Microbiome Analysis Reveals the Attenuation Effect of Lactobacillus From Yaks on Diarrhea via Modulation of Gut Microbiota

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Domestic yaks (Bos grunniens) are indigenous to the Tibetan Plateau and display a high diarrhea rate due to poor habitat and husbandry conditions. Lactobacillus has been shown to exert beneficial effects as antimicrobial, growth promotion, and gut microbiota in humans and/or murine models, but the relevant data regarding Lactobacillus isolated from yaks was unavailable. Therefore, this study aimed to investigate the effects of Lactobacillus from yaks on the intestinal microbial community in a mouse model and determine whether Lactobacillus supplementation contributed in alleviating diarrhea by modulating gut microbiota. A total of 12 ileac samples from four groups were collected for 16S rRNA gene amplicon sequencing of V3-V4 region. Results revealed that although Lactobacillus supplementation did not change the diversity of gut microbiota in mice, the proportion of some intestinal microbiota significantly changed. Specifically, the proportion of Lactobacillus and Sphingomonas in the Lactobacillus treated-group (L-group) were increased as compared to control group (C-group), whereas Pantoea, Cutibacterium, Glutamicibacter, Turicibacter, Globicatella, Microbacterium, Facklamia, unidentified_Corynebacteriaceae, Brachybacterium, and Staphylococcus were significantly decreased in the L-group. In contrast, Escherichia coli (E. coli) infection significantly decreased the proportion of beneficial bacteria such as Globicatella, Acinetobacter, Aerococcus, and Comamonas, while loads of pathogenic bacteria significantly increased including Roseburia and Megasphaera. Interestingly, Lactobacillus administration could ameliorate the microbial community structure of E. coli-induced diarrheal mice by reducing the relative abundance of pathogenic bacteria such as Paenibacillus, Aerococcus, Comamonas, Acinetobacter, Corynebacterium, Facklamia, and Globicatella. Results in this study revealed that Lactobacillus supplementation not only improved the gut microbiota but also alleviated diarrhea in...
INTRODUCTION

Animal gut microbiota is one of the largest and most complicated existing micro-ecosystems that provides an important barrier to bacterial infections (Lynch and Pedersen, 2016; Li et al., 2020; Liu et al., 2020b). Additionally, it helps in providing mucosal immunity, material metabolism, and nutrient absorption and regulation (Wu and Wu, 2012; Yue et al., 2020). Generally, ongoing competition and interaction of microorganisms may gradually change in microbial community structure from simple to a complicated and eventually a dynamic and balanced ecosystem (Jami et al., 2013; Zhao et al., 2015). This community’s consistency is a precondition for maintaining normal physiological functions (Li et al., 2018a; Ritz et al., 2020). Previous research has shown that constipation, colitis, diabetes, and obesity may be related to alteration in intestinal microbiota. Moreover, this study is expected to provide a new theoretical basis for the establishment of a preventive and treatment system for diarrhea in yaks.

Keywords: Tibet Plateau, yak, gut microbiota, Lactobacillus, Escherichia coli

MATERIALS AND METHODS

Animal Experiments and Sample Collection

Twenty-five-day-old healthy Kunming mice (n=40, initial weight 30 ± 3 g) were obtained from an experimental animal center, South China Agricultural University (Guangzhou, China). The study was permitted by the ethics committee of Tibet Agriculture & Animal Husbandry University. The mice used in this study were self-propagated and showed a higher degree of genetic uniformity. The selected mice were randomly divided into four groups, each comprising 10 mice (n=10) viz. control group (C-group), Lactobacillus-treated group (L-group), E. coli-induced group (E-group), and prevention group (EL-group). The mice were raised in plastic cages for 14 days under a recommended standard illumination time (12 h/12 h light/dark cycle), breeding temperature (33°C–35°C), and humidity (53%–57%). Furthermore, sufficient water and feed were provided ad libitum for all groups throughout the entire experiment. The E group was provided with same diet as control group but with the addition of E. coli at 1 × 10⁹ CFU/day on day 14 post-hatch to induce diarrhea. The L and EL group were treated with Lactobacillus at 1 × 10⁹ CFU/day from day 1 to day 14, but mice in the EL group were compulsively supplemented with E. coli at 1 × 10⁹ CFU/day on day 14. Mice in the C group were provided with the same volumes of saline as the L group to minimize stress response. Moreover, the overall performance of mice was recorded three times a day and death, diarrhea, dullness, and tiredness were considered abnormal. Three mice from each group were euthanized by injecting pentobarbital (25 mg/kg). Subsequently, the intestines were removed from the abdominal cavity, and the mesentery was stripped using a sterilized surgical knife. The intestines including duodenal, ileum, jejunal, and cecum were knotted using cotton.
ropes to minimize the potential cross-contamination among different intestine samples. The contents in the ileum were immediately collected, snap-frozen using liquid nitrogen, and finally stored at -80°C until further study.

**gDNA Extraction**

Bacterial DNA from all samples was extracted by using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) as per manufacturer’s guidelines. The quality of DNA was evaluated by 0.8% (w/v) agarose gel electrophoresis. A Nanodrop™ Spectrophotometer (Thermo Scientific, Massachusetts, USA) was used to quantify the DNA.

**16S rRNA Gene Amplification and Sequencing**

Universal V3/V4 16S rRNA gene primers (338F: ACTCCTACGGGAGGCAGCAG and 806R: GGAACACHVGGGTWTCTAAT) were used along with the barcode sequences for amplification of the conserved regions of the bacteria followed by a 2% agarose gel electrophoresis procedure to evaluate the quality of the polymerase chain reaction (PCR) products. The PCR product was then purified and recycled by using AxyPrep DNA Gel Extraction Kit (Axygen, Corning, New York, USA). The fluorescent quantitation of PCR-recycled products was conducted on FLx800™ Multi-Detection Microplate Reader (BioTek Instruments, Inc., Winooski, Vermont, USA) by using Quant-iT PicoGreen™ dsDNA Assay Kit (Invitrogen, Carlsbad, California, USA). The sequencing library was prepared by using TruSeq Nano DNA Low Throughput Library Prep Kit (Illumina, Inc., San Diego, California, USA) following the manufacturer’s protocol. The amplified products were repaired by End Repair Mix. Simultaneously, a magnetic bead screening procedure was used for removing the self-connecting fragments of the linker, and the sequencing library was purified. The PCR amplification was performed, and the library enrichment was performed by AMPure XP Beads (Beckman Coulter Inc., Brea, California, USA). The final fragment-selection and purification of the library were conducted on 2% agarose gel electrophoresis.

The libraries’ quality was assessed by using Agilent High Sensitivity DNA Kit on Agilent Bioanalyzer (Agilent Technologies, Inc., Santa Clara, California, USA) prior to the sequencing process. Moreover, the libraries having only one single peak without a linker was selected. The libraries were quantified by using Quant-it™ PicoGreen™ dsDNA Assay Kit (Invitrogen, Carlsbad, California, USA) on the QuantFluor® RNA System (Promega Corporation, Madison, Wisconsin, USA), with a concentration >2 nM. The qualified libraries were gradient diluted and mixed in a proportion to the required amount of sequencing. Finally, the MiSeq Reagent Kit V3 (600 cycles) was used to perform the 2x300 bp paired-end sequencing on the MiSeq sequencing system (Illumina, Inc., San Diego, California, USA).

**Bioinformatics and Statistical Analysis**

QIIME software (Qiime1.9.1) was used to screen and analyze the 16S rRNA preliminary data quality. The interrogative and short sequences (<200 bp) were removed by using QIIME software. The obtained sequences were clustered and operational taxonomic unit (OTU) were partitioned at ≥97% sequence similarity by program VSEARCH (1.9.6). The Ribosomal Database Program (RDP) classifier was used to classify the representative sequences of each OTU at confidence threshold of 0.8. The MUSCLE software was used for phylogenetic analysis and multiple sequence alignments of each OTU. The multiple alpha diversity indices including Shannon, Simpson, Chao1, and Good’s coverage were calculated to evaluate the alpha diversity. Moreover, the sparse curves were used for assessing the sequencing depth of each sample prior to the evaluation of alpha and beta diversity. The beta diversity was also calculated to assess the similarity of community structure in the samples. GraphPad Prism (version 6.0c) and R (v3.0.3) software were used to perform the statistical analysis. In addition, the criterion of significance was conducted at p-values <0.05. The values were expressed as means ± standard deviation (SD).

**RESULTS**

**Clinical Symptoms**

Clinical observation results showed that the mice in the C and L group had a normal feed intake and displayed active behavior. However, mice in the E group showed dullness, messy hair, watery feces, and pasting. Conversely, mice in the EL group and the control group were in good mental state and without diarrhea symptoms.

**DNA Sequences Analyses**

In the microbiome analysis, a total of 274,247, 265,341, 263,510, and 253,825 original sequences were acquired from C, L, E, and EL group, respectively (Table 1). After eliminating the unqualified data, a total number of 961,904 high-quality reads were achieved from all the samples, with an average of 80,158 (ranging from 75,598 to 90,528) reads per sample. Following the proper sequencing depth of each sample prior to the evaluation of alpha and beta diversity. Moreover, the sparse curves were used for assessing the sequencing depth of each sample prior to the evaluation of alpha and beta diversity. The beta diversity was also calculated to assess the similarity of community structure in the samples. GraphPad Prism (version 6.0c) and R (v3.0.3) software were used to perform the statistical analysis. In addition, the criterion of significance was conducted at p-values <0.05. The values were expressed as means ± standard deviation (SD).

**TABLE 1** | The sequence information of each sample.

| Sample | Raw reads | Combined reads | Qualified Reads | Effective (%) |
|--------|-----------|----------------|-----------------|---------------|
| C1     | 87,037    | 79,283         | 76,082          | 87.41%        |
| C2     | 89,587    | 85,791         | 82,857          | 92.49%        |
| C3     | 97,623    | 81,093         | 76,128          | 77.98%        |
| L1     | 85,953    | 83,188         | 80,494          | 93.65%        |
| L2     | 94,888    | 92,730         | 90,528          | 95.42%        |
| L3     | 84,500    | 80,291         | 77,475          | 91.69%        |
| E1     | 83,003    | 78,273         | 76,118          | 91.71%        |
| E2     | 84,412    | 81,212         | 78,706          | 93.24%        |
| E3     | 96,095    | 90,371         | 87,082          | 90.62%        |
| EL1    | 89,464    | 85,212         | 82,185          | 91.86%        |
| EL2    | 80,217    | 77,592         | 75,598          | 94.24%        |
| EL3    | 84,144    | 81,065         | 78,651          | 93.47%        |
and EL group was 87, 122, 67, and 68, respectively and 16 core OTUs were recognized in all the samples (Figure 1F). Both rarefaction and species accumulation curves for all samples tend to be stable. The number of qualified sequences reached over 10,000 and 50,000, respectively, suggesting that sequencing’s depth and quantity met the demands for sequencing and analysis (Figures 1G, H). Furthermore, the rank abundance curve is wide and falling relaxedly, showing excellent abundance and evenness (Figure 1I).

**Microbial Diversity Index in Different Groups**

To assess the differences in intestinal microbial community diversity among the four groups, the qualified sequences obtained in the sequencing were aligned to estimate alpha and beta indices. The alpha diversity of gut microbiota can be reflected by community abundance (Chao1 and ACE), diversity index (Simpson), and sequencing depth (Good’s coverage). Good’s coverage estimates in all the samples were

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**FIGURE 1** | Venn diagrams and sample feasibility analysis. Venn diagrams for bacterial OTUs compositions in C (A), L (B), E (C) and EL (D) groups. (E) Venn diagram for unique and shared bacterial OTUs in four groups. (F) Venn diagrams for core OTUs compositions. The rarefaction (G) and species accumulation curve (H) and rank abundance curve (I) were used to assess the adequacy, evenness and richness of sequencing of each sample. Each curve with a different color shown in the figures indicates a sample.
approximately 100%, indicating excellent coverage (Figure 2A). The control mice showed the highest Chao1 and ACE indexes as compared to other groups, whereas the Chao1 and ACE indexes in mice infected with *E. coli* were the lowest (Figures 2B, C). The average of Chao1 and ACE indices in the L-group ranged from 218.71 to 277.02 and 226.022 to 289.15, respectively, while Simpson index ranged from 0.22 to 0.62. The analysis of alpha diversity indicated no statistically significant differences in the Chao1, ACE, and Simpson between the C and L groups, which indicated that *Lactobacillus* administration had no effect on the diversity and richness of the gut microbiota of mice (Figure 2D). However, intergroup analysis of alpha diversity intuitively indicated that gut microbiota’s richness and diversity in EL-group mice were higher than those in E-group, indicating that supplementing with *Lactobacillus* alleviated the gut microbiota imbalance of mice induced by *E. coli*. The beta diversity analysis showed that samples in the C, L, E, and EL groups were clustered closely, indicating that gut microbiota in the four groups was not different (Figure 3).

**Figures 2 and 3**

**Figure 2** | Comparison of alpha diversity of mice gut microbiota in different groups. Four indices such as Good’s coverage (A), Chao1 (B), ACE (C), and Simpson (D) were used to assess the alpha diversity of gut microbiota. The data used in this study were expressed as the mean ± SD.

**Figure 3** | Principal coordinate (PCoA) analysis of gut microbiota in different groups. (A, B) represent PCoA map based on unweighted and weighted uniFrac distance, respectively. Each colored point indicates one sample and the difference in the different groups can be evaluate by the distance between the points.

**Alterations in the Composition of Gut Microbiota in Different Groups**

The proportion of dominant phyla and genera were assessed by microbial taxa assignment in C, L, E, and EL groups (Figure 4). According to the phylum assignment result, phyla *Firmicutes*
FIGURE 4 | Relative abundance of the most preponderant (top 10 and 30) gut microbial taxa at phylum (top 10) and genus (top 30) levels for bacteria among four groups. (A, C) Relative abundance of gut microbiota in each sample at the phylum and genus levels. (B, D) Relative abundance of gut microbiota on the basis of the average number of each subfamily at the phylum and genus levels.

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(91.08%, 97.82%, and 91.58%) and Actinobacteria (7.64%, 1.04%, and 5.51%) were the most preponderant bacteria in the mice of C, L, and E group, which accounted for approximately 97% of the taxonomic groups identified (Figures 4A, B). Remarkably, the predominant phylum in the EL-group was Firmicutes (72.01%), whereas phylum Proteobacteria was subsidiary (26.69%), slightly different from the other groups. Other phyla such as Fusobacteria, Acidobacteria, Chloroflexi, unidentified_Bacteria, and Tenericutes were represented with a lower abundance. At the genus level, Lactobacillus (68.30% and 91.98%) was the most predominant bacterium in the mice of C-group and L-group followed by the Staphylococcus (17.92% and 4.07%), which together made up 85% and 95% of the overall bacterial composition, respectively (Figures 4C, D). Moreover, Nosocomicoccus (43.94%) and Lactobacillus (34.97%) were the most prevalent bacteria in the E-group, whereas Lactobacillus (63.23%) and Ralstonia (24.47%) were observed to be predominant in the EL-group. The relative richness of these bacteria was also displayed by a heat map produced by clustering analysis (Figure 5).

To further compare the differences in intestinal microflora among the four groups, Linear discriminant analysis effect size (LEiSe) analysis coupled with Linear discriminant analysis (LