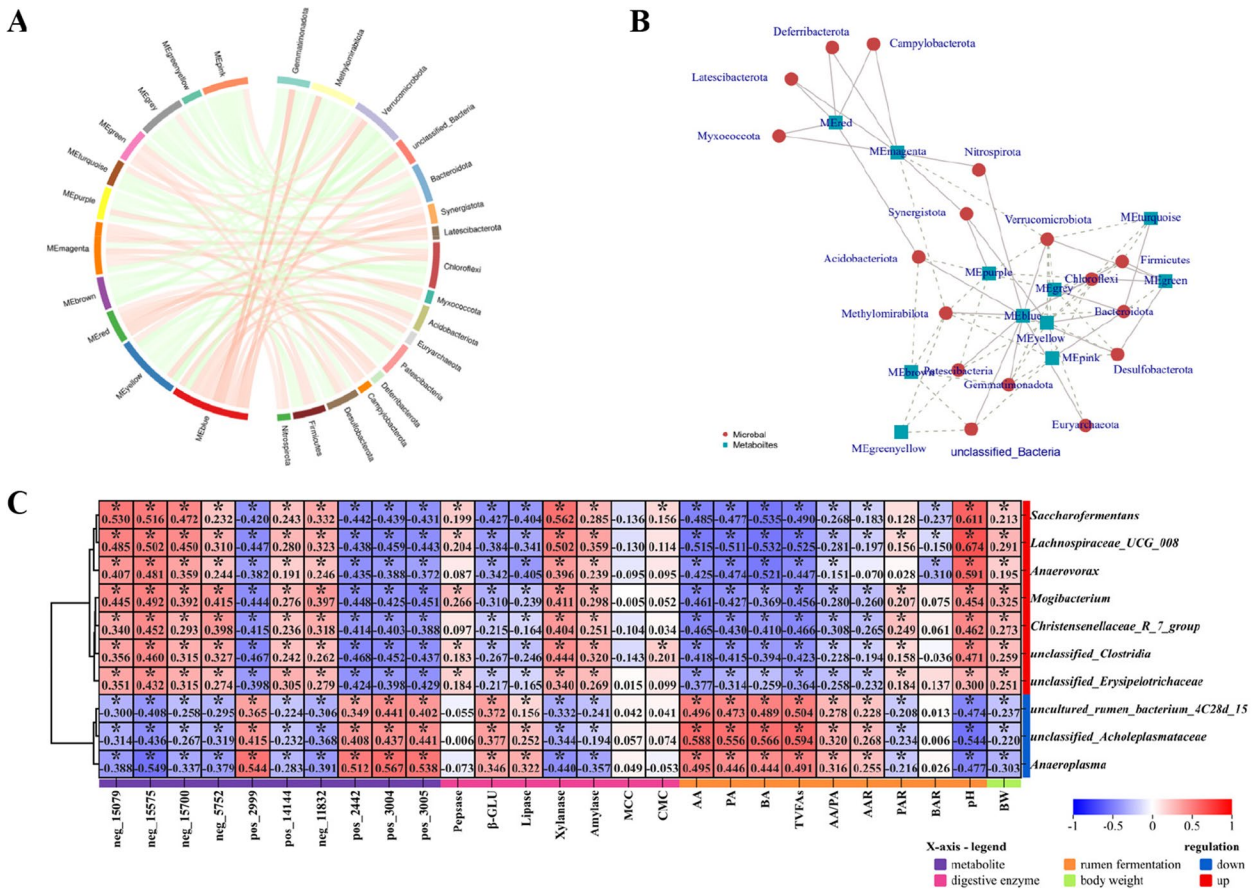

Fig. 11 Rumen microbial and metabolite correlation analysis. Metabolite cluster-microbe chord diagrams (**A**) and correlation network diagrams (**A**). **C** Heat map of rumen microbial markers correlating with metabolic markers and host phenotypes. Note: neg_15079: 1,3-Propane sultone, neg_15575: Flibanserin, neg_15700:3-Amino-5-mercapto-1,2,4-triazole, neg_5752:4-Coumarate, pos_2999: Reduced riboflavin, pos_14144: Fingolimod phosphate ester, S-, neg_11832:C75, pos_2442: Cowanin, pos_3004: PG (18:2(9Z,12Z)/0:0), pos_3005: Mozenavir. HF: Hu female lamb, HM: Hu male lamb. The * in the correlation heat map indicates P < 0.05. The values in the heat map represent correlation coefficients

showed that xylanase, amylase, PAR, pH, and BW were significantly negatively correlated with down-regulated microbial markers in the HM group ($P < 0.05$), but they were significantly positively correlated with up-regulated microbial markers in the HM group ($P < 0.05$) (Fig. 11 C). However, β-GLU, lipase, AA, PA, BA, TVFAs, AA/PA, and AAR were significantly positively correlated with down-regulated microbial markers in HM group ($P < 0.05$), but they were significantly negatively correlated with up-regulated microbial markers in HM group ($P < 0.05$) (Fig. 11 C).

Discussion

Animal growth is defined by the increase in weight and volume of body tissues and the accumulation of components like protein, lipids, water, and ash, among males generally have greater growth potential than females [42]. Of note, body weight is a key economic factor in sheep production, affected by genetic and environmental interactions, including host genetics and rumen microecology-metabolites [10, 23]. The results showed that HM lambs had significantly larger body size indices than HF lambs, with a notable positive correlation among the indices (body weight, body height, hip height, circumference of cannon bone, and chest circumference) of the lambs. This result is consistent with previous studies that found a significant positive correlation between body size indicators in lambs [28], as well as the observation that male lambs exhibit greater growth potential [42]. The study found that body weight was significantly positively correlated with *Succiniclasticum* and *NK4 A214_group*. Meanwhile, *NK4 A214_group* were significantly positively correlated with body height, circumference of cannon bone, and chest circumference. Thus, we hypothesized that the significant weight gain in male lambs may be related to sex-induced changes in serum indices, rumen microbes, metabolite profiles, and VFAs in lambs [10, 42, 43].

Serum is the main carrier of nutrients and waste in the body, crucial for assessing health conditions and overall health status. The study found no significant differences between HF and HM in GLU, AKP, PK, CK, TG, CRE, HDL, BCA albumin, UN, CCK and GLP-1. This outcome is generally consistent with prior research indicating that sex has weak effects on the serum biochemical indices of the animals [43, 44]. However, HM significantly increased serum hormones, such as GPT, GOT, GH, ghrelin, and IGF-1. GH and IGF-1 stimulate the release of growth inhibitory hormone, which negatively regulates GH secretion from the anterior pituitary gland [45]. Overall, correlation coefficients $|R| < 0.3$ were found between serum biochemistry and hormones and the top 10 microbial genus. The research demonstrated that GH is secreted by the anterior pituitary gland and is regulated by hypothalamic hormones, specifically growth hormone-releasing hormone and somatostatin, as well as by ghrelin [45, 46].GH promotes bone growth, enhances lipolysis in adipocytes, and increases amino acid uptake and nitrogen retention in muscle tissue, thereby maintaining muscle mass and strength [47]. Additionally, it plays a regulatory role in carbohydrate metabolism, nitrogen balance, mineral homeostasis, and electrolyte equilibrium [47]. Mantel's r analysis showed the strongest correlations with GH in rumen microbiota, fermentation parameters, enzyme activities, and differential metabolites. Moreover, GH was significantly negatively correlated with *Prevotella*, but positively correlated with *NK4 A214_group*, *Veillonellaceae_UCG_001* and *Butyrivibrio*. The significantly higher GH significant serum in male lambs may be related to sex influencing rumen microbial and metabolite enrichment pathways (such as amino acid metabolism) in lambs [47, 48], but further clarification is needed. Furthermore, rumen microbiota was integral to the rumen fermentation process, which significantly influences the growth and feed efficiency of ruminant animals [9, 10, 49]. Rumen microbiota generate VFAs and nutrients through fermentation, providing up to 70% of the host's energy [10, 15, 16]. This study showed that male lambs had lower ruminal VFAs, a reduced AA/PA, lower acetate molar percentage and diminished β-glucosidase and lipase activities than females. Conversely, the overall molar percentage of VFAs and digestive enzyme (pepsase, xylanase, amylase, and CMC) was found to be significantly higher in male lambs. Research indicates that higher VFAs concentrations improve pancreatic sensitivity, boosting insulin secretion, fat oxidation, and activate free fatty acid receptor 2, promote peptide YY and GLP-1 secretion, enhance anorexigenic signaling, and leading to orexigenic signaling decrease, ultimately affecting food intake and leading to a negative energy balance, reducing fat deposition and body weight [50, 51]. Therefore, high concentrations of VFAs before feeding negatively affect lamb appetite and growth. This result supports findings that females have higher intestinal acetate and propionate levels than males, likely due to sex differences in VFA metabolism [28, 42, 51]. Wang et al. indicating that the average daily gain in 180 days of age goats is positively correlated with the levels of PA, BA, and PAR in rumen fluid, while showing a negative correlation with AAR and AA/PA [49]. Consequently, in the context of livestock production, the enhancement of growth and development in fattening lambs can be achieved by elevating the molar ratio of propionic acid relative to butyric acid, while simultaneously decreasing the ratio of acetic acid to propionic acid. Moreover, we speculated that the changes of body weight, serum index,

VFA molar content, molar ratio and enzyme activity in rumen might be related to the changes of rumen microbial community caused by sex [10, 28, 42, 44, 52, 53].

Subsequently, we conducted an analysis of the influence of gender on the rumen microbiota of lambs. Distinct rumen microbial β-diversity was found between sexes, with male lambs showing elevated α-diversity. The observed increase in microbial diversity within the rumen of lambs may enhance their ability to adapt to environmental fluctuations and maintain homeostasis within the intra-rumen ecosystem [54]. The research indicated that the predominant microbiota in the lamb rumen consisted of Firmicutes and Bacteroidota, aligning with findings from earlier investigations into rumen microbiota in various ruminant species, including goat [53], sheep [28, 55], dairy cows [56], cattle [57]. Research has proven that Verrucomicrobiota and Synergistota are abundant in genes related to lignocellulosic polymer decomposition and the fermentation of by-products into VFAs, could enhancing the conversion of cellulose degradation into usable VFAs [58–60]. However, Synergistota in ewe lambs is enriched in the rumen and contributes to the alleviation of acute rumen toxicity [61], and negatively with residual feed intake [62]. Research has proven that Chloroflexi degrade plant compounds, including cellulose, starch, long-chain sugars, and pyrogallol [63]. Verrucomicrobiota is rich in fucoidanases, including glycoside hydrolases, sulfurylases, and carbohydrate esterases [64]. Thus, male lambs significant enhancing rumen xylanase, amylase, carboxymethyl cellulase enzyme, Desulfobacterota, Verrucomicrobiota, and Chloroflexi abundance may promotes nutrient conversion [58–60, 63, 64]. However, it is worth noting on this basis that Desulfobacterota may contribute to intestinal inflammation [65], positively correlate with lipid concentration [66], and liver inflammation [67]. Notably, rumen Bacteroidota and Firmicutes are key player for feed digestion (such as carbohydrate and protein degradation), energy metabolism, and resistance to external pathogens, whereas a reduced Firmicutes/Bacteroidota (F/B) is associated with weight loss and reduced feed efficiency [28, 68, 69]. This study found that Bacteroidota associated with carbohydrate and protein degradation decreased significantly, while Firmicutes related to energy metabolism increased in male Hu sheep lambs. Male lamb higher F/B ratio indicates superior energy utilization [52, 70, 71], with low prefeeding VFAs further boosting metabolic efficiency [28, 50, 51].

Additionally, it was found that rumen *Prevotella* was significantly lower in male lambs of Hu sheep. *Prevotella* was significantly negatively correlated with rumen IVA, IBAR, IVAR, but positively correlated with AA, PA, BA, and TVFAs, which is due to the fact that *Prevotella* is characterized as a dominant genus in the rumen fermenting fibers into acetate and succinate [28, 52, 53, 55, 72]. Studies have shown that *Prevotella* has been linked to elevated milk production [73, 74], but it was negatively correlated with goat growth rate [49]. It has been demonstrated that *Succiniclasticum* converts rumen fiber-derived succinic acid into propionic acid, promoting gluconeogenesis, enhancing fiber utilization, improving feed conversion ratio for animal growth and production [75]. Meantime, the male lambs *Ruminococcaceae_NK4 A214_group* were significantly higher than female lambs [53]. Interestingly, study showed that dysbiosis in sheep intestinal flora decreases the *NK4 A214_group* [76], and *NK4 A214_group* were positively associated with feed efficiency [77]and Xylanase. Moreover, fat-soluble vitamin absorption is influenced by bile acid levels, and significantly correlate with the *NK4 A214_group* [76]. *Veillonellaceae_UCG_001* has been negatively correlated to the butyrate proportion [78], but it was positively correlated with xylanase and amylase. This study found that *Butyrivibrio* was significantly positively correlated with pepsase, xylanase, amylase, and carboxymethyl cellulase, but negatively correlated with β-glucosidase and lipase, while *Butyrivibrio*, a hemicellulose-degrading bacterium, ferments complex carbohydrates to produce VFAs and is abundant in healthy individuals [79–81]. Interestingly, Hu sheep male lambs significantly increased *Succiniclasticum*, *uncultured_rumen_bacterium*, *NK4 A214_group*, *Veillonellaceae_UCG_001* and *Butyrivibrio*. This finding indicates that male lambs demonstrate a higher production potential, which may be attributed to sex-related alterations in microbial. These changes potentially enhance animal energy enrichment capacity and improve feed conversion efficiency, thereby supporting the growth and development of lambs [52, 70, 71, 75, 77]. Furthermore, the findings from the ROC curve analysis of rumen biomarkers in lake sheep indicated that *Anaeroplasma* exhibited the highest contribution among the top ten biomarkers. The study found that *Anaeroplasma* is a group of bacteria that boosts TGF-β and IgA expression in the T-dependent mucosal immune response, making fecal anaerobes effective adjuvants or probiotics for enhancing mucosal immunity [82]. Moreover, the results of correlation analysis by body weight, rumen parameters and top 10 rumen microbial markers correlation showed that xylanase, amylase, PAR, pH, and body weight were significantly negatively correlated with down-regulated microbial markers in the HM group, but they were significantly positively correlated with up-regulated microbial markers in the HM group. However, β-glucosidase, lipase, AA, PA, BA, TVFAs, AA/PA, and AAR were significantly positively correlated with down-regulated microbial markers in HM group but they were significantly negatively correlated with up-regulated microbial markers in HM group.

Additionally, in the intricate ecosystem of the rumen, interactions among microbiota may play an equally outstanding role in physiological functions. Although the physiological roles of dominant bacterial populations are frequently highlighted, low-abundance colonies, despite their limited presence, exhibit greater taxonomic diversity and may exert a substantial influence on host functions [49, 83, 84]. It was also found that Firmicutes dominated the correlation network at the level of the top 80 genus of sheep rumen microorganisms, a result that is consistent with previous studies [85].

KEGG functional analysis showed that rumen microbial functions were top 5 enriched in the global and overview maps, carbohydrate metabolism, amino acid metabolism, metabolism of cofactors and vitamins, energy metabolism, and nucleotide metabolism pathway, which is consistent with previous findings [28]. Notably, carbohydrate metabolism (such as glyoxylate and dicarboxylate, TCA cycle, glycolysis/gluconeogenesis, fructose and mannose, galactose, et al.) was significantly higher in ewe lambs, whereas membrane transport (such as ABC transporters) and signal transduction (such as two-component system) were significantly higher in male lambs. It is noteworthy that ABC transporters use the energy generated by ATP hydrolysis to transport substrates across membranes in a reverse concentration gradient for small molecules (e.g., metal ions, sugars, amino acids, nucleotides and vitamins) and organic compounds (e.g., peptides, proteins, oligonucleotides, cellular metabolites, etc.) [86, 87]. The two-component system is a versatile signaling mechanism that regulates cellular responses to environmental signals, influenced by the bacteria's ecological niche [88]. Thus, enrichment of these functions of microbial may impact nutrient transport and utilization, leading to variations in rumen VFA content and molar ratios, but further clarification is needed. Mantel's r analysis indicated significant associations between rumen microbiota genus levels, differential metabolites, and rumen digestive enzymes, VFA molar content and ratios, especially Xylanase, TVFAs, and IVAR. Although Mantel's r reveals solely linear relationships, the observed weak correlations might underlie critical functional associations [10, 23].

Ruminal metabolites are essential for the physiological metabolism of ruminants and the interaction between rumen microbiota and host [23, 49]. The study found that sex significantly influences rumen metabolites profiles in lambs. Meantime, Up-regulated different metabolites showed positive correlations with each other, while as did down-regulated different metabolites. In contrast, up-regulated and down-regulated different metabolites were mainly negatively correlated. This result aligns with earlier studies on sheep rumen metabolites [23]. Additionally, Mantel's results showed that sheep rumen microbiota and rumen differential metabolites were significantly correlated [23], especially caryophyllene oxide and capric acid, which were significantly reduced in the rumen of male lambs. The results indicate that caryophyllene oxide is not only anti-inflammatory activity [89]. Moreover, capric acid reduces inflammation and oxidative stress by influencing NF-κB, MAPK/ERK, p38, and Nrf2 signaling pathways [90, 91].Consequently, male lambs should exhibit greater concern regarding the adverse effects of inflammation and stress on their growth during feeding management, in comparison to ewe lambs. Furthermore, the enrichment of rumen metabolites was conducted utilizing the KEGG database, revealing that these metabolites were predominantly associated with amino acid metabolism, digestive system, lipid metabolism, which was similar to the previous results of rumen metabolite enrichment in sheep [85]. Meantime, Mantel's results showed that rumen metabolites was significantly correlated with rumen VFA molar content (AA, PA, BA, IVA, VA, and TVFAs), VFA molar ratios (AA/PA, AAR, PAR, IBAR, BAR, IVAR, and VAR), and rumen digestive enzyme (pepsase, β-GLU, lipase, xylanase, amylase, MCC, and CMC). This observation suggests that there exists a more robust association between metabolites and rumen fermentation than with the rumen microbiota. Additionally, Mantel's r analysis revealed significant correlations between rumen microbial genus and differential metabolites in the KEGG top 5 pathways. Next, the correlation analysis of rumen enzyme activities with KEGG top 5 enriched differential metabolites revealed that lipase, β-GLU, AAR, AA/PA, BA, PA, TVFAs and AA were basically positively correlated with alpha-Linolenic acid metabolism, porphyrin metabolism, and taste transduction, respectively, but negatively correlated with monobactam biosynthesis and TCA cycle pathway. In addition, BW, pH, xylanase, amylase, PAR, and VAR were largely negatively correlated with alpha-linolenic acid metabolism and taste transduction, respectively, but they were positively correlated with monobactam biosynthesis and TCA cycle pathway. Therefore, rumen metabolites are key small molecules that significantly influence the physiological functions of animals [23, 49, 81]. Study has confirmed that amino acid metabolism is primarily achieved through the oxidative functions of the tricarboxylic acid cycle, as well as its involvement in the conversion of glycolipids, purines, pyrimidines, and other metabolites [92], while aromatic amino acid transaminases and arginine deaminases were mainly found in Firmicutes and Actinomycetota, whereas branched-chain amino acid transaminases were only present in Bacteroidota and Firmicutes [25]. This study showed that male lambs rumen up-regulated metabolites

were mainly linked to amino acid metabolism, whereas down-regulated metabolites were associated with lipid metabolism. Research indicates that, amino acid metabolism (such as glycine, serine and threonine metabolism, tyrosine metabolism, arginine and proline metabolism, cysteine and methionine metabolism, phenylalanine metabolism) contributes to the formation of precursors for biomolecular metabolism and is implicated in epigenetic modifications, among other functions [92]. Moreover, phenylalanine metabolism and purine metabolism were specific to the microbial community [93]. Thus, the observed enrichment of phenylalanine metabolic pathways in the rumen of male lambs may be attributed to the influence of sex on the structure of rumen microbiota [93]. Our analysis of the correlation of up-regulated differential metabolites of amino acid metabolic pathways with rumen fermentation parameters and body weight revealed that rams up-regulated amino acid metabolic pathways were significantly and positively correlated with body weight, VAR, PAR, amylase, and xylanase, but significantly negatively correlated with AA, TVFAs, PA, BA, AA/PA, and AAR. Furthermore, lipids are fundamental metabolites that serve multiple functions, including energy sources, structural components, and signaling mediators, while lipid metabolites are crucial as signaling molecules in the regulation of energy and immune homeostasis [94]. The study found that arachidonic acid metabolism pathway is vital for regulating inflammation and liver glucose and lipid balance, involving prostanoids, leukotrienes, epoxyeicosatrienoic acids, arachidonate, lipoxin, dihydroxyeicosatetraenoic acid, eicosatetraenoic acids, lipoxins, et al. [95, 96]. However, in ewes up-regulated lipid metabolic pathways positively correlated with lipase, β-GLU, AAR, AA/PA, TVFAs, PA, BA, and AA, but significantly negatively correlated with body weight, VAR, PAR, amylase, and xylanase. Research has demonstrated that the glycine, serine, and threonine metabolic pathways are thought to provide the main energy metabolism precursor substance for the TCA cycle [97], and these metabolic pathways closely related to arginine and proline metabolism, cysteine and methionine metabolism, pyruvate, folate, propionate, butyrate, and PUFAs metabolism, etc [98]. Meantime, the conversion of L-citrulline, facilitated by intestinal flora, to L-arginine can triggers a NO-Ca^{2+} positive feedback loop in osteoblasts thereby enhancing skeletal mechanical responsiveness [99]. Moreover, arginine and proline metabolism and muscle development play an important role in the regulation of sheep meat quality [100], while proline-degrading bacteria are found only in the in Firmicutes [25]. Additionally, the male lamb up-regulated tyrosine metabolism pathway enriched for fumarate, 4-Coumarate, vanillylmandelic acid, dopamine, 3-Amino-3-(4-hydroxyphenyl) propanoate, 4- Hydroxyphenylacetaldehyde, and L-Tyrosine. Notably, L-Tyrosine is an aromatic amino acid required for protein synthesis in all organisms is closely related to 4-Coumarate,3-Amino-3-(4-hydroxyphenyl) propanoate, 4- Hydroxyphenylacetaldehyde, and fumarase [101], and tyrosine phosphorylation is one of the major mechanisms regulating signal transduction pathways and key cellular functions, such as TCA cycle, pentose phosphate pathway, and lipid metabolism [102]. Therefore, the enhancement of amino acid metabolism promotes the growth and development of lambs, and there is a significant correlation between the enrichment of rumen metabolites and microbial activity [28, 93]. The relationship between the microbiota and the metabolome is closely associated with the physiological state of the host. The regulation of nutritional homeostasis within the host is determined by the interactions between the microbiota and the host's metabolic framework. Additionally, the microbiota plays a significant role in modulating the host's metabolome, thereby aiding in the maintenance of homeostasis across peripheral organs and tissues [14, 23, 25]. The study demonstrated a robust association between microbial and metabolic modules, particularly a significant correlation between the predominant (Firmicutes and Bacteroidota) phyla and metabolic modules. Further by analyzing the correlation of microbial-metabolite markers in rumen fluid, it was found that significantly up-regulated microbial markers were significantly positively correlated with significantly up-regulated rumen metabolites markers in the HM group, but significantly negatively correlated with significantly down-regulated rumen metabolites markers in the HM. In addition, significantly down-regulated microbial markers in the HM group were significantly negatively correlated with significantly up-regulated metabolites markers in the HM group, but significantly positively correlated with significantly down-regulated microbial markers. This outcome may be associated with the interactions among microbial communities, wherein the exchange of metabolites between these communities plays a significant role in regulating the trophic homeostasis of the host [25, 103, 104].

Conclusions

The sex of lambs has been shown to affect changes in rumen metabolic profiles and the composition of rumen microbiota, which in turn supports their growth and developmental needs. Notably, significant correlations were observed among rumen metabolites, microbial communities, and the interactions between microorganisms and metabolites, especially Firmicutes and Bacteroidota as dominant phylum in the sheep rumen with significant differences in correlation with

rumen metabolic modules. Furthermore, the relationship between metabolites and rumen fermentation was determined to be more robust than that between rumen microbiota and rumen fermentation. Meanwhile, the rumen microbial markers and metabolic markers in lambs exhibited a highly significant correlation with each other, as well as a significant correlation with lamb body weight and rumen fermentation parameters. Additionally, lamb rumen microbes markers (*Lachnospiraceae_UCG_008*, *Saccharofermentans*, *unclassified_Clostridia*, *Christensenellaceae_R_7_group*, *Anaerovorax*, *Mogibacterium*, and *unclassified_Erysipelotrichaceae*) exhibited a significant positive correlation with body weight, propionic acid ratio, xylanase, amylase, and metabolites markers (such as 1,3-Propane sultone, 4-Coumarate, 3-Amino-5-mercapto-1,2,4-triazole, et al.), while demonstrating a negative correlation with β-GLU, lipase, acetic acid, propionic acid, butyric acid, TVFAs, acetic acid/propionic acid and metabolites markers (such as Reduced riboflavin, Cowanin, PG (18:2(9Z,12Z)/0:0), and Mozenavir). However, lamb rumen microbes markers (such as *Anaeroplasma*, *unclassified_Acholeplasmataceae*, and *uncultured_rumen_bacterium_4 C28 d_15*) exhibited a significant positive correlation with β-GLU, lipase, acetic acid, propionic acid, butyric acid, TVFAs, acetic acid/propionic acid, acetic acid ratio, and metabolites markers (such as Reduced riboflavin, Cowanin, PG (18:2(9Z,12Z)/0:0), and Mozenavir), while demonstrating a negative correlation with body weight, propionic acid ratio, xylanase, amylase, and metabolites markers (such as 1,3-Propane sultone, 4-Coumarate, 3-Amino-5-mercapto-1,2,4-triazole, et al.). Furthermore, lamb body weight demonstrated a significant positively correlated with rumen metabolites related to amino acid metabolism pathway (glycine, serine and threonine metabolism, tyrosine metabolism, arginine and proline metabolism, cysteine and methionine metabolism, and phenylalanine metabolism) and demonstrated a significant negatively correlated with those linked to lipid metabolism pathway (arachidonic acid metabolism, alpha-Linolenic acid metabolism, steroid hormone biosynthesis, linoleic acid metabolism, primary bile acid biosynthesis, biosynthesis of unsaturated fatty acids, and cutin, suberine and wax biosynthesis). Thus, key microbial metabolites in the rumen can significantly influence lamb phenotype, and sex induces changes in the rumen microbial-metabolic profile of sheep, which modulates rumen function and adapts to the growth needs of lambs. This finding offers valuable insights into the management of lamb health and the development of targeted microbiota-metabolism management strategies regulating and optimizing rumen function in sheep to enhance productivity.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12866-025-04049-6.

Supplementary Material 1: Supplementary Figure 1. Rumen microbial beta diversity UPGMA analysis.

Supplementary Material 2: Supplementary Figure 2. Heatmaps were analyses to correlate the most enriched pathway metabolites with rumen fermentation parameters and BW in rams and ewes

Supplementary Material 3: Supplementary Table 1. Overview of 16S rRNA gene sequencing data

Supplementary Material 4: Supplementary Table 2. Information on rumen fluid metabolism module and KEGG differential enrichment

Supplementary Material 5: Supplementary Table 3. Rumen microbes and metabolic module correlations

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Authors' contributions
H.W., S.Z., and J.H. conceived and designed the research. H.W., J.Z., HY. J., HB. J., Y.P., and X.Z. collected samples and performed the experiment. H.W. and HY. J. analyzed the data. HY. J. and J.Z. helped with the bioinformatics and statistical analysis. H.W. wrote the manuscript, and all other authors revised the manuscript. All authors read and approved the final manuscript.

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Data availability
Sequence data that support the findings of this study have been deposited in the NCBI Sequence Read Archive (SRA) under accession numbers PRJNA1180906 and PRJNA1180905.

Declarations

Ethics approval and consent to participate
The experiment was conducted according to the guidelines approved (2010−JAAS−XM−01) by the Ethics Committee (Institute of Animal Husbandry and Veterinary, Jiangxi Academy of Agricultural Sciences) on 2021.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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