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RESEARCH ARTICLE

Revealing the Fungi Microbiome Difference of Suffolk Cross with Tibetan Sheep on Plateau

Yue Ren[1,2*] Yangzhong Zhaxi[1,2], Mengjun Liu[1,2], Asif Idrees[3] and Kun Li[4,5]

[1]Institute of Livestock Research, Tibet Academy of Agricultural and Animal Husbandry Sciences, Lhasa 850000, China
[2]Key Laboratory of Animal Genetics and Breeding on Tibetan Plateau, Ministry of Agriculture and Rural Affairs, China
[3]KBCMA, College of Veterinary and Animal Sciences, Narowal, Pakistan
[4]Institute of Traditional Chinese Veterinary Medicine, College of Veterinary Medicine, Nanjing Agricultural University, Nanjing 210095, PR China
[5]MOE Joint International Research Laboratory of Animal Health and Food Safety, College of Veterinary Medicine, Nanjing Agricultural University, Nanjing 210095, PR China
*Corresponding author: renyue34@126.com (Yue Ren)

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ABSTRACT

Sheep plays a vital role as ruminant animal, which contributes to human needs by supplying milk, mutton and cashmere. As lifestyle of people is improved, there is an increasing need for mutton and its products. Fungi are important component of the microbiota and may affect immune and inflammatory responses, digestion, and nutrients absorption. However, little information is available about the fungal microbiota in sheep on the plateau. Forty-five sheep were divided into five groups equally (n = 9) (AL, BL, CL, DL and EL), sheep in CL, DL and EL groups were given grain feeds twice daily. Sheep in AL were fed with alfalfa and oat grasses, and BL was offered with equal amount of alfalfa grass, oat grass and grain feeds. Samples of rumen fluid were collected from sheep for microbiome analysis after a four-month raising period. The microbiota analysis of collected rumen fluid samples revealed 2 333 664 filtered sequences and 3810 amplicon sequence variants (ASVs). Alpha diversity analysis showed obvious different chao1, Shannon_entropy and Simpson in sheep. A total of 7 phyla and 56 genera including potential beneficial fungi like *Penicillium*, *Torula*, *Filobasidium*, *Tomentella* and pathogenic *Rhodotorula,* etc. were significantly different in sheep. Our findings provide new insights of the diverse diets on the fungal microbiome of sheep and contribute for better feeding practices of sheep reared on the plateau.

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INTRODUCTION

Sheep plays a vital role as ruminant animal, which contributes to human needs by supplying milk, mutton and cashmere (Purgatorio *et al.,* 2022). As lifestyle of people is improved, there is an increasing need for mutton and its products. In plateau regions of China, ruminants like yaks and sheep are critical important food resources for local resident and travelers (Guo *et al.,* 2020; Lu *et al*., 2023). Therefore, efficient breeding of these food animals is necessary and meaningful to fulfil the need of people at plateau (Lan *et al.,* 2021).

Mammals are evolved in such environment that is dominated by microbes (Cholewińska *et al.,* 2020). The amount and variety of microbes with their functions and metabolites have important impression on animals (Zhang et al., 2024). These microbes are composed of numerous microorganisms including fungi, protists, bacteria, viruses and archaea (Martino *et al.,* 2022). Among them, particular attention was paid to intestine microbiota due to its confidant relationship with host health (Goyal *et al.,* 2021; Zhang *et al*., 2024). A prior study indicated that gut microbiota was greatly involved in digestion and absorption of nutrients, intestinal barrier integrity, and immunity (Goyal *et al.,* 2021; Ren *et al.,* 2023). In addition, the anaerobic fungi in the rumen of ruminant are important for degrading cellulose and hemicellulose to promote digestion and first stomach function (Li *et al.,* 2022). Besides bacteria, fungi also occupy an integral part of the microbiota (Dong *et al.,* 2023). The host gut microbiota could influence the inflammatory and immunological responses, interact with bacteria, regulate homoeostasis and physiology (Cholewińska *et al.,* 2020). The dynamic microbiota could be affected by diet types, age, antibiotics,

and diseases. Previous studies reported fungal microbiota dysbiosis in colorectal cancer, Crohn's disease, diarrheal ruminants (Lei *et al.,* 2023).

Due to the development of techniques for metagenomic sequencing, microbiota of intestine, skin, reproductive tract and other parts from host were deeply understood (Han *et al.,* 2017; Lei *et al*., 2023). Diet significantly influences microbiota composition; animals fed various forages exhibit distinct gut microbiota profiles. (Ogu and Madukwe, 2021; Tulu UT, 2022; Dong *et al.,* 2023). However, limited knowledge is available about the fungal microbiota of plateau sheep with different feeds. Therefore, we conducted this research to analyze the difference of fungi microbiome in Suffolk cross with Tibetan sheep consuming diverse forage grasses.

MATERIALS AND METHODS

Experiment design: Three months old plateau cross (PC) sheep (n=45) were brought from a local farm in Lhasa and acclimatized for three days. Afterwards, the sheep were divided into five experimental groups (n=9) equally (AL, BL, CL, DL and EL), sheep in CL, DL and EL groups were given grain feeds twice daily. Oat grass and Alfalfa feeds were given to sheep in group AL, while BL group was offered with equal amount of alfalfa grass, oat grass and grain feeds. The sheep were raised for four months, and after that rumen fluid samples were collected from sheep for further study.

Microbiome sequencing of cross sheep: Commercial stool genomic DNA extraction kits (Solarbio Science & Technology Co., Ltd, China) were employed to extract DNA samples from rumen fluid of PC sheep. Then the qualitative and quantitative examination of all the DNA products from PC sheep was performed via NanoDrop One Microultraviolet-visible spectrophotometer and 0.8% agarose gel electrophoresis (Thermo Scientific, USA), respectively as per previously reported study (He *et al.,* 2023). The target region of the total internal transcribed spacer in all PC sheep were amplified using universal primers pairs of ITS1 (F: 5'-CTTGGTCATTTAGAG GAAGTAA3'; R:5'-GCTGCGTTCTTCATCGATGC3') (Monard *et al.,* 2013). Then, the products were used for library construction utilizing Hieff NGS® OnePot II DNA Library Prep Kit for Illumina® (Yeasen, China). Finally, all the library products were used for sequencing through Illlumina HiSeq platform.

Microbiome analysis of cross sheep: All the raw sequences of PC sheep were first filtered by DADA2 to generate ASVs, and then used to produce taxonomy table via aligning with database of unite (Nilsson *et al.,* 2019; Barnes *et al.,* 2020). Diversities of alpha indexes (Shannon, Chao1, Faith's phylogenetic and observed OTUs) and beta indexes (unweighted pair-group method with nonmetric multidimensional scaling, principal coordinate analysis and arithmetic means) were calculated (Zhang *et al.,* 2023). The distinguished fungi among the five sheep groups were examined via ANOVA, DEseq2 (Shanmugam *et al.,* 2021). The fungi microbiota function of KEGG Ortholog in different PC sheep groups was estimated using PICRUSt (Ijoma *et al.,* 2021).

Bioinformatics analysis: The "Atacama soil microbiome tutorial" of Qiime2docs, along with customized program scripts was followed for the analysis. The first step included bringing in the raw data FASTQ files into a format compatible with QIIME2 using the qiime tools import program. After demultiplexing, the sequences from each sample underwent quality filtering, trimming, denoising, merging, and removal of chimeric sequences using the QIIME2 dada2 plugin, resulting in the creation of the feature table of ASVs. The QIIME2 feature-classifier plugin was employed to align ASV sequences to a pre-trained GREENGENES 13_8 99% database to create the taxonomy table. Contaminating mitochondrial and chloroplast sequences were then filtered using the QIIME2 feature-table plugin. Various methods, including ANCOM, ANOVA, Kruskal Wallis, LEfSe, and DEseq2, were used to identify fungi with different abundance among samples and groups. The core-diversity plugin within QIIME2 was utilized to calculate diversity metrics. Alpha diversity indices were computed to evaluate microbial diversity within individual samples. Beta diversity distance measurements, including Bray Curtis, unweighted UniFrac, and weighted UniFrac, were employed to investigate structural variations in microbial communities across samples, visualized through principal coordinate analysis (PCoA) and nonmetric multidimensional scaling (NMDS). Additionally, potential functional profiles of microbial communities in terms of KEGG Orthologs (KO) were predicted using PICRUSt.

Statistical analysis: All the results of PC sheep data were analyzed by employing ANOVA and Dunn's test via IBM SPSS (26.0). Data depicted as means ± SD and statistically significant is recognized when $P < 0.05$.

RESULTS

Sequencing data in PC sheep in different groups: In the PC sheep groups, more obtained raw and filtered sequences were found in AL (668 451, 557 081) and BL (672 660, 568 649), while less data was achieved in CL (472 093, 388 405), DL (459 815, 397 541) and EL (492 936, 421 988) (Table 1). Those sequences were aligned to 3810 ASVs (AL=488, BL=574, CL=880, DL=836 and EL=988), with 44 shared ASVs among the five PC sheep groups. Group AL shared 165, 187, 73 and 179 ASVs with BL, CL, DL and EL, respectively (Fig 1a). The distribution of ASVs in phylum (AL=5, BL=5, CL=6, DL=6 and EL=6), class (AL=12, BL=11, CL=14, DL=14 and EL=15), order (AL=64, BL=55, CL=79, DL=68 and EL=86), family (AL=104, BL=101, CL=155, DL=129 and EL=159) and genus (AL=144, BL=139, CL=224, DL=200 and EL=256) are shown in Fig 1b.

Comparing analysis of the fungal microbiota in PC sheep: Alpha diversity analysis showed that chao1 in EL was obviously higher than BL ($P<0.01$). Shannon entropy in CL, DL and EL were observably higher. Simpson in DL was significantly higher than AL ($P<0.01$) and BL ($P<0.05$) (Fig 2). Beta diversity analysis did not reveal

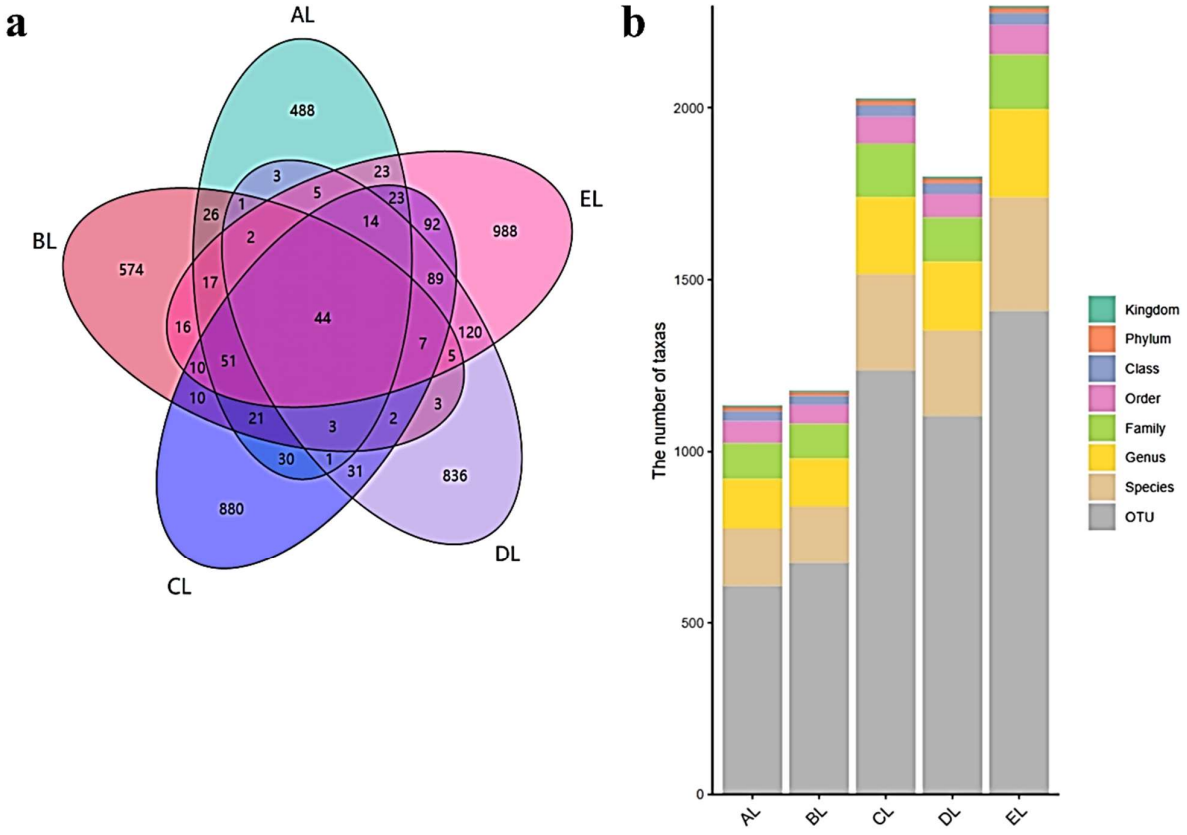

Fig. 1: Analysis of sequencing data in different sheep groups. a: Venn map, b; Annotation statistics.

Table 1: The sequencing data in PC sheep.

Sample ID	Input	Filtered	Percentage of input passed filter	Denoised	Merged	Percentage of input merged	Non-chimeric	Percentage of input non-chimeric
AL1	132279	111866	84.57	111111	89191	67.43	89186	67.42
AL2	134292	113579	84.58	113135	94007	70.00	93524	69.64
AL3	147377	119048	80.78	118303	92717	62.91	92653	62.87
AL4	122169	100985	82.66	97657	81845	66.99	81385	66.62
AL5	132334	111603	84.33	109214	88139	66.60	88074	66.55
BL1	135916	115482	84.97	114354	102214	75.20	101594	74.75
BL2	135334	113864	84.14	112614	97535	72.07	96876	71.58
BL3	134571	114186	84.85	112916	95573	71.02	95285	70.81
BL4	132751	112672	84.87	111645	100039	75.36	98595	74.27
BL5	134088	112445	83.86	111899	92786	69.20	90777	67.70
CL1	131682	98168	74.55	95803	82087	62.34	81259	61.71
CL2	77417	65042	84.02	61903	56769	73.33	56054	72.41
CL3	102163	85785	83.97	83690	75140	73.55	74939	73.35
CL4	107622	94745	88.03	93692	89996	83.62	86015	79.92
CL5	53209	44665	83.94	43949	40598	76.3	40405	75.94
DL1	162941	141182	86.65	140255	116210	71.32	114038	69.99
DL2	74848	66253	88.52	65059	62574	83.6	61503	82.17
DL3	55219	47005	85.12	46492	45840	83.01	45352	82.13
DL4	88979	74637	83.88	73702	72046	80.97	70788	79.56
DL5	77828	68464	87.97	67322	61462	78.97	60333	77.52
EL1	132389	111344	84.10	108634	99355	75.05	98247	74.21
EL2	60459	52124	86.21	51378	48443	80.13	45882	75.89
EL3	80181	69505	86.69	68253	60307	75.21	59086	73.69
EL4	75320	65087	86.41	64341	61231	81.29	60089	79.78
EL5	144587	123928	85.71	122954	108353	74.94	108181	74.82

obvious difference among PC sheep groups; however, group distance analysis found dramatically difference between AL and BL ($P<0.05$), CL ($P<0.05$), DL ($P<0.01$) and EL ($P<0.05$), respectively. Likewise, the distance between BL and CL ($P<0.05$), DL ($P<0.01$) and EL ($P<0.01$) was memorably longer (Fig 3).

Next, we examined the alteration in microbiota composition in different taxa. At the phylum level, the main phyla in AL and BL were Neocallimastigomycota (AL=74.46%, BL=64.08%), Ascomycota (AL=6.15%, BL=4.13%) and Chytridiomycota (AL=1.94%, BL=6.68%), while Ascomycota (CL=30.72%, DL=32.30%, EL=37.95%), Neocallimastigomycota (CL=30.49%, DL=6.35%, EL=16.92%) and Basidiomycota (CL=14.10%, DL=23.60%, EL=17.30%) were the primary phyla in CL, DL and EL groups (Fig 4a). At the class level, the main classes in group AL and group BL were Neocallimastigomycetes (AL=74.46%,

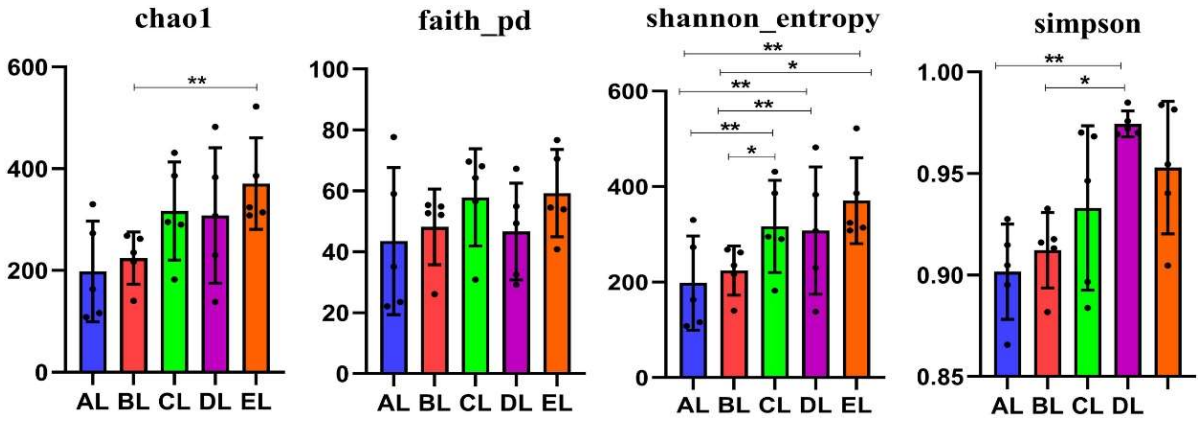

Fig. 2: Comparing analysis of alpha diversity index in PC sheep.

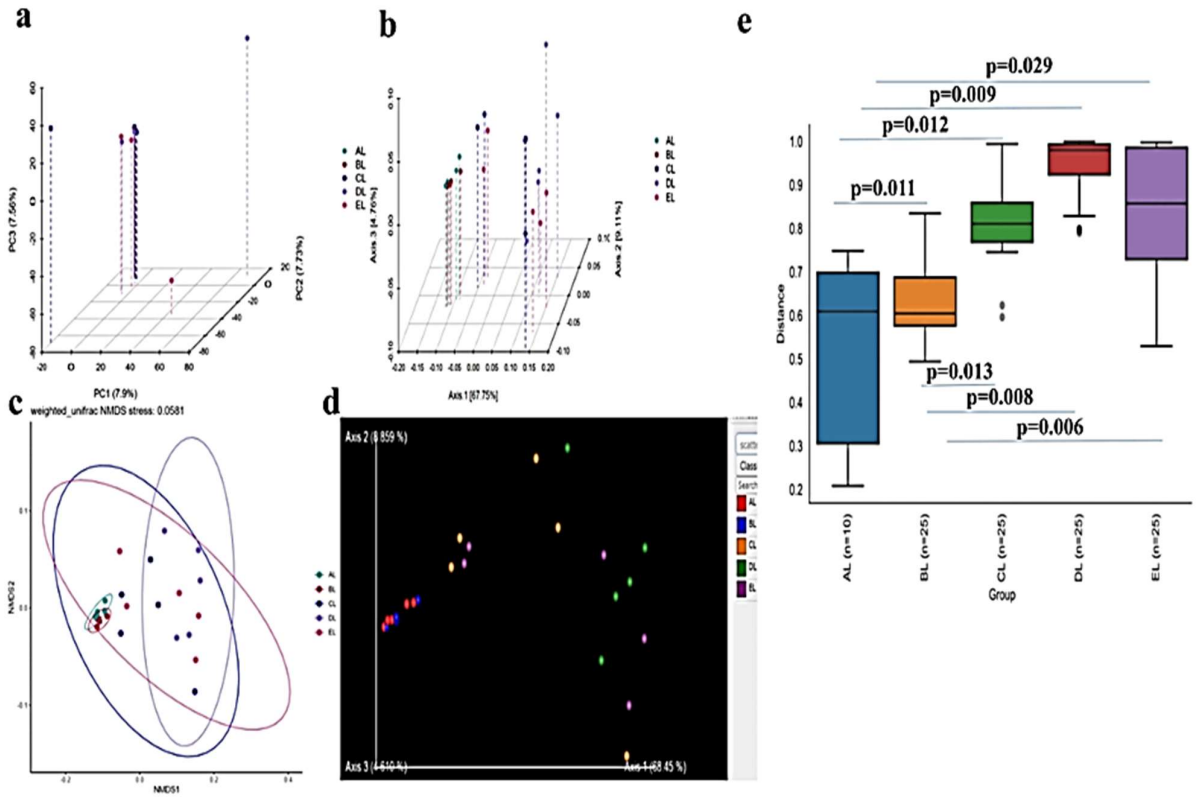

Fig. 3: Comparing analysis of beta diversity index in the ST sheep groups. a: PCA; b: PCoA, c: NMDA, d: qiime2.

BL=64.08%), Saccharomycetes (AL=3.67%, BL=1.20%) and Sordariomycetes (AL=0.82%, BL=1.00%). The classes of Neocallimastigomycetes (30.49%), Agaricomycetes (11.87%) and Eurotiomycetes (9.18%) were dominated in CL, Eurotiomycetes (12.71%), Agaricomycetes (10.19%) and Tremellomycetes (8.76%) were staple in DL, and Neocallimastigomycetes (16.92%), Agaricomycetes (10.58%) and Dothideomycetes (10.16%) were prime in EL (Fig 4b). At the order level, the staple orders found in PC sheep were Neocallimastigales (74.46%), Saccharomycetales (3.67%), and Hypocreales (0.43%) in AL, Neocallimastigales (64.08%), Saccharomycetales (1.20%), and Pleosporales (0.53%) in BL, Neocallimastigales (30.49%), Eurotiales (8.93%) and Pleosporales (5.84%) in group CL, while Eurotiales (12.42%), Neocallimastigales (6.35%) and Hypocreales (5.37%) in DL, and Neocallimastigales (16.92%), Pleosporales (9.18%) and Saccharomycetales (9.05%) in EL (Fig 4c). At the family level, the dominating families in each group were Neocallimastigaceae (74.46%), Debaryomycetaceae (2.99%) and Phaffomycetaceae (0.58%) in AL, Neocallimastigaceae (64.08%), Debaryomycetaceae (0.92%) and Trichocomaceae (0.39%) in BL, Neocallimastigaceae (30.49%), Aspergillaceae (7.60%) and Sebacinaceae (4.42%) in CL, Aspergillaceae (10.46%), Neocallimastigaceae (6.35%) and Sebacinaceae (4.11%) in DL, and Neocallimastigaceae (16.92%), Aspergillaceae (7.00%) and Phaffomycetaceae (6.57%) in EL (Fig 4d). At the genera level, the main genera in PC sheep were *Caecomyces* (40.41%), *Piromyces* (8.95%) and *Pecoramyces* (6.03%) in AL, *Piromyces* (28.37%),

