Animal Microbiome

RESEARCH

Open Access

Effect of *Lactiplantibacillus plantarum* N-1 and isomaltose-oligosaccharide on promoting growth performance and modulating the gastrointestinal microbiota in newborn Hu sheep

Xinyi Xu[1], Zhiwei Zhou[1], Zhiqiang Zhou[2], Yudong Ma[1], Dongmei Luo[1], Senlin Zhang[2], Pinggui Yang[3], Tianwu An[3*] and Qun Sun[1*]

Abstract

Background Diarrhea is usually observed in newborn Hu lambs, while severe diarrhea may lead to the stunted growth and even death in lambs, necessitating the common practice of antibiotic administration to newborns. In order to explore the application of the effective probiotics and/or prebiotic treatment in animal feed to lessen the recline on antibiotics, 27 newborn of Hu lambs were equally allocated into three groups: control group (Con), probiotics group (Pro) receiving *Lactiplantibacillus plantarum* N-1 (LPN-1), and synbiotics group (Syn) receiving LPN-1 combined with isomaltose-oligosaccharide (IMO), and raised till 60 days of age.

Results Compared with the Con, the incidence of severe diarrhea was lower in both two treatment groups, accompanied by a significant reduction in terramycin administration frequency ($P < 0.05$). The daily feed intake in newborns significantly increased after probiotics or synbiotics treatment ($P < 0.05$), leading to the substantial increment in average daily gain by 48.28% and heart girth ($P < 0.05$), as well as enhancements in height ($P < 0.01$) at 60 days of the age in synbiotics treatment group. Applying probiotics and synbiotics exhibited the enhanced rumen weight ($P < 0.05$), and synbiotics further promoted the spleen development ($P < 0.05$). The inclusion of probiotics and synbiotics significantly modified the gut microbial composition of Hu lambs ($P < 0.01$), with an increase in *Butyrivibrio proteoclasticus* and *Pseudoruminococcus massiliensis*, which were associated with starch and sucrose metabolism. Additionally, the Syn group exhibited an upsurge in the number of species associated with amino acid metabolism and cellulolysis, as well as the raised short-chain fatty acids levels in the newborn gut ($P < 0.05$).

*Correspondence:
Tianwu An
antianwu@126.com
Qun Sun
qunsun@scu.edu.cn

Full list of author information is available at the end of the article

Conclusions This study demonstrated that LPN-1 and IMO had an enhanced effect to improve the growth performance and decrease the reliance on antibiotics by promoting the feed intake, balancing the gut microbiota and increasing the short-chain fatty acids content in Hu lambs.

Keywords Newborn Hu sheep, *Lactiplantibacillus plantarum*, Probiotics, Synbiotics, Growth performance, Gastrointestinal microbiota

Introduction

Since the last century, the addition of antibiotics to animal feed has brought tremendous benefits to husbandry, its extensive use. However, the widespread use of antibiotics has also led to adverse effects such as antibiotic resistance and residue issues [1]. Long-term antibiotic use leads to not only the accumulation of antibiotic residues in meat and meat products, but also the accumulation of antibiotic-resistant bacteria (ARBs) and genes (ARGs) in the environment, posing a potential threat to ecosystems and human health [2, 3]. To date, the European Union, China have forbade the usage of antimicrobial growth promoters in animal nutrition [4].

Diarrhea in newborn lambs is a globally significant issue in sheep husbandry, contributing to up to 46% of lamb mortalities as evidenced by a study by the American Lambs Experiment Station [5]. In certain developing regions, veterinarians may prescribe antibiotic as a prophylactic measure for newborn lambs [6]. Since 2020, Hu sheep have been introduced to Neijiang, Sichuan, due to their high commercial value and robust reproductive capabilities, making them a preferred choice among local farmers. However, due to the inadequate sanitary conditions and the immature feeding management, diarrhea has become a common occurrence among newborn Hu lambs, often necessitating the use of antibiotics such as terramycin. It is necessary to find an effective antibiotic alternative to reduce the use of antibiotics and lamb mortality, meanwhile improve lamb growth performance, help farms reduce breeding costs and increase economic benefits. Finding low-cost antibiotic alternatives would be more beneficial to reduce the misuse of antibiotics in the developing regions and farms with poor breeding conditions.

Lactiplantibacillus plantarum, a commonly utilized probiotic, has shown to decrease the incidence of diarrhea through modulating the gut microbiota [7], reducing inflammation [4], improving gut barrier functions [8], producing the extracellular polysaccharides [9] and improving the short-chain fatty acids (SCFAs) levels in the gut microbiota [10]. The probiotic effects of different strains vary greatly [11]. It is necessary to screen out the strains with both diarrhea-preventing and growth-promoting properties to help farms reduce breeding costs. *L. plantarum* N-1 (LPN-1), a lactic acid bacterium isolated from traditional yak milk cheese in Inagi Aden by our team, is shown to be tolerant to acidity and bile salts, with the capability to modulate the gut microbiota, enhance gut barrier function and promote the production of the gut SCFAs [12–14]. It remains unclear whether high populations of acid-producing bacteria entering the rumen have impacts on the rumen environment of newborn lambs. Further experiments are needed to determine whether LPN-1 can successfully colonize the gut, help balance the gut microbiota, promote the production of SCFAs in the gut, and improve the diarrhea of lambs.

Isomaltose-oligosaccharide (IMO) is a low-cost prebiotic derived from starch, which has been demonstrated widely to promote the growth of various probiotics, including *L. plantarum* and *Lactobacillus rhamnosus* GG [15, 16]. IMO has found extensive application in the feed for pig, poultry, and fish with significant advances of promoting the growth performance [17, 18]. However, IMO alone appeared not to have the significant effect on the growth performance of sheep [19]. Recent studies have shown that IMO can serve as an adjuvant to enhance the effectiveness of substances like probiotics [20], green tea [21], and cranberry polyphenols [22]. Considering its proven capacity to enhance the growth and survival of *L. plantarum* [16], with evidence that synbiotics is more effective in promoting animal growth [23], we hypothesized that the combination of IMO and LPN-1 might be more effective and assessed their impact on preventing diarrhea and promoting growth in newborn Hu lambs.

Materials and methods

Animals, diets and experimental design

The study was carried out in Sichuan Wanghu Animal Husbandry located in Zizhong County in the east of Sichuan Province, China. During the experiment, the average ambient temperature was 17℃, the average relative humidity was 77.8%, and the ventilation was adequate. Twenty-seven healthy male Hu lambs of 0–5 days old with similar genetic background (mean body weight: 4.71 ± 0.83 kg) were randomly divided into three groups: control (Con, $n = 9$), probiotics (Pro, $n = 9$), and synbiotics (Syn, $n = 9$). Ovine/Caprine Clostridial Diseases Vaccine was administered to all lambs. The composition of the initial feed and the ewe feed was formulated in accordance with the nutrient requirements for meat sheep (NY/T 816–2021), the ingredients and dietary components and chemical compositions can be found in Table S1 and Table S2. All indicators of nutritional composition were tested following the prescribed methods outlined in

the Chinese national standards. LPN-1 freeze-dried powders (Hehe Bioengineering Technology Company, China) were administered at a rate of 10^9 CFU/kg initial BW/day to the Pro group. The Syn group received the same amount of LPN-1 along with 80 mg IMO/kg initial BW/day. The LPN-1 powder and IMO were dissolved in the appropriate amount of water and then administered to the lambs' mouths with a 2 mL syringe. The Con group received 2 mL of drinking water per day using the same method. Three separate pens (4 m × 4 m) were arranged to accommodate the three groups of Hu lambs. Lambs could move and drink breast milk freely in their respective pens, which had the same number of ewes. Starting from day 7, the newborn Hu lambs were consistently fed a fixed quantity of initial feed. The experiment lasted for 60 days, during which the body weight and size (body length, height, and heart girth) were measured every 15 days.

Diarrhea rate

During the experiment, the diarrhea experienced by Hu lambs was monitored, and the related data was recorded. According to Casper et al.'s method [24], we scored and evaluated the daily feces of Hu lambs, and the standard for evaluation was outlined in Table S3. The average fecal score was calculated using the following formula: Average Fecal Scores = Sum of the Fecal Scores / Total Number of Lambs. No intervention was made in the cases where Hu lambs experienced diarrhea for less than a day. However, instances where the Hu lambs experienced diarrhea for more than two consecutive days, exhibited signs of listlessness or showed no improvement in their diarrhea were treated with terramycin under the guidance of veterinary professionals.

Sample collection

Blood sample collection occurred on days 30 and 60 of the experiment, where 5 mL of venous blood was collected from the lambs to prepare serum samples. Blood samples were placed in a disposable vacuum container and left to rest for 4 h. Afterwards, the samples were centrifuged at 3000 ×g for eight minutes at 4 °C, and the supernatant was separated for later analysis. On day 60 of the experiment, five lambs were randomly chosen from each of the three groups for slaughter. The thymus and spleen of each lamb were weighed and recorded first. Then, a tissue block measuring approximately 4 cm^2 was taken from the same location of the rumen and placed in paraformaldehyde for storage, while being kept away from light. Rumen contents were filtered using four layers of sterile gauze and then separated into tubes for frozen storage, which were then stored in liquid nitrogen. After sampling, the rumen, reticulum, omasum, and abomasum were cleaned, dried, and weighed. Ileum contents were collected into cryopreservation tubes and stored in liquid nitrogen till use.

Detection of blood indexes

The serum levels of Immunoglobulin A (IgA), Immunoglobulin G (IgG), Immunoglobulin M (IgM), and lipopolysaccharide (LPS) were determined using enzyme-linked immunosorbent assay (ELISA) kits manufactured by Shanghai Meimian Biotechnology Limited Company in China.

Determination of rumen morphology

The rumen tissue, which had an area of approximately 4 cm^2, was immersed in a 4% paraformaldehyde fixative solution and then left in the dark for more than 24 h. After that, the tissue was removed and trimmed using a scalping-knife. The trimmed tissue then underwent dehydration with multiple gradients of alcohol and was embedded in paraffin, followed by cutting the wax blocks into thin sections which had a thickness of 4 μm using a paraffin microtome. Following HE staining, we utilized Image-Pro Plus 6.0 (Media Cybemetics, USA) to analyze the rumen papilla height and mucosal layer thickness.