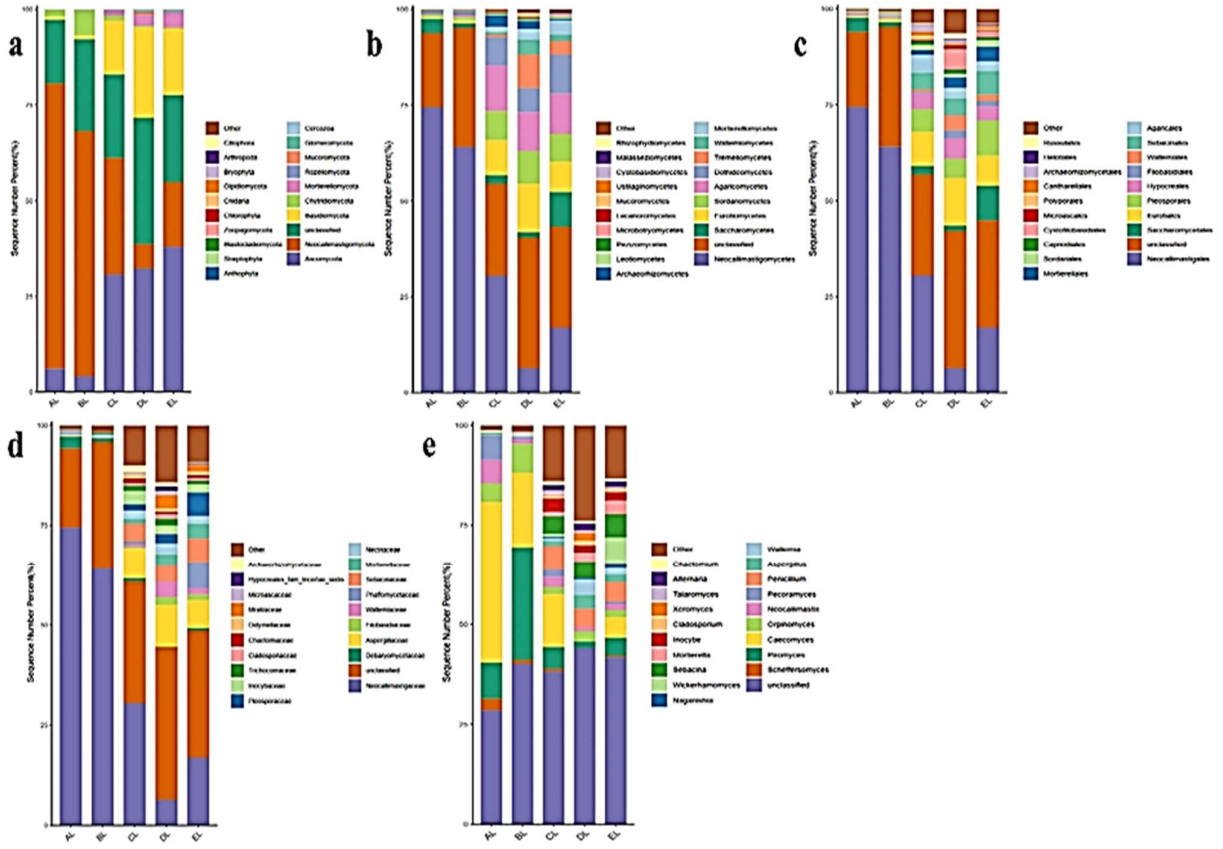

Fig. 4: Microbiota structure analysis of PC sheep at different taxa. a: Phylum, b: Class, c: Order, d: Family, f: Genera.

Caecomyces (18.69%) and *Orpinomyces* (7.32%) in BL, *Caecomyces* (13.24%), *Penicillium* (6.12%) and *Piromyces* (5.38%) in CL, *Penicillium* (4.63%), *Sebacina* (4.10%) and *Wallemia* (3.91%) in DL, and *Wickerhamomyces* (6.57%), *Sebacina* (5.88%) and *Caecomyces* (5.13%) in EL (Fig 4e).

Detection of distinguished fungi species in PC sheep in different groups: The analysis of DESeq2 Volcano map indicted that compared with the abundance of phyla in group AL, Basidiomycota (P<0.05) and Mortierellomycota (P<0.05) were remarkably higher in CL, Mortierellomycota (P<0.001), Basidiomycota (P<0.001), Mucoromycota (P<0.05) and Glomeromycota (P<0.05) were meaningfully higher in DL, and Mortierellomycota (P<0.0001), Basidiomycota (P<0.001) and Glomeromycota (P<0.05) were significantly higher in EL. Whereas, the abundance of Chytridiomycota (P<0.05) and Neocallimastigomycota (P<0.05) were significantly lower in CL, Neocallimastigomycota (P<0.0001), Chytridiomycota (P<0.001) and Anthophyta (P<0.05) were markedly lower in DL, and Chytridiomycota (P<0.0001) and Neocallimastigomycota (P<0.001) were also significantly lower in EL, respectively (Fig 5a). Compared with the abundance of genus in AL, *Penicillium* (P<0.0001), *Inocybe* (P<0.0001), *Sebacina* (P<0.0001), *Mortierella* (P<0.0001), *Clavulina* (P<0.0001), *Lophiostoma* (P<0.0001), *Dolichousnea* (P<0.001), *Ilyonectria* (P<0.001), *Candida* (P<0.01), *Leccinum* (P<0.01), *Alternaria* (P<0.01), *Fusarium* (P<0.01), *Torula* (P<0.01), *Gibberella* (P<0.01), *Melanogaster* (P<0.01), *Trichoderma* (P<0.05), *Hirsutella* (P<0.05), *Xeromyces* (P<0.05), *Scopulariopsis* (P<0.05), *Aspergillus* (P<0.05), *Tausonia* (P<0.05), *Amphinema* (P<0.05) and *Archaeorhizomyces* (P<0.05) were significantly higher in CL, *Xeromyces* (P<0.0001), *Tausonia* (P<0.0001), *Mortierella* (P<0.0001), *Saitozyma* (P<0.0001), *Sarocladium* (P<0.0001), *Aspergillus* (P<0.0001), *Kazachstania* (P<0.0001), *Candida* (P<0.0001), *Rhizopus* (P<0.0001), *Penicillium* (P<0.0001), *Alternaria* (P<0.0001), *Pithoascus* (P<0.0001), *Sebacina* (P<0.0001), *Thermomyces* (P<0.001), *Itersonilia* (P<0.001), *Malassezia* (P<0.001), *Filobasidium* (P<0.001), *Inocybe* (P<0.001), *Lophiostoma* (P<0.001), *Acremonium* (P<0.001), *Wallemia* (P<0.001), *Ilyonectria* (P<0.001), *Russula* (P<0.001), *Cladosporium* (P<0.001), *Monascus* (P<0.01), *Clonostachys* (P<0.01), *Xenopolyscytalum* (P<0.01), *Fusarium* (P<0.01), *Clavulina* (P<0.01), *Sporobolomyces* (P<0.01), *Leccinum* (P<0.05), *Glomus* (P<0.05), *Entoloma* (P<0.05), *Melanogaster* (P<0.05), *Amphinema* (P<0.05) and *Clavaria* (P<0.05) were markedly higher in DL, and *Mortierella* (P<0.0001), *Sebacina* (P<0.0001), *Itersonilia* (P<0.0001), *Kazachstania* (P<0.0001), *Penicillium* (P<0.001), *Lophiostoma* (P<0.001), *Alternaria* (P<0.001), *Malassezia* (P<0.001), *Inocybe* (P<0.001), *Tausonia* (P<0.001), *Gibberella* (P<0.001), *Clavulina* (P<0.001), *Ilyonectria* (P<0.01), *Fusariella* (P<0.01), *Leccinum* (P<0.01), *Aspergillus* (P<0.01), *Hirsutella* (P<0.01), *Saitozyma* (P<0.01), Glomus (P<0.05), *Clavaria* (P<0.05), *Cladosporium* (P<0.05), *Thermomyces* (P<0.05), *Pichia* (P<0.05), *Monascus* (P<0.05), *Xeromyces* (P<0.05),