Rumen microbial DNA extraction and quantitative real-time PCR (qPCR) analysis

DNA was extracted from rumen contents utilizing the TB601 fecal DNA extraction kit (Tianmo, China) in accordance with the instructions provided by the manufacturer. The relative abundance of various common microorganisms present in the rumen was detected using S1000TM Thermal Cycler PCR (Bio-Rad, USA). The reaction volume was 20 μL, consisting of 1.2 μL primers (10 μM), 7.8 μL of deionized water, 1 μL cDNA, and 10 μL TB Green ® Premix Ex Taq™ (TaKaRa, Japan). Table S3 displays the rumen microbial primers utilized.

Ileal microbial DNA extraction, 16 S rRNA amplification, sequencing, analyzing

Microbial DNA was extracted from the ileal contents using the E.Z.N.A.® stool DNA Kit (Omega, USA) according to manufacturer's protocols. The DNA concentration was determined by using Qubit Flex (Thermo Fisher Scientific, USA). Universal primers 27 F and 1492R were utilized to amplify the 16 S rRNA V1-V9 sequences. The amplified DNA was extracted from 2% agarose gels and purified via the AxyPrep DNA Gel Extraction Kit (Axygen, USA). According to the Pacific Biosciences manual, the SMRTbell library was constructed and sequenced on the PacBio Sequel II platform. All sequencing was performed by Biozeron (Lingen, Shanghai, China). The PacBio raw data were processed using SMRT Link Analysis (version 11.0) to obtain circular consensus sequences (CCS). Sequences shorter than 1000 bp or longer than

1800 bp were filtered out using SMRT Portal, and barcode and primer sequences were removed using lima (https://lima.how/). The optimized sequences were noise cancelled by using DADA2 in QIIME2. The phylogenetic affiliation of each 16 S rRNA gene sequence was analyzed by uclust algorithm (https://github.com/topics/uclust) against the Silva (SSU138.1) 16 S rRNA database (http://www.arb-silva.de) using confidence threshold of 80% [9]. The rarefaction analysisbased on Mothur v.1.21.1 [25] was conducted to reveal the diversity indices, including the Chao and Shannon diversity indices. LEfSe was performed using the tools at http://huttenhower.sph.harvard.edu/lefse/ [26], and the LDA threshold was indicated under the legends. The functional changes of microbiota in different samples were predicted using FAPROTAX 1.2.5 (http://www.loucalab.com/archive/FAPROTAX/lib/php/index.php?section=Download.) and Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2) (http://picrust.github.io/picrust/tutorials/genome_prediction.html) based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database.

Extraction and determination of short chain fatty acid content

200 µL of ileum contents were added to a centrifuge tube along with 1800 µl of 0.5% phosphoric acid solution then centrifuged at 13,000 × g for 10 min. An equal volume of ethyl acetate was added to the supernatant and mixed thoroughly. The mixture was centrifuged again to obtain the supernatant organic phase, which was then filtered into a chromatographic sample bottle using a 0.22 µm filter head. The resulting liquid was subjected to analysis using an Agilent7890B gas chromatograph (Agilent, USA). Using a DB-WAX ultra-inert gas chromatography column, the procedure begins by maintaining a temperature of 80 °C for 3 min, followed by a ramp-up to 200 °C at a rate of 20 °C per minute, and then holding for 11 min.

Table 1 Statistics of diarrhea in Hu lambs

Item	Treatments			SEM	*P*-value
	Con	Pro	Syn		
The number of lambs that had diarrhea	6	4	6	-	0.693
The number of lambs that had continuous diarrhea[1]	4	2	1	-	0.418
The number of lambs that had severe diarrhea[2]	3^{b}	0^{a}	0^{a}	-	0.086
Total number of terramycin treatment	7^{B}	1^{A}	1^{A}	-	0.040

[1] Continuous diarrhea: Diarrhea for 2 or more consecutive days

[2] Severe diarrhea: Diarrhea for 3 or more consecutive days

[a, b] Mean values within a row with different superscripts have a tendency to differ ($P < 0.10$)

[A, B] Mean values within a row with different superscripts are significantly different ($P < 0.05$)

Statistical analysis

SPSS 25.0 was utilized to conduct data analysis. For the body weight, body measurements, physiological and biochemical parameters, and organ weights of Hu sheep, we evaluated normality using the Shapiro-Wilk test and assessed homogeneity of variances using Levene's test. After confirming that the data followed a normal distribution and met the assumption of homogeneity of variances, we performed statistical analysis using one-way analysis of variance (ANOVA), with Tukey's test for post hoc comparisons. For the fecal scores, feed intake, ileal SCFAs content, rumen microbiota, and ileal microbiota data of Hu sheep, we analyzed significant differences using the Kruskal-Wallis test, followed by Bonferroni correction for post hoc testing. When comparing differences between two specific groups, the Wilcoxon rank-sum test was applied. Fisher's exact test was used to compare the diarrhea rate, and Spearman's correlation was used for correlation analysis. Graphs were generated with Graphpad Prism 8 (GraphPad Software, CA, USA), OmicStudio tools (https://www.omicstudio.cn/tool) and the Biozeron tools at (http://www.cloud.biomicroclass.com/CloudPlatform). The data was presented as mean ± SEM. "#"was used to represent tendency ($0.05 < P < 0.10$). "*", "**", and "***" were used to represent the level of statistical significance ($P < 0.05$, $P < 0.01$, $P < 0.001$).

Results

Behavioral observation

During the experiment, we conducted preliminary behavioral statistics on three groups of lambs, as shown in Table S5. Starting from the 16th day of the experiment, the lambs in two experimental groups not only finished the initial feed but also competed with their mothers for feed. However, this phenomenon was not observed in the Con group. We estimated the weight of the feed intake by the lambs based on the volume of feed they consumed, as shown in Table 1.

Diarrhea rate

During the experiment, 16 out of 27 Hu lambs (59.26%) experienced diarrhea, with 6 of them suffering from severe diarrhea, accounting for 22.22% of the total number of Hu lambs, which is also 37.50% of those that had diarrhea. Tables 1 and 2 showed the statistics of diarrhea in Hu lambs. The total number of terramycin treatment in Pro and Syn groups was significantly lower than Con group ($P < 0.05$), and the average fecal scores of the Pro group was significantly lower than Con Group ($P < 0.05$).

Table 2 Statistics of diarrhea rate in Hu lambs

Item	Treatments			SEM	*P*-value
	Con	Pro	Syn		
Total days of fecal scores					
Score = 0	57.89[b]	59.22[a]	58.78 [ab]	0.256	0.094
Score = 1	0.22	0.22	0.22	0.082	1.000
Score = 2	1.89 [B]	0.56 [A]	1 [AB]	0.231	0.049
Average fecal scores	4.33 [B]	1.33 [A]	2.22 [AB]	0.515	0.044

[a, b] Mean values within a row with different superscripts have a tendency to differ ($P < 0.10$)

[A, B] Mean values within a row with different superscripts are significantly different ($P < 0.05$)

Table 3 Changes in body weight and body size of lambs from 0 to 60 days

Item	Treatments			SEM	*P*-value
	Con	Pro	Syn		
ADFI[1], *g/d*					
Initial feed	100.00	100.00	100.00	5.97	1.000
Ewe feed	0.00 [A]	49.60 [B]	50.20[B]	5.07	< 0.001
Total	100.00 [A]	149.60 [B]	150.20 [B]	8.90	0.023
BW[2], *kg*					
D 0	4.70	4.70	4.74	0.42	0.993
D 15	6.91	7.46	7.88	0.65	0.339
D 30	9.08	9.47	10.97	0.99	0.153
D 45	11.15[a]	11.81[ab]	14.19[b]	1.30	0.068
D 60	13.13[A]	14.76[AB]	17.25[B]	1.69	0.038
ADG[3], *g/d*					
D 0–15	147.56	183.93	209.44	28.21	0.109
D 16–30	144.81[ab]	133.7[a]	205.93[b]	30.74	0.059
D 31–45	137.78	155.93	214.44	40.14	0.158
D 46–60	132.22	196.67	204.07	40.05	0.164
D 0–60	140.59[A]	167.56[AB]	208.47[B]	25.85	0.046
BL[5], *cm*					
D 0	34.98	33.60	33.60	1.61	0.621
D 15	42.99	42.28	43.88	1.26	0.454
D 30	46.26	47.54	48.32	1.87	0.544
D 45	51.97	50.72	52.14	1.23	0.462
D 60	53.10	55.37	57.12	1.78	0.145
BH[6], *cm*					
D 0	39.22	38.51	39.33	1.29	0.727
D 15	42.94	43.59	45.02	1.19	0.230
D 30	46.93[AB]	45.41[A]	48.14[B]	1.05	0.017
D 45	49.77	50.16	51.87	1.07	0.142
D 60	51.43[A]	53.63[AB]	56.03[B]	1.37	0.010
HG[7], *cm*					
D 0	40.20	39.60	40.89	1.41	0.664
D 15	45.32	46.27	49.18	1.83	0.112
D 30	52.23	51.69	53.97	2.09	0.533
D 45	54.74[a]	54.80[a]	59.23[b]	2.26	0.096
D 60	57.83[A]	58.70[AB]	64.74[B]	2.45	0.025

[1]*ADFI* Average daily feed intake (includes feed only), [2]*BW* Body weight; [3]*ADG* the ADG; [4]*FCR* feed conversion ratio; [5]*BL* Body length; [6]*BH* Body height; [7]*HG* Heart girth

[a, b] Mean values within a row with different superscripts have a tendency to differ ($P < 0.10$)

[A, B] Mean values within a row with different superscripts are significantly different ($P < 0.05$)

Growth performance

Following the typical farming method of the farm, all three groups of lambs were fed equal amounts of initial feed, as shown in Table 3. On the 60th day of the experiment, no significant difference was observed in the average body weight, average daily gain (ADG), body length, and the body height of the Con group compared to the Pro group ($P > 0.05$). After supplementation with IMO, the body weight ($P = 0.032$) and the ADG ($P = 0.038$) of lambs in the Syn group were significantly higher than those in the Con group. There was also a significant increase in the body height ($P < 0.01$) and the heart girth ($P = 0.027$) in the Syn group compared to the Con group.

Immune function

Results from Table 4 indicated that for all three groups, there were no significant differences in levels of physical and chemical indexes ($P > 0.05$). While the Syn group showed significantly higher spleen weight ($P = 0.018$) and spleen index ($P = 0.044$) in comparison to the other two groups.

Rumen development

The results in Table 5 indicated that the rumen weight in two treatment groups were significantly greater than those in the Con group ($P = 0.027$). The rumen index in the pro group was significantly higher than other two groups ($P < 0.01$). However, there was no significant change in rumen morphology (rumen papillae height and mucosal layer thickness) among the three groups ($P > 0.05$). Additionally, compared with the other two groups, the ruminal pH value of the lambs in the Pro group was significantly decreased ($P = 0.023$).

Relative abundance of rumen cellulose degrading microbiota

The results in Table 6 indicated that the relative abundance of *B. fibrisolvens* in the Pro group was significantly lower ($P = 0.024$), while that of *Lactobacillus* was higher ($P = 0.028$) than the Con group. The study found no significant difference in the relative abundance of rumen cellulose degrading microbiota between the Syn and Con groups ($P > 0.05$). Significantly lower levels of ruminal *Methanogenus* were found in the Pro group compared to the Syn group ($P = 0.049$). Figure 1 illustrated the correlation between rumen indexes and rumen cellulose degrading microbiota. The results indicated a significant negative correlation between the relative abundance of *Lactobacillus* and ruminal pH (Spearman's correlation, $P < 0.01$), a significant positive correlation between the relative abundance of *Butyrivibrio fibrisolvens* and ruminal pH (Spearman's correlation, $P = 0.031$), and a

Table 4 Differences in immune function of Hu lambs

Item	Treatments			SEM	*P*-value
	Con	Pro	Syn		
D 30					
Serum IgA, μg/mL	317.51	290.77	322.25	7.37	0.173
Serum IgM, μg/mL	1812.92	1882.37	1809.98	28.18	0.507
Serum IgG, μg/mL	6146.39	6446.19	6529.26	104.87	0.281
D 60					
Serum IgG, μg/mL	5666.85	5990.00	5942.78	124.10	0.535
Rumen IgA, μg/mL	199.75	190.00	185.00	5.00	0.506
Ileal IgA, μg/mL	163.70	145.12	160.68	7.43	0.583
Ileal LPS, pg/mL	8.98	7.39	7.94	0.37	0.215
Spleen weight, g	20.90^{A}	24.54^{A}	32.64^{B}	1.90	0.020
Spleen index, %	0.162^{A}	0.150^{A}	0.194^{B}	0.007	0.024
Thymus weight, g	5.34	6.14	5.66	0.30	0.586
Thymus index, %	0.044	0.037	0.034	0.003	0.272

[a, b] Mean values within a row with different superscripts have a tendency to differ ($P < 0.10$)

[A, B] Mean values within a row with different superscripts are significantly different ($P < 0.05$)

Table 5 Stomachic development in 60-day-old Hu lambs

Item	Treatments			SEM	*P*-value
	Con	Pro	Syn		
Organ weight, g					
Rumen	151.98^{A}	246.46^{B}	224.6^{B}	16.01	0.026
Reticulum	30.94	34.06	37.3	1.96	0.448
Omasum	18.82	21.86	26.28	1.93	0.304
Abomasum	55.32	73.86	71.64	4.34	0.166
Organ index, g/kg					
Rumen	11.78^{A}	16.96^{B}	13.26^{A}	0.72	0.002
Reticulum	2.38	2.36	2.23	0.08	0.858
Omasum	1.45	1.54	1.58	0.11	0.891
Abomasum	4.29^{a}	5.10^{b}	4.25^{a}	0.18	0.063
Rumen physico-chemical indexes					
Papilla height, mm	0.149	0.129	0.141	0.01	0.438
Mucosal thickness, mm	1.36	1.29	1.34	0.09	0.959
Ruminal pH	6.24^{A}	5.87^{B}	6.15^{A}	0.05	0.023

[a, b] Mean values within a row with different superscripts have a tendency to differ ($P < 0.10$)

[A, B] Mean values within a row with different superscripts are significantly different ($P < 0.05$)

significant positive correlation between the relative abundance of protozoa and rumen weight (Spearman's correlation, $P = 0.043$).

Ileal microbiota

Microbial composition in the ileum

The results of the study on the ileum microbiota of 60-day-old Hu lambs were presented in Fig. 2. The Shannon-Wiener curves depicted in Fig. 2A appeared to be flat, indicating that the sequencing data volume was sufficient to capture the microbial information present

Table 6 Differences in the relative abundance of rumen functional microbiota (%)

Item	Treatments			SEM	*P*-value
	Con	Pro	Syn		
Ruminococcus albus	0.0355	0.0384	0.0377	0.0067	0.931
Ruminococcus flavefaciens	0.3134	0.3228	0.3417	0.0425	0.932
Fibrobacter succinogenes	4.3377	2.7802	1.4175	0.8437	0.476
Butyrivibrio fibrisolvens	0.4247^{B}	0.0468^{A}	0.3052^{AB}	0.2500	0.024
Lactobacillus	0.0005^{A}	0.0529^{B}	0.0019^{AB}	0.0126	0.028
Total methanogen	0.3462^{A}	0.1481^{B}	0.4586^{A}	0.0646	0.049
Protozoa	2.8678	4.8288	4.5752	1.0486	0.651

[A, B] Mean values within a row with different superscripts are significantly different ($P < 0.05$)

in the samples. As shown in Fig. 2B, the ileum microbiota of the three groups was clearly distinguishable from one another. Analysis of similarities (Anosim) indicated that there were significant differences among the groups ($P < 0.01$). Figure 2C and D respectively showed the differences in alpha diversity among the three groups. Compared to the Con group, the Shannon index and the Chao1 index of the ileal microbiota in the Pro group were significantly lower ($P < 0.01$). There was no significant difference in the Shannon index and the Chao1 index between the Syn group and the Con group ($P > 0.05$). The structural composition of lamb ileum microbiota at the phylum and genus levels was shown respectively in Fig. 2E and F. As seen in the figures, the most common phyla were Firmicutes, Candidatus Saccharibacteria, Actinobacteria, and Bacteroidetes, and the most common genera were *Candidatus Saccharimonas*, *Bulleidia*, *Eisenbergiella*, *Phocea* and *Lactobacillus*. Using the Random Forest model, 15 genera were screened out (Fig. 2G), which may serve as key biomarkers for distinguishing the three groups. The relative abundances of these 15 genera are provided in Table S6. LEfSE analysis in Fig. 2H-I demonstrated a significant increase in the relative abundance of 9 genera such as *Olsenella*, *Absicoccus*, *Anaerotruncus*, *Howardella* and *Peptococcus* in the Pro group, while the relative abundance of 16 genera such as *Eisenbergiella*, *Christensenella*, *Enterocloster*, *Lawsonibacter* and *Lacrimispora* decreased significantly compared to the Con group. In the Syn group, there were significant increases in the relative abundance of 10 genera such as *Pseudoruminococcus*, *Schaedlerella*, *Agathobacter*, *Anaerotruncus*, and *Chelatococcus*, while the relative abundance of 6 genera such as *Bulleidia*, *Desnuesiella*, *Rubripirellula*, *Cutibacterium*, *and Anaerosacchariphius* decreased significantly compared to the Con group. The relative abundances of microbial taxa associated with LEfSE results

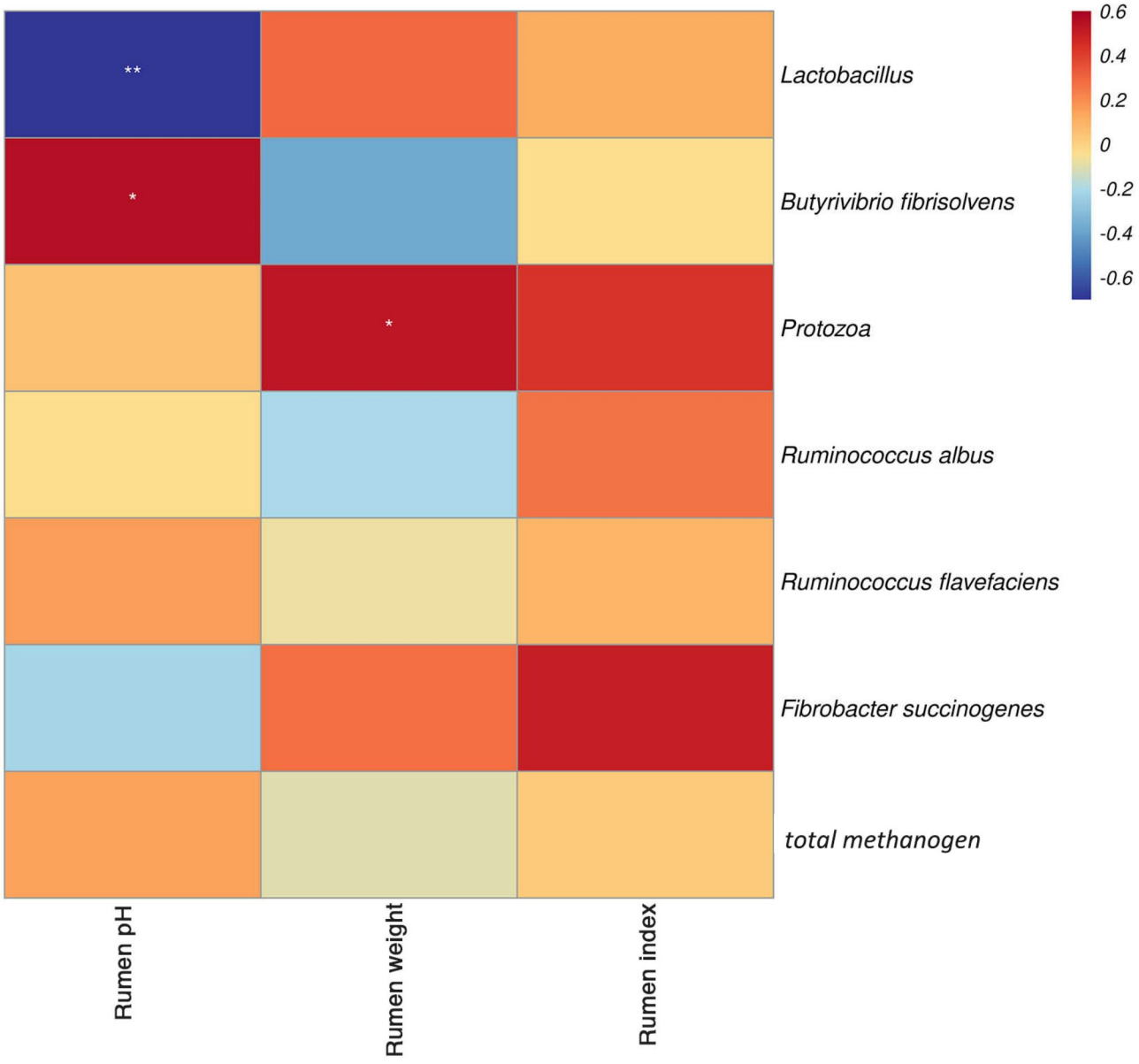

Fig. 1 Correlation analysis between rumen functional microbiota and rumen indexes of 60-day-old Hu lambs (Spearman's correlation, *$P < 0.05$, **$P < 0.01$)

were shown in the Additional file 2 and 3. Based on the correlation analysis, we found 15 bacterial genera that exhibited significant correlations with production performance (Fig. 3).

PICRUSt 2 and FAPROTAX functional prediction

To investigate the impact of LPN-1 and IMO on the ileal microbiota of Hu lambs, we analyzed the functional composition of their ileal microbiota using both PICRUSt2 method (Fig. 4A and C) and FAPROTAX method (Fig. 4D and F). We observed significant alterations in 22 KEGG metabolic pathways in the Pro group and 16 metabolic pathways in the Syn group, in contrast to the Con group, as detected by the PICRUSt2 method. The FAPROTAX method also revealed that the Pro group showed significant changes in 6 metabolic pathways, and the Syn group showed significant changes in 8 metabolic pathways when compared to the Con group.

SCFAs

Figure 5A showed a highly significant increase in ileum valeric acid content in the Pro group compared to the Con group ($P < 0.01$). Additionally, the content of acetic acid ($P = 0.038$) and the total SCFAs ($P = 0.017$) in the Syn group was significantly higher than the Con group. Figure 5B demonstrated the results of the correlation

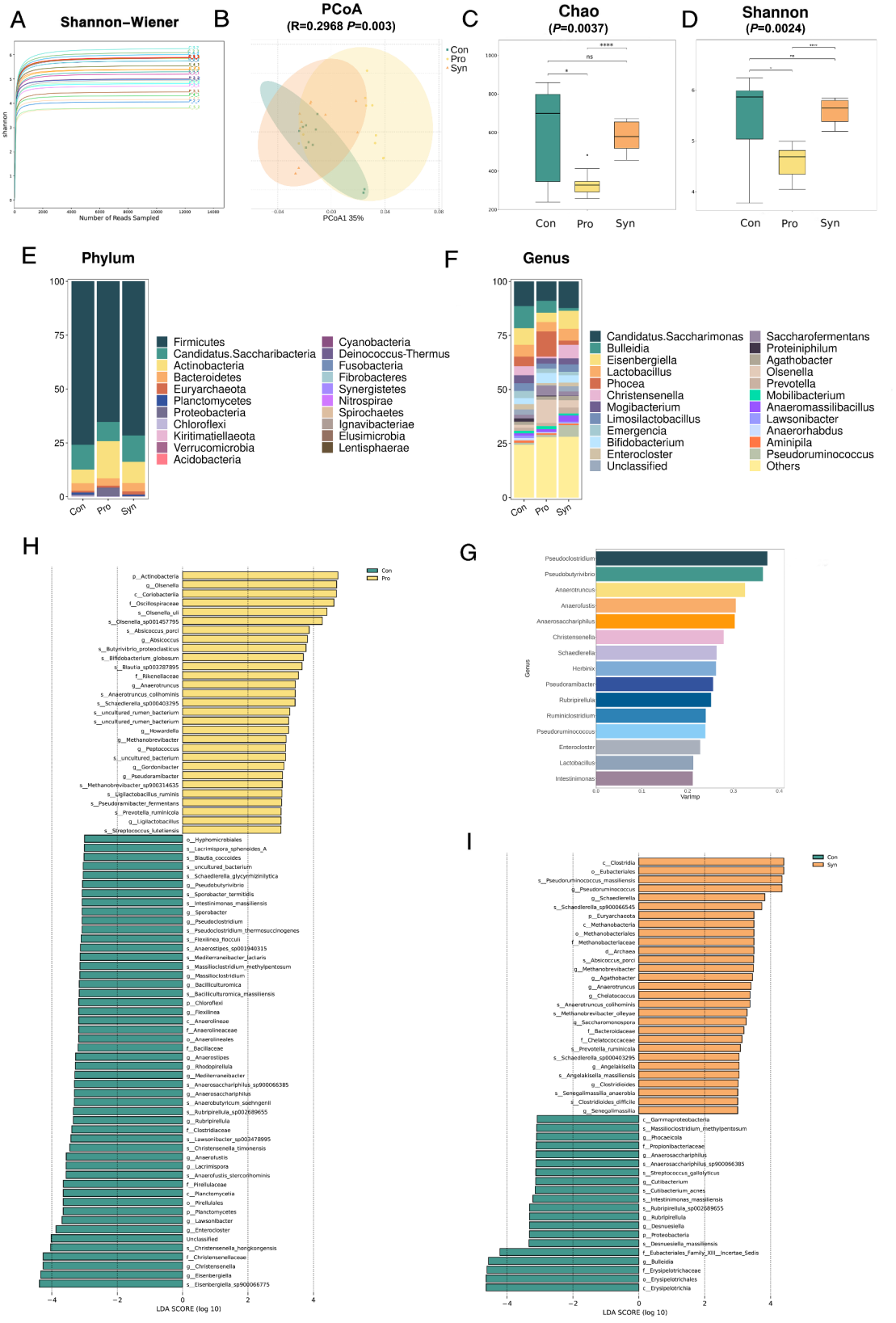

Fig. 2 Changes of ileal microbiota in 60-day-old Hu lambs. **A**: Shannon curve; **B**: β-diversity analysis (PCoA); **C**: Chao index; **D**: Shannon index; **E**: Relative abundance of the lambs' ileal microbiota in level phylum; **F**: Relative abundance of the lambs' ileal microbiota in level genus; **G**: Random forest analysis; **H**: LEfSe analysis between the Con group and Pro group (LDA threshold = 3.0); **I**: LEfSe analysis between Con group and Syn group (LDA threshold = 3.0). *$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$

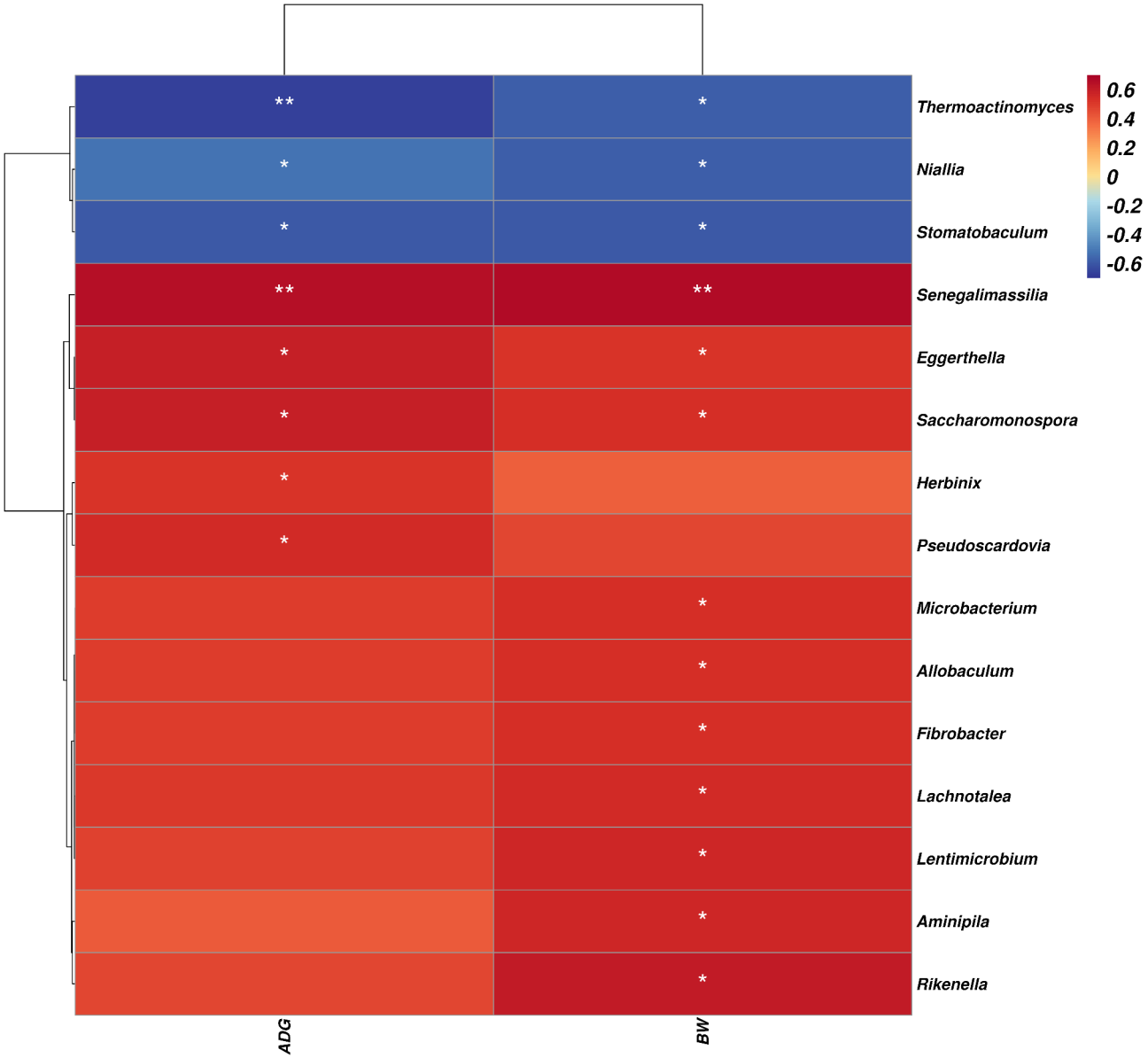

Fig. 3 Statistics of bacteria genera with significant correlation with growth performance (Spearman's correlation, *$P < 0.05$, ** $P < 0.01$)

analysis between the SCFAs content and ileum microbiota. Nineteen bacteria showed a significant positive correlation with the SCFAs content, while nineteen bacteria showed a significant negative correlation. Figure 5C demonstrated the results of the correlation analysis between the SCFAs content and microbial community function prediction. The results showed that 9 KEGG metabolic pathways were significantly positively correlated with the SCFAs content, and 13 KEGG metabolic pathways were significantly negatively correlated with the SCFAs content.

Discussion

Agricultural sector consumes nearly two-thirds of the world's antibiotic supply, exacerbating the antimicrobial resistance crisis and posing a serious threat to global public health security [27]. Terramycin is one of the commonly used antimicrobials to treat diarrhea in young ruminants [5, 28]. It is of great significance to find an effective non-drug-resistant method for diarrhea control. This study found that the combination of LPN-1 and IMO demonstrated enhanced efficacy, which can promote the growth and development of newborn Hu sheep and reduce the frequency of Terramycin use by balancing gut microbiota and increasing gut SCFAs content. This method is low-cost and efficient, not only reducing

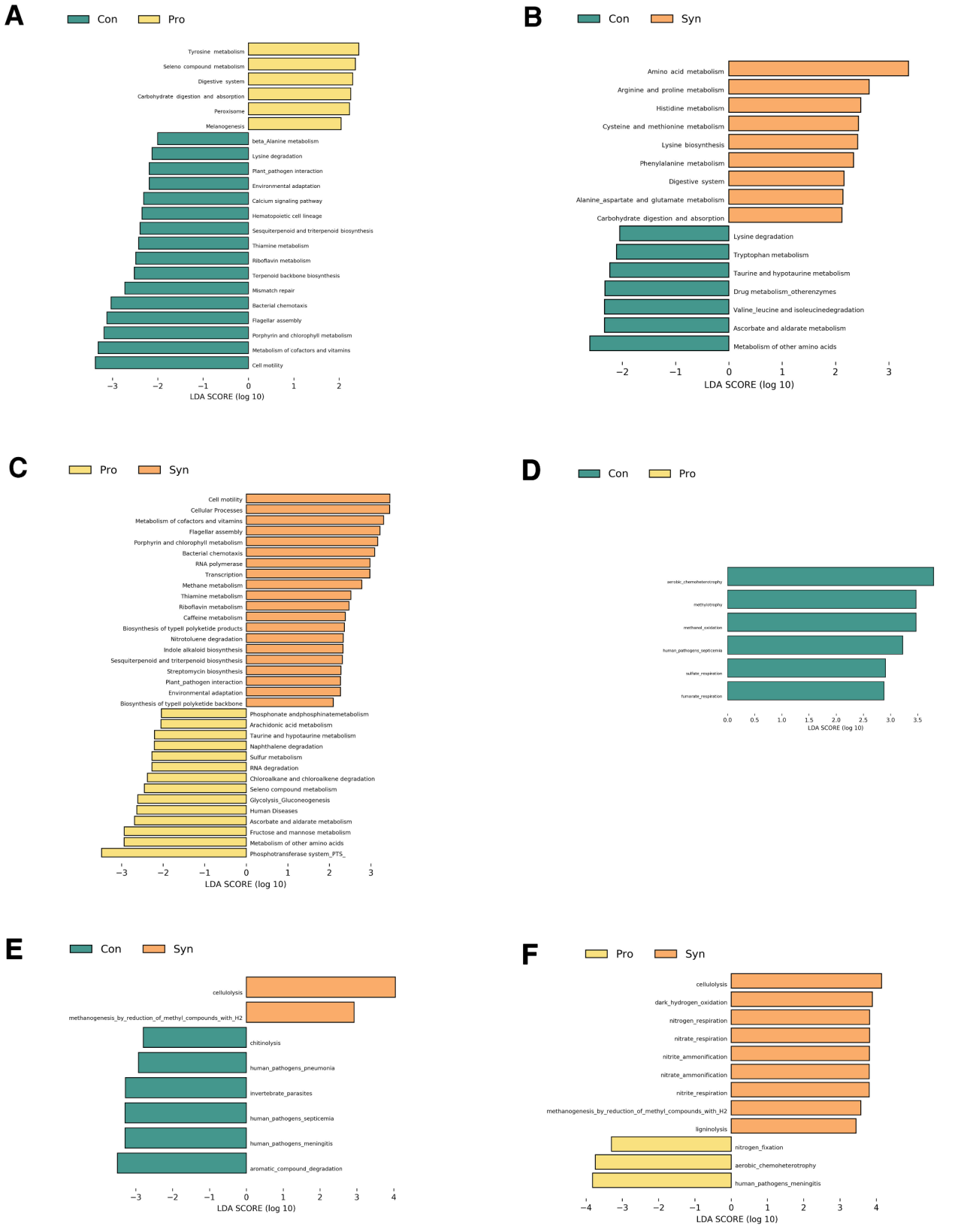

Fig. 4 Functional prediction of ileal microbiota (LEfSe analysis, LDA threshold = 2.0). **A-C**: PICRUSt2 functional prediction; **D-F**: FAPROTAX functional prediction

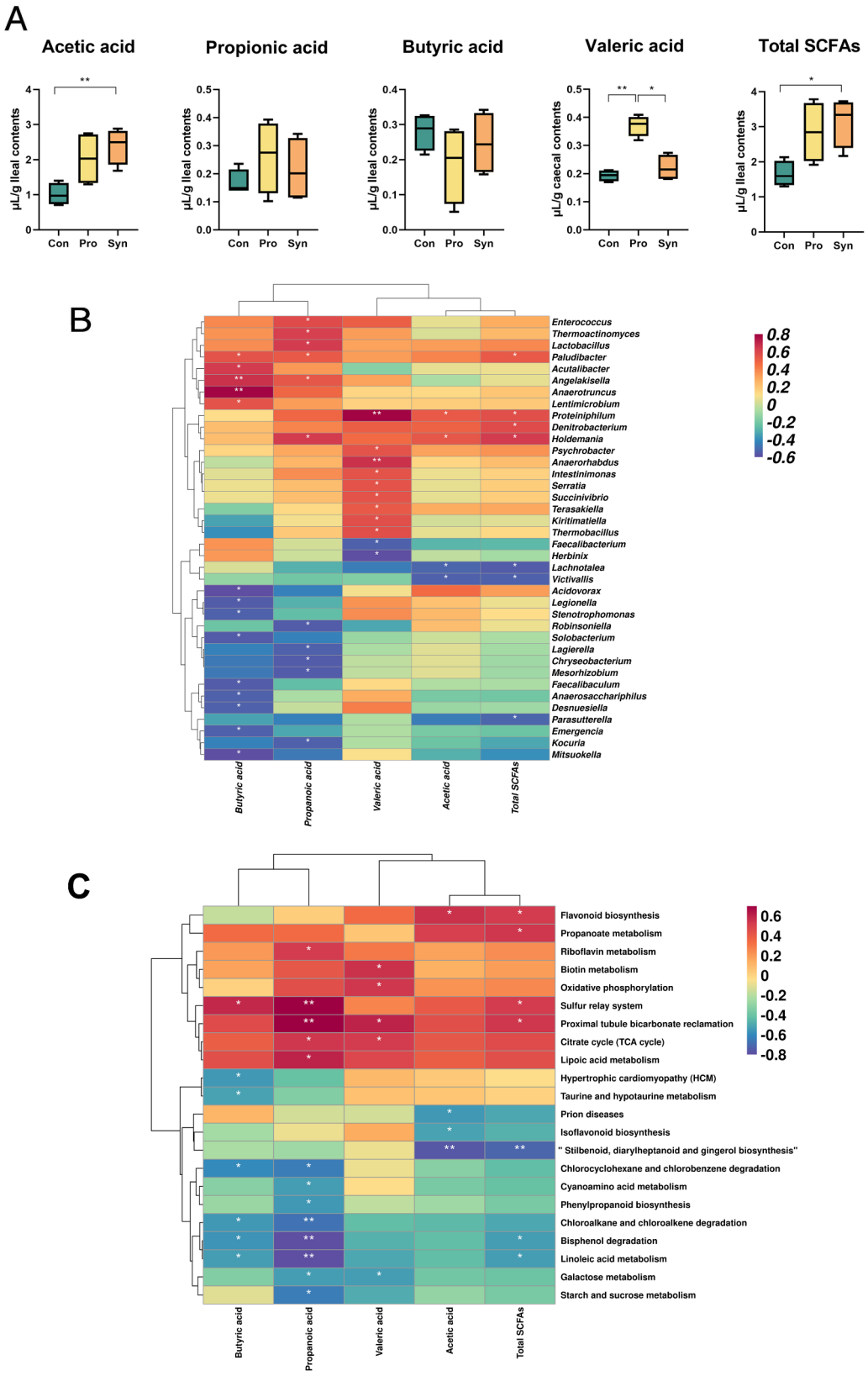

Fig. 5 Correlation analysis of SCFAs content and ileal microbiota. **A**: Ileal SCFAs content; **B**: Correlation analysis of SCFAs and ileal microbiota (Spearman's correlation). Data were presented as the mean ± SEM. *$P < 0.05$, ** $P < 0.01$. **C**: Correlation analysis of SCFAs and microbial community function prediction (Spearman's correlation). Data were presented as the mean ± SEM. *$P < 0.05$, ** $P < 0.01$

antibiotic dependence in newborn Hu sheep but also enhancing economic benefits.

Diarrhea rate is an essential indicator of gut health [29]. In this study, all lambs were vaccinated against Ovine/Caprine Clostridial Diseases Vaccine, and no mortality cases attributable to severe diarrhea were observed throughout the experimental period. The results revealed that 59.26% of the Hu lambs exhibited diarrhea symptoms, confirming that diarrhea is a common health issue in pre-weaning Hu lambs. Further analysis showed that 56.25% of the cases presented with mild diarrhea, characterized as transient diarrhea, which resolved spontaneously without the need for antibiotic treatment. This transient diarrhea may be associated with non-infectious factors such as chilling and dyspepsia. The farm is semi-open, where significant diurnal temperature variations can easily lead to chilling in lambs. Due to the constraints of the experimental site, the lambs have limited room to move around, which may cause them to suffer from dyspepsia. 7 lambs (43.75% of the total diarrhea cases) exhibited continuous diarrhea symptoms, characterized by yellowish watery mucoid stool. Prompt treatment with Terramycin effectively controlled all cases, with no mortality reported. Furthermore, we found that 89.47% of diarrhea occurred in lambs aged 0–30 days. This may be due to the fact that before 30 days of age, the diversity and richness of the gut microbiota in Hu sheep are relatively low, resulting in weaker resistance to external pathogens. Previously, Huang et al. [30] found that the Chao1 index of feces in 35-day-old Hu lambs was twice as high as the 7-day-old one. In summary, the period from 0 to 30 days of age is a high-incidence phase for diarrhea in Hu sheep. Implementing effective intervention measures to reduce lamb diarrhea during this period can effectively decrease the use of antibiotics. Our results showed that there was no significant difference in the occurrence of diarrhea and continuous diarrhea among the three groups of Hu lambs, but the number of severely diarrhea lambs in the two treatment groups showed a decreasing trend compared to the Con group ($P < 0.10$). The use of terramycin in Hu lambs in the two treatment groups significantly decreased ($P < 0.05$), indicating that the use of terramycin for treating severe diarrhea in pre-weaned Hu lambs can be effectively reduced by single or combined administration of IMO LPN-1. In addition, we also found that the fecal scores of the Pro group was significantly decreased ($P < 0.05$), which may be related to the fact that LPN-1 produced lactic acid or other antibacterial products, and reduced ruminal pH value, which made a variety of acid-resistant bacteria unable to survive [4]. Previous studies have found that probiotics can reduce the diarrhea rate by increasing the diversity and richness of rumen microbiota [31]. This study found that after feeding probiotics, the fecal score of Hu sheep significantly decreased, and the richness and diversity of the gut microbiota also significantly decreased. The results of this study indicate that there is no absolute correlation between diarrhea incidence and the diversity, richness of gastrointestinal microbiota.

For the statistics of pre-weaning feed intake in animals, breast-milk substitute feeding is a favorable approach [32]. The purpose of this experiment was to investigate the preventive effects of LPN-1 and IMO on diarrhea in newborn lambs. Due to the significant impact of ewes on the establishment of the microbial community in the gut of lambs, we did not separate ewes from lambs in this experiment. In order to minimize the difference in total nursing volume between the three groups as much as possible, we arranged an equal number of nursing ewes in each pen. Based on the farming experience of this farm, we started feeding each lamb 100 g/day of initial feed from the 7th day. Surprisingly, we observed that from the 16th day onwards, both the Pro group and the Syn group of Hu lambs began to consume additional ewe feed, and we estimated their additional feed intake based on the volume of feed they consume. The drawback of this method is that the data on additional feed intake is not precise, but since all three groups of ewes and lambs were raised in the same way, and only the Pro group and the Syn group showed active additional feeding behavior, it still proves the contribution of LPN-1 and IMO in promoting feed intake in Hu lambs.

The impact of probiotics and prebiotics on ruminant growth performance is currently a topic of debate. Previous studies have shown that *L. plantarum* can greatly enhance calve growth [24, 33]. In contrast, Zhang et al. [34] found that administration of *L. plantarum* increased feed conversion rates in calves, but did not significantly increase average daily gain. Wang et al. [35] found that the administration of compound probiotics to newborn calves had no effect on growth performance. Wang et al. [19] demonstrated that supplementing 4.5 g IMO to the diets of weaned lambs did not significantly impact ADG or average daily feed intake (ADFI). Our results showed that the growth promotion effect of LPN-1 alone was not significant, but combining LPN-1 and IMO significantly enhanced both body weight and size in Hu lambs ($P < 0.05$). Similar results in fattening lambs were obtained by Estrada-Angulo et al. [23].

The spleen, the largest lymphoid organ by volume, not only plays a crucial role in both antibacterial and antifungal immune responses, but also has the function of blood storage, hematopoietic, and removal of senescent red blood cells. It is in this organ that innate and adaptive immune responses can effectively take place [36]. The weight of the lymphatic organs can reflect the ability of the body to supply lymphocytes in the immune response, so the spleen index is often used to assess the strength

of immunity [37]. The Syn group showed a significant increase in the spleen index ($P < 0.05$), and no anatomical abnormalities were observed in the appearance of the spleen, indicating that the combination of LPN-1 and IMO can boost spleen development and enhance the immunity of newborn lambs. Song et al. [38] also found similar results in newborn broilers when they administered a combination of *L. plantarum* and FOS, but the specific mechanism needs to be further explored.

The rumen, one of the important digestive organs of lambs, plays a crucial role in the metabolism, immunity, and health of the host. Newborn Hu lamb's rumen lacks the ability to ferment feeds [39]. As the rumen develops and microbiota colonizes, Hu lambs were able to digest and absorb vegetable fibers. The rapid development of rumen and rumen microbiota of newborn Hu lambs could facilitate the acquisition of digesting and absorbing plant food, promote the growth of Hu lambs, and ease weaning stress. The experimental results revealed that both the Pro and Syn groups showed a significant increase in rumen weight and rumen index ($P < 0.05$). The findings suggest that LPN-1, both alone and in combination with IMO, may stimulate rumen development, thereby indirectly promoting feed intake and growth in Hu lambs. Lin et al. [40] previously noted that the microbiome-driven production of acetate and butyrate mediated the regulation of growth-related signaling pathways in rumen epithelium by growth-related genes. Based on our experimental results, it is hypothesized that LPN-1 could stimulate rumen development by promoting the synthesis of organic acids or by stimulating the rumen microbiota to produce organic acids. However, further studies are needed to validate the hypothesis.

The gut plays a crucial role in the digestion and absorption of nutrients as well as serving as an important defense barrier against pathogenic factors from the external environment of the gut [41]. Firmicutes were found to be the most abundant phylum in the ileum microbiota of Hu lambs, which is consistent with the findings of Tanca et al. [42]. The relative abundance of potential pathogens such as Proteobacteria, *Erysipelotrichaceae*, *Cutibacterium* and *Gammaproteobacteria* in the Syn group showed a significant decrease. According to the results of the random forest model, we found that compared with the Con group, the relative abundance of *Christensenella* and *Pseudobutyrivibrio* in the Pro group was significantly decreased, while those in the Syn group were significantly increased ($P < 0.05$). *Christensenella* is considered a potential probiotic, while *Pseudobutyrivibrio* can produce butyrate through the fermentation of various carbohydrates, contributing to gut health. Herbinix was significantly elevated in the Syn group, and correlation analysis revealed a significant positive association with average daily gain, suggesting its potential unique functionality that warrants further investigation. Additionally, the abundance of *Pseudoruminococcus* significantly increased in the Syn group, which may help promote the digestion and absorption of cellulose. In summary, the combination of LPN-1 and IMO can modulate the ileal microbiota of Hu sheep by reducing the relative abundance of harmful bacteria and increasing the abundance of beneficial bacteria, thereby promoting the overall health of Hu sheep. Zhang et al. reported that probiotics could enhance the apparent digestibility of nutrients in Holstein calves [34]. Dong et al. [43] found that xylo-oligosaccharides and exogenous enzymes can improve energy use efficiency of Jersey cows. PICRUSt2 functional analysis revealed the enrichment of bacteria that related to digestive system and carbohydrate digestion and absorption in the ileum of Hu lambs in both treatment groups. Moreover, in the Syn group, the ileum of Hu lambs exhibited an enrichment of bacteria that related to amino acids metabolism and cellulolysis. We discovered a significant increase in the content of amino acids in Syn group Hu mutton via mutton quality detection [44], which was consistent with the predicted results. These findings suggest that LPN-1 and IMO can enhance the proliferation of bacteria that aid in digestion function in the ileum, which could potentially promote the growth of Hu lambs.

Short-chain fatty acids (SCFAs) are crucial for the growth and development of pre-weaning Hu lambs. SCFAs cannot only serve as substrates for energy production, lipogenesis, gluconeogenesis, and cholesterol synthesis to promote growth and weight gain [45], but they are also essential for the development of gut barrier function in lambs [12, 46]. They help enhance gut integrity and reduce the risk of diarrhea and infections. As the length of carbon chains varies among different SCFAs, they perform varied functions. Among them, acetate is a practical physiological fuel, which can be directly utilized by peripheral tissues for Hu lambs [47]. It can also serve as a precursor for fat synthesis, being converted into acetyl-CoA to participate in fat metabolism [45, 48], and thereby enhancing body fat deposition of animals. Propionate can be absorbed by epithelial cells and then transported to the liver for glycogen synthesis [49]. Some microbes in the gut can used both lactate and acetate to synthesize butyrate [50]. Butyrate is the primary energy source for colon epithelial cells, enhancing gut barrier function, maintaining gut mucosal integrity, and alleviating inflammatory bowel diseases [48, 51]. Current research on valerate is limited, Onrust et al. [52] found that valerate can improve broiler growth performance, significantly reduce the incidence of necrotic enteritis, and has the potential to maintain gut health. In summary, we found that LPN-1 could significantly increase valerate content in the ileum of Hu lambs, thereby indirectly

maintaining gut health and reducing the occurrence of diarrhea. The combination of LPN-1 and IMO could significantly increase acetate content in the ileum of Hu lambs ($P<0.05$), thereby supplying the host with more energy, improving the growth performance of lambs, and promoting body fat deposition [44]. Butyrate plays a crucial role in the gastrointestinal development, immune function of newborn Hu sheep [53]. Our previous studies have found that LPN-1 can effectively increase gut butyrate levels [13, 14]. However, in this study, we did not observe a positive impact of LPN-1 or its combination with IMO on ileal butyric acid content. This may be related to the decrease in ruminal pH after LPN-1 administration, which led to a reduction in the abundance of butyrate-producing bacteria such as *Butyrivibrio fibrisolvens*.

Previous studies have shown that synbiotics promote the growth and development of chickens and sheep more effectively than the use of probiotics or prebiotics alone [23, 54]. The possible mechanism is that prebiotics help oral probiotics to colonize in the gut, allowing them to work more effectively [55, 56]. Different results were obtained in the experiment with rumen microbiota of Hu lambs, speculating that it may be related to the specific characteristics of the ruminant digestive system. The administration of LPN-1 alone effectively reduced fecal scores and antibiotic use in Hu lambs, increased their feed intake, and promoted rumen development. However, it didn't significantly improve the growth performance of Hu sheep and instead reduced the diversity and richness of the ileal microbiota. In newborn lambs, the gastrointestinal microbiota is not yet fully established. The influx of a large number of *L. plantarum* into the rumen may lead to a decrease in ruminal pH, subsequently reducing the relative abundance of acid-sensitive bacteria such as *Butyrivibrio fibrisolvens* and diminishing cellulase activity [57]. Additionally, a decrease in the relative abundance of methanogens, which play a positive role in fiber degradation, was observed in the rumen of the Pro group ($P<0.05$), potentially leading to reduced rumen fiber degradation efficiency [58]. Previously, Johnstone et al. [59] reported adverse events in critically ill patients administered *L. rhamnosus* GG, including its colonization in sterile sites or dominance in non-sterile niches. Therefore, when administering probiotics to newborn animals with immature gut microbiota, careful attention to dosage is essential. Interestingly, the inclusion of IMO not only reserved the positive effects of LPN-1 on Hu lambs but also mitigated the disruptive impact of LPN-1 on gastrointestinal microbiota. It further enriched functional bacteria associated with carbohydrate metabolism and amino acid metabolism, increased ileal SCFAs content, and thereby significantly enhanced the growth performance of Hu lambs. This study may reveal a novel synbiotic mechanism distinct from previous reports [60, 61], providing valuable insights for the application of probiotics additives in ruminant feed.

To ensure the parallelism of the experiment, we exclusively selected male lambs born within a 5-day window as experimental subjects. In subsequent experiments, we plan to increase the number of animals to enhance the statistical power and validity of the results. Considering the low abundance of gut microbiota in the very limited amount of fecal from the newborn lambs, and the potential stress responses of lambs caused by fecal sampling by human, we did not perform the initial baseline testing. To minimize the impact of initial differences on the experimental results, we selected ewes and newborn lambs from the same breeding farm, and randomly divided them into three groups. Throughout the whole study, all animals were maintained under identical feeding and management protocols. During this study, ewes and lambs were not kept apart. LPN-1 powder and IMO were dissolved in drinking water and administered daily using a 2 mL syringe. Although this method is closer to the farm's traditional breeding approach, it may cause stress to Hu lambs. According to Huang et al. [62], the application of compound probiotics through spraying in the delivery room and piglet activity areas can profoundly alter the microbiota composition in the delivery environment, resulting in significantly improved daily gain and higher weaning weight in piglets. This approach offers a promising solution to the challenge of high breast milk replacer costs and stress experienced by lambs.

Conclusion

This study demonstrated that LPN-1 combined with IMO had an enhanced effect on improving the growth performance and reducing the use of antibiotics in pre-weaning Hu lambs. Accordingly, the combination of LPN-1 and IMO has the potential to be developed into a substitute for antibiotic growth promoters thus reducing the antibiotics dependence and improving the economic benefits of Hu lambs.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42523-025-00392-1.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Acknowledgements

The authors would like to thank Sichuan Wanghu Animal Husbandry for providing the experimental site. Thanks to Qing Li, Yongming Ding, Yuhan Zhang, Qiurui Tian, Jiaqi Huang for their help on sample collecting. Thanks to ChatGPT3.5 and Deepseek for improving the language and readability of this manuscript.

Author contributions
Xinyi Xu: Methodology, Investigation, Visualization, Writing - Original draft preparation. Zhiwei Zhou: Investigation, Visualization, Writing- Reviewing and Editing. Zhiqiang Zhou: Methodology, Investigation. Yudong Ma: Investigation. Dongmei Luo: Investigation. Senlin Zhang: Investigation. Pinggui Yang: Resources. Tianwu An: Methodology, Investigation, Resources, Writing - Reviewing and Editing. Qun Sun: Conceptualization, Methodology, Resources, Writing - Reviewing and Editing. All authors reviewed the manuscript.

Funding
This research was supported by Sichuan Livestock Mutton Innovation Team "Breeding and Demonstration and Promotion of High-quality Sheep Breeds in Tibetan Areas" (SCCXTD-2024-14) and Sichuan Province Science and Technology Program (2022YFQ0100).

Data availability
The sequence data have been submitted to the NCBI sequence reads archive (SRA) under accession number SUB14906953.

Declarations

Ethics approval and consent to participate
The experiment received approval from the Ethics Committee at the College of Life Science, Sichuan University (Project number SCU230319001).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Key Laboratory of Bio-resources and Eco-environment of the Ministry of Education, College of Life Sciences, Sichuan University, Chengdu 610065, P. R. China
[2]College of Biomass Science and Engineering, Sichuan University, Chengdu 610065, P. R. China
[3]Sichuan Academy of Grassland Sciences, Chengdu, P. R. China

Received: 10 December 2024 / Accepted: 2 March 2025
Published online: 12 March 2025

References

1. Su Y, Sun X, Zhao S, et al. Dietary alfalfa powder supplementation improves growth and development, body health, and meat quality of Tibetan sheep. Food Chem. 2022;396:133709. https://doi.org/10.1016/j.foodchem.2022.133709.
2. Wang J, Deng L, Chen M, et al. Phytogenic feed additives as natural antibiotic alternatives in animal health and production: A review of the literature of the last decade. Anim Nutr. 2024;17:244–64. https://doi.org/10.1016/j.aninu.2024.01.012.
3. Perez-Bou L, Gonzalez-Martinez A, Gonzalez-Lopez J, Correa-Galeote D. Promising bioprocesses for the efficient removal of antibiotics and antibiotic-resistance genes from urban and hospital wastewaters: potentialities of aerobic granular systems. Environ Pollut. 2024;342:123115. https://doi.org/10.1016/j.envpol.2023.123115.
4. Ali M, Xu C, Hina Q, Li A, Li K. Interrelations between probiotics, gut microbiota, intestinal barrier, and immune response; focusing on diarrhea in dairy Calves1. J Integr Agric. 2024. https://doi.org/10.1016/j.jia.2024.05.022.
5. Li S, Cui D, Wang S, et al. Efficacy of an herbal granule as treatment option for neonatal Tibetan lamb diarrhea under field conditions. Livest Sci. 2015;172:79–84. https://doi.org/10.1016/j.livsci.2014.11.010.
6. Lovatt F, Duncan J, Hinde D. Responsible use of antibiotics on sheep farms: application at farm level. Practice. 2019;41(1):23–33. https://doi.org/10.1136/inp.k5370.
7. Yue Y, He Z, Zhou Y, et al. Lactobacillus plantarum relieves diarrhea caused by enterotoxin-producing Escherichia coli through inflammation modulation and gut microbiota regulation. Food Funct. 2020;11(12):10362–74. https://doi.org/10.1039/d0fo02670k.
8. Yang KM, Jiang ZY, Zheng CT, Wang L, Yang XF. Effect of Lactobacillus plantarum on diarrhea and intestinal barrier function of young piglets challenged with enterotoxigenic Escherichia coli K88. J Anim Sci, 2014. 92(4): pp. 1496– 503.https://doi.org/10.2527/jas.2013-6619
9. Kim. rRNA Gene-Based identification of Bacteria and Archaea using the EzTaxon server. Methods Microbiol. 2014;61–74. https://doi.org/10.1016/bs.mim.2014.08.001.
10. Yang B, Yue Y, Chen Y, et al. Lactobacillus plantarum CCFM1143 alleviates chronic diarrhea via inflammation regulation and gut microbiota modulation: A Double-Blind, randomized, Placebo-Controlled study. Front Immunol. 2021;12:746585. https://doi.org/10.3389/fimmu.2021.746585.
11. Tian L, Zhao R, Xu X, et al. Modulatory effects of Lactiplantibacillus plantarum on chronic metabolic diseases. Food Sci Hum Wellness. 2023;12(4):959–74. https://doi.org/10.1016/j.fshw.2022.10.018.
12. Liu Y, Wang X, Chen Q, et al. Camellia sinensis and Litsea Coreana ameliorate intestinal inflammation and modulate gut microbiota in dextran sulfate Sodium-Induced colitis mice. Mol Nutr Food Res. 2020;64(6):e1900943. https://doi.org/10.1002/mnfr.201900943.
13. Wei Z, Cui Y, Tian L, et al. Probiotic Lactiplantibacillus plantarum N-1 could prevent ethylene glycol-induced kidney stones by regulating gut microbiota and enhancing intestinal barrier function. Faseb J. 2021;35(11):e21937. https://doi.org/10.1096/fj.202100887RR.
14. Tian L, Liu R, Zhou Z, et al. Probiotic characteristics of Lactiplantibacillus plantarum N-1 and its Cholesterol-Lowering effect in hypercholesterolemic rats. Probiotics Antimicrob Proteins. 2022;14(2):337–48. https://doi.org/10.1007/s12602-021-09886-1.
15. Soto, Carmen. Electron J Biotechnol. 2013;16(4):206–11. https://doi.org/10.2225/vol16-issue4-fulltext-9.
16. Siang SC, Lai KW, Lin NK, Phing PL. Ciência E Tecnologia De Aliment. 2019;39(1). https://doi.org/10.1590/fst.27518.
17. Aftabgard M, Salarzadeh A, Mohseni M, Bahri Shabanipour AH, Zorriehzahra MEJ. The combined efficiency of dietary isomaltooligosaccharides and Bacillus spp. On the growth, Hemato-Serological, and intestinal microbiota indices of Caspian brown trout (Salmo trutta caspius Kessler, 1877). Probiotics Antimicrob Proteins. 2019;11(1):198–206. https://doi.org/10.1007/s12602-017-9361-z.
18. Deng JPWH, Li F, X L. Research progress and application of isomaltooligosaccharide in animal husbandry. Feed Ind. 2020;41(23):5. https://doi.org/10.13302/j.cnki.fi.2020.23.001.
19. Wang XF, Zheng DL, Zhu YL, Liu YF. Effects of oligosaccharides on the morphology and structure of small intestinal mucosa in weaned lambs. Anim Husb Veterinary Med. 2010;42(12):48–50.
20. Li J, Tan B, Mai K. Dietary probiotic Bacillus OJ and isomaltooligosaccharides influence the intestine microbial populations, immune responses and resistance to white spot syndrome virus in shrimp (Litopenaeus vannamei). Aquaculture. 2009;291(1):35–40. https://doi.org/10.1016/j.aquaculture.2009.03.005.
21. Singh DP, Singh J, Boparai RK, et al. Isomalto-oligosaccharides, a prebiotic, functionally augment green tea effects against high fat diet-induced metabolic alterations via preventing gut dysbacteriosis in mice. Pharmacol Res. 2017;123:103–13. https://doi.org/10.1016/j.phrs.2017.06.015.
22. Singh DP, Singh S, Bijalwan V, et al. Co-supplementation of isomalto-oligosaccharides potentiates metabolic health benefits of polyphenol-rich cranberry extract in high fat diet-fed mice via enhanced gut butyrate production. Eur J Nutr. 2018;57(8):2897–911. https://doi.org/10.1007/s00394-017-1561-5.
23. Estrada-Angulo A, Zapata-Ramírez O, Castro-Pérez BI, et al. The effects of single or combined supplementation of probiotics and prebiotics on growth performance, dietary energetics, carcass traits, and visceral mass in lambs finished under subtropical climate conditions. Biology. 2021;10(11):1137. https://doi.org/10.3390/biology10111137.
24. Casper DP, Hultquist KM, Acharya IP. Lactobacillus plantarum GB LP-1 as a direct-fed microbial for neonatal calves. J Dairy Sci. 2021;104(5):5557–68. https://doi.org/10.3168/jds.2020-19438.
25. Schloss PD, Westcott SL, Ryabin T, et al. Introducing Mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol. 2009;75(23):7537–41. https://doi.org/10.1128/aem.01541-09.
26. Segata N, Izard J, Waldron L, et al. Metagenomic biomarker discovery and explanation. Genome Biol. 2011;12(6):R60. https://doi.org/10.1186/gb-2011-12-6-r60.

27. Leistikow KR, Beattie RE, Hristova KR. Probiotics beyond the farm: benefits, costs, and considerations of using antibiotic alternatives in livestock. Front Antibiot. 2022;1:1003912. https://doi.org/10.3389/frabi.2022.1003912.
28. Zhang X, Yi X, Zhuang H, Deng Z, Ma C, Invited Review. Antimicrobial use and antimicrobial resistance in pathogens associated with diarrhea and pneumonia in dairy calves. Anim (Basel). 2022;12(6). https://doi.org/10.3390/ani12060771.
29. Pierce KM, Callan JJ, McCarthy P, O'Doherty JV. Performance of weanling pigs offered low or high lactose diets supplemented with avilamycin or inulin. Anim Sci. 2005;80(3):313–8. https://doi.org/10.1079/ASC40900313.
30. Huang Y, Wang G, Li C, et al. Periodical changes of feces microbiota and its relationship with nutrient digestibility in early lambs. Anim (Basel). 2022;12(14). https://doi.org/10.3390/ani12141770.
31. Guo YQ, Hu YR, Liu SR, et al. Effects of the oat hay feeding method and compound probiotic supplementation on the growth, antioxidant capacity, immunity, and rumen Bacteria community of dairy calves. Antioxid (Basel). 2023;12(10). https://doi.org/10.3390/antiox12101851.
32. Quijada NM, Bodas R, Lorenzo JM, et al. Dietary supplementation with sugar beet fructooligosaccharides and Garlic residues promotes growth of beneficial Bacteria and increases weight gain in neonatal lambs. Biomolecules. 2020;10(8):1179. https://doi.org/10.3390/biom10081179.
33. Jiang X, Xu HJ, Cui ZQ, Zhang YG. Effects of supplementation with Lactobacillus plantarum 299v on the performance, blood metabolites, rumen fermentation and bacterial communities of preweaning calves. Livest Sci. 2020;239:104120. https://doi.org/10.1016/j.livsci.2020.104120.
34. Zhang R, Zhou M, Tu Y, et al. Effect of oral administration of probiotics on growth performance, apparent nutrient digestibility and stress-related indicators in Holstein calves. J Anim Physiol Anim Nutr (Berl). 2016;100(1):33–8. https://doi.org/10.1111/jpn.12338.
35. Wang H, Yu Z, Gao Z, et al. Effects of compound probiotics on growth performance, rumen fermentation, blood parameters, and health status of neonatal Holstein calves. J Dairy Sci. 2022;105(3):2190–200. https://doi.org/10.3168/jds.2021-20721.
36. Mebius RE, Kraal G. Structure and function of the spleen. Nat Rev Immunol, 2005. 5(8): pp. 606– 16.https://doi.org/10.1038/nri1669
37. Cheng L, Zhang W, Jin Q, et al. The effects of dietary supplementation with lotus leaf extract on the immune response and intestinal microbiota composition of broiler chickens. Poult Sci. 2021;100(3):100925. https://doi.org/10.1016/j.psj.2020.12.023.
38. Song D, Li A, Wang Y, et al. Effects of synbiotic on growth, digestibility, immune and antioxidant performance in broilers. Animal. 2022;16(4):100497. https://doi.org/10.1016/j.animal.2022.100497.
39. Zhang J, Xu XF. Research progress of rumen flora in young ruminants. Pratacultural Sci. 2020;37(02):363–9. https://doi.org/10.11829/j.issn.1001-0629.2019-0278.
40. Lin L, Xie F, Sun D, et al. Ruminal microbiome-host crosstalk stimulates the development of the ruminal epithelium in a lamb model. Microbiome. 2019;7(1):83. https://doi.org/10.1186/s40168-019-0701-y.
41. Li Q, Li L, Chen Y, et al. Bacillus licheniformis PF9 improves barrier function and alleviates inflammatory responses against enterotoxigenic Escherichia coli F4 infection in the Porcine intestinal epithelial cells. J Anim Sci Biotechnol. 2022;13(1):86. https://doi.org/10.1186/s40104-022-00746-8.
42. Tanca A, Fraumene C, Manghina V, et al. Diversity and functions of the sheep faecal microbiota: a multi-omic characterization. Microb Biotechnol. 2017;10(3):541–54. https://doi.org/10.1111/1751-7915.12462.
43. Dong L, Zhao L, Li B, et al. Dietary supplementation with xylooligosaccharides and exogenous enzyme improves milk production, energy utilization efficiency and reduces enteric methane emissions of Jersey cows. J Anim Sci Biotechnol. 2023;14(1):71. https://doi.org/10.1186/s40104-023-00873-w.
44. Zhou Z, Xu X, Luo D, et al. Effect of dietary supplementation of Lactiplantibacillus plantarum N-1 and its synergies with oligomeric isomaltose on the growth performance and meat quality in Hu sheep. Foods. 2023;12(9). https://doi.org/10.3390/foods12091858.
45. den Besten G, Lange K, Havinga R, et al. Gut-derived short-chain fatty acids are vividly assimilated into host carbohydrates and lipids. Am J Physiol Gastrointest Liver Physiol. 2013;305(12):G900-10. https://doi.org/10.1152/ajpgi.00265.2013.
46. Mann ER, Lam YK, Uhlig HH. Short-chain fatty acids: linking diet, the Microbiome and immunity. Nat Rev Immunol. 2024;24(8):577–95. https://doi.org/10.1038/s41577-024-01014-8.
47. Yamashita H, Kaneyuki T, Tagawa K. Production of acetate in the liver and its utilization in peripheral tissues. Biochim Et Biophys Acta (BBA) - Mol Cell Biology Lipids. 2001;1532(1):79–87. https://doi.org/10.1016/S1388-1981(01)00117-2.
48. Ma J, Piao X, Mahfuz S, Long S, Wang J. The interaction among gut microbes, the intestinal barrier and short chain fatty acids. Anim Nutr. 2022;9:159–74. https://doi.org/10.1016/j.aninu.2021.09.012.
49. den Besten G, van Eunen K, Groen AK, et al. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. J Lipid Res. 2013;54(9):2325–40. https://doi.org/10.1194/jlr.R036012.
50. Duncan SH, Barcenilla A, Stewart CS, Pryde SE, Flint HJ. Acetate utilization and Butyryl coenzyme A (CoA):acetate-CoA transferase in butyrate-producing bacteria from the human large intestine. Appl Environ Microbiol. 2002;68(10):5186–90. https://doi.org/10.1128/aem.68.10.5186-5190.2002.
51. Cui YQ, Wei ZT, Cao Y, et al. Effects of sodium butyrate on intestinal flora and inflammatory factors in rats with renal calcium oxalate stones. J Sichuan Univ (Natural Sci Edition). 2020;57(04):818–24. https://doi.org/10.3969/j.issn.0490-6756.2020.04.031.
52. Onrust L, Van Driessche K, Ducatelle R, et al. Valeric acid glyceride esters in feed promote broiler performance and reduce the incidence of necrotic enteritis. Poult Sci. 2018;97(7):2303–11. https://doi.org/10.3382/ps/pey085.
53. Liu L, Sun D, Mao S, Zhu W, Liu J. Infusion of sodium butyrate promotes rumen papillae growth and enhances expression of genes related to rumen epithelial VFA uptake and metabolism in neonatal twin lambs. J Anim Sci. 2019;97(2):909–21. https://doi.org/10.1093/jas/sky459.
54. Awad WA, Ghareeb K, Abdel-Raheem S, Böhm J. Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights, and intestinal histomorphology of broiler chickens. Poult Sci. 2009;88(1):49–56. https://doi.org/10.3382/ps.2008-00244.
55. Frece J, Kos B, Svetec IK, et al. Synbiotic effect of Lactobacillus helveticus M92 and prebiotics on the intestinal microflora and immune system of mice. J Dairy Res. 2009;76(1):98–104. https://doi.org/10.1017/S0022029908003737.
56. Abd El-Hack ME, El-Saadony MT, Shafi ME, et al. Probiotics in poultry feed: A comprehensive review. J Anim Physiol Anim Nutr. 2020;104(6):1835–50. https://doi.org/10.1111/jpn.13454.
57. Zhao PT, Liu DC, Gao M, et al. Effects of different dietary NFC/NDF on the contents of Vibrio fibrobutyricoides, Streptococcus bovis and Megalococcus ebergii in rumen of dairy goats. Chin J Anim Nutr. 2011;23(10):1716–24. https://doi.org/10.3969/j.issn.1672-5190.2011.09.019.
58. Stumm CK, Gijzen HJ, Vogels GD. Association of methanogenic bacteria with ovine rumen ciliates. Br J Nutr. 1982;47(1):95–9. https://doi.org/10.1079/BJN19820013.
59. Johnstone J, Meade M, Lauzier F, et al. Effect of probiotics on incident Ventilator-Associated pneumonia in critically ill patients: A randomized clinical trial. JAMA. 2021;326(11):1024–33. https://doi.org/10.1001/jama.2021.13355.
60. Ling W, Sha-sha T, Rong S, Bin L. Synergistic effect of Konjac Oligosaccharides/Isomalto-oligosaccharide complex on the growth of Lactobacillus acidophilus modern food. Sci Technol. 2015;31(10):151–5. https://doi.org/10.13982/j.mfst.1673-9078.2015.10.026.
61. Ouwehand AC, Tiihonen K, Mäkivuokko H, Rautonen N. 9 - Synbiotics: combining the benefits of pre- and probiotics, in Functional Dairy Products, M. Saarela, Editor. 2007, Woodhead Publishing. pp. 195–213. https://doi.org/10.1533/9781845693107.2.195
62. Huang W, Ma T, Liu Y, et al. Spraying compound probiotics improves growth performance and immunity and modulates gut microbiota and blood metabolites of suckling piglets. Sci China Life Sci. 2022. https://doi.org/10.1007/s11427-022-2229-1.

Publisher's note