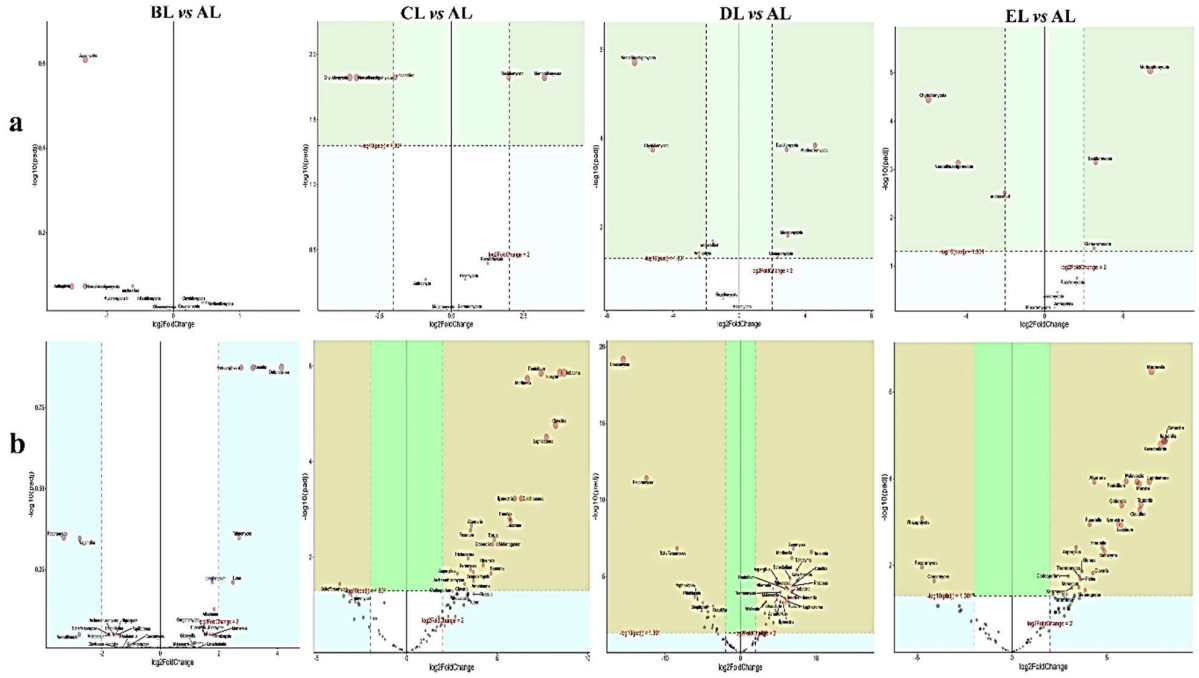

Fig. 5: Examination of distinguished fungi species among the ruminant groups by DESeq2 Volcano map a: Phylum, b: Genera.

Melanogaster (P<0.05), *Tomentella* (P<0.05), *Wallemia* (P<0.05), *Ustilaginoidea* (P<0.05) and *Amphinema* (P<0.05) were significantly higher in EL. Whereas, the abundance of *Scheffersomyces* (P<0.05) was significantly lower in CL, *Caecomyces* (P<0.0001), *Pecoramyces* (P<0.0001), *Scheffersomyces* (P<0.0001), *Phlebiopsis* (P<0.001), *Hyphopichia* (P<0.001), *Simplicillium* (P<0.001), *Occultifur* (P<0.001), *Rhizophlyctis* (P<0.001), *Setophoma* (P<0.01), *Curvularia* (P<0.01), *Hannaella* (P<0.01), *Sporisorium* (P<0.01), *Wongia* (P<0.01), *Galerella* (P<0.05), *Neocallimastix* (P<0.05), *Rhodotorula* (P<0.05), *Achroiostachys* (P<0.05), *Keissleriella* (P<0.05) and *Dokmaia* (P<0.05) were obviously lower in DL, and *Rhizophlyctis* (P<0.001), *Pecoramyces* (P<0.05), *Caecomyces* (P<0.05), *Occultifur* (P<0.05) and *Scheffersomyces* (P<0.05) were remarkably lower in EL (Fig 5b).

Network analysis and functional prediction of fungal microbiota in PC sheep: Network analysis indicated that phyla of Ascomycota, Glomeromycota, Basidiomycota, Mortierellomycota, Olpidiomycota, Arthropoda and Nematoda were positively contributed to microbiota in the sheep, while Blastocladiomycota, Chytridiomycota and Neocallimastigomycota were negatively phyla (Fig 6a). Similarly, genus of *Caecomyces*, *Piromyces*, *Orpinomyces*, *Neocallimastix*, *Pecoramyces*, *Wickerhamomyces*, *Scheffersomyces* and *Phlebiopsis* were negatively related to the sheep microbiota, while *Penicillium*, *Sebacina*, *Inocybe*, *Mortierella*, *Aspergillus*, *Wallemia*, *Alternaria*, *Tausonia*, *Archaeorhizomyces*, *Fusarium*, *Talaromyces*, *Cladosporium*, *Xeromyces* and *Naganishia* were positive genera (Fig 6b).

Functional prediction revealed 34 obvious different metaCys pathways among the five PC sheep groups, which including 4-amino-2-methyl-5-phosphomethyl-pyrimidine biosynthesis, chitin degradation to ethanol, D-galactose degradation V, fatty acid beta-oxidation V, etc., (Fig 7).

DISCUSSION

Due to the continuous revival in living standards of the people, high quality meat and milk products are becoming popular and demanding (Dixit *et al.,* 2023). Sheep is an important food animal, and finding efficient feeds for this ruminant on the plateau is necessary and meaningful. In this study we compared the fungal microbiota in plateau sheep with different feeds. 2,765,955 raw data and 2,333,664 filtered sequences were generated in PC sheep. Though higher sequences obtained in AL and BL, less ASVs were aligned in these two groups. These results were in line with alpha diversity analysis with higher indexes of chao1, Shannon_entropy and Simpson in sheep fed with grain feeds (Guo *et al.,* 2020; Undugoda and Kannangara, 2022.). The current results is not in accordance to the findings in dairy cattle in different feeds with similar alpha diversity (Li *et al.,* 2020), but partly in agreement with beef cattle with different diets (Cui *et al.,* 2022).

Beta diversity analysis found significant difference the group distance between AL and BL (P<0.05), CL (P<0.05), DL (P<0.01) and EL (P<0.05), respectively. Similarly, the distance between BL and CL (P<0.05), DL (P<0.01) and EL (P<0.01) was memorably longer, which were in keeping with the different microbiota structures among the PC ruminant groups different taxa. The different fungi microbiota eventually affected their functions in PC animals with 34 obvious different metaCys pathways.

The ratio of Basidiomycota/Ascomycota in AL (0.11) and BL (0.22) was also lower than CL (0.46), DL (0.73) and EL (0.46), which indicated the microbiota shifts in host (Acar *et al.,* 2023). Further, we explored the

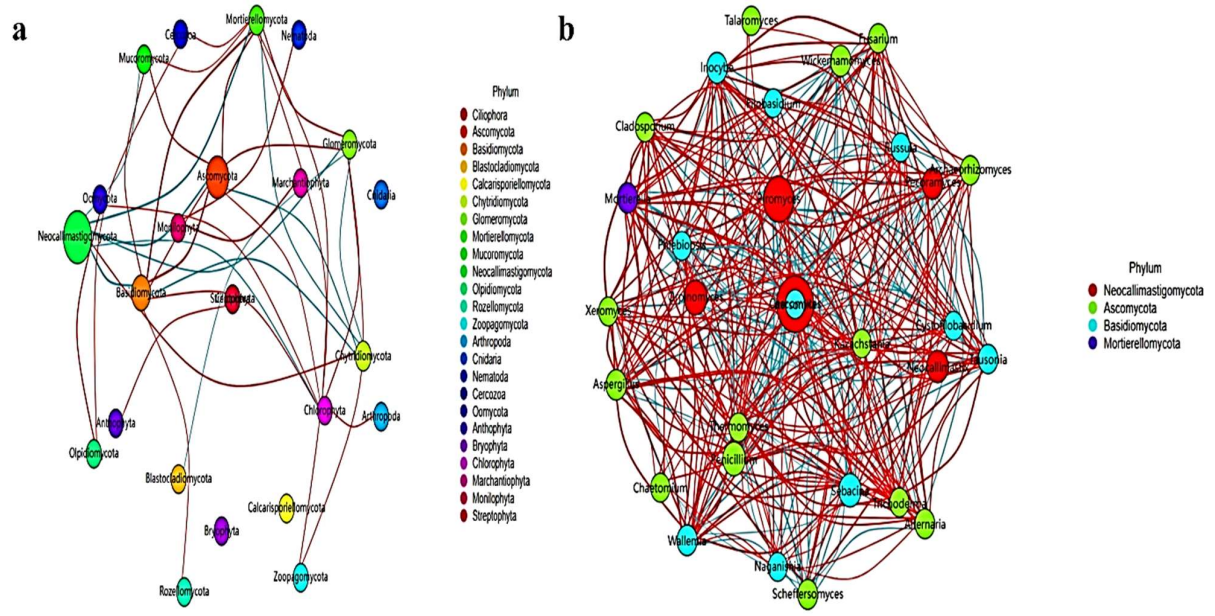

Fig. 6: Network analysis of fungi microbiome of PC sheep. a: Phylum, b: Genera.

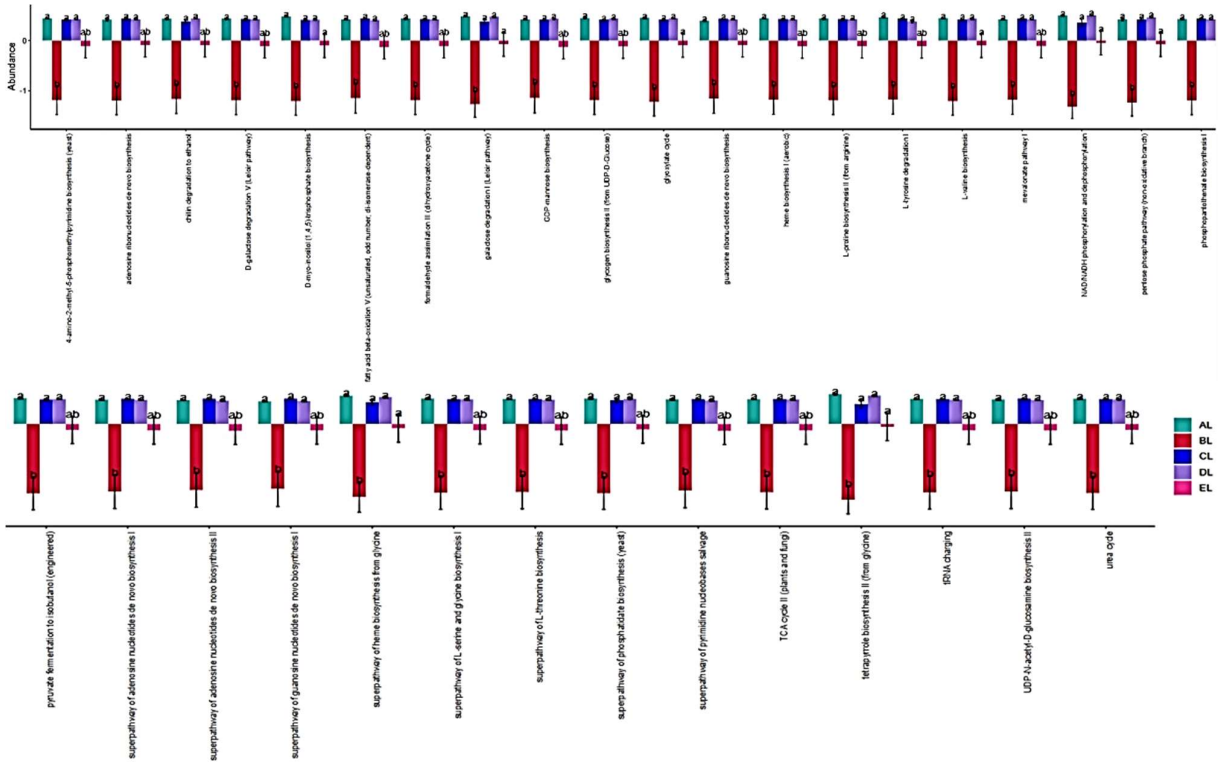

Fig. 7: Microbiota function comparing analysis of metaCys pathways in PC sheep.

distinguished fungal species among the PC sheep groups and found 7 phyla and 56 genera via DESeq2 Volcano map. A greater occurrence of phyla Chytridiomycota, Neocallimastigomycota, Bryophyta, Chlorophyta, Olpidiomycota, Cnidaria, Anthophyta, Rozellomycota, Ascomycota, Cercozoa and Blastocladiomycota were found in sheep feeding on various pasture and concentrates (Ren *et al.,* 2023).

Among genera, *Penicillium* was closely related to intestinal health and a previous study found lower abundance of this genera in mice exposed to fluoride (Cao *et al.,* 2020), lower level of *Mortierella* was reported in diarrheal yaks caused by *Cryptosporidium parvum* (Lu *et al.,* 2023), and lower abundance of *Aspergillus* was reported in Parkinson's disease (Weis *et al.,* 2021). The higher abundance of those genera in CL, DL and EL may indicate that grain foods could make positive contrition to the health of PC sheep. *Torula* can maintain the intestinal health and enhance immunity in pigs (Espinosa *et al.,* 2023), the greater occurrence of this genera in CL showed

that grain feed could improve intestinal health in PC sheep. Previous study reported that the metabolites of *Trichoderma* have anti-inflammatory, antimicrobial and anticancer responses (Li *et al.,* 2019), the greater occurrence of this genera in CL may showed that this feed could decrease intestine inflammation in ruminants. *Saitozyma* is negatively related to gastric carcinogenesis (Zhong *et al.,* 2021), and *Thermomyces* is strongly associated with weight gain in mice (Mims *et al.,* 2021). The greater occurrence of these two genera in DL and EL indicate that concentrated foods may promote health and growth in PC sheep. Genera includes *Spor, Pichia, Rhodotorula, Cordyceps, Kazachstania* and *Xeromyces,* were found higher in animals feeding on concentrates (Ren *et al.,* 2023). *Filobasidium* was positively related to the remission of ulcerative colitis (Van Thiel *et al.,* 2022), and the abundance of *Sporobolomyces* was negatively related to ulcerative colitis (Qiu *et al.,* 2017), the higher abundance of these genus in DL may show that the feed used in this group could promote intestine health in sheep. *Tomentella* can generate acetate and butyrate (Li *et al.,* 2020), while acetate and butyrate have function of protecting intestinal barrier and anti-inflammatory responses (Deleu *et al.,* 2023). The greater occurrence of this genus in EL reveals that grain feed in this group could promote gut health in PC sheep. Higher abundance of *Curvularia* was found in citizens with nonalcoholic fatty liver disease (You *et al.,* 2021) and *Rhodotorula* is a pathogenic genera (Hof, 2019), the lower abundance of these two genera in EL indicate that this grain feed promote the sheep heath. The *Inocybe*, *Sebacina*, *Clavulina*, *Alternaria*, *Gibberella genera* are widely found in environment (Kim *et al.,* 2020; Robinson *et al.,* 2022; Sanz-Benito *et al.,* 2023), which may have a little relationship with the health of PC sheep on the plateau.

Conclusions: In this study, we compared the fungal microbiota of sheep on the plateau fed with different forages and found the abundance of 7 phyla and 56 genera including potential beneficial fungi like *Penicillium*, *Torula*, *Filobasidium*, *Tomentella* and pathogenic *Rhodotorula,* were significantly different in sheep. Our findings contribution to the better feeding of sheep on the plateau.

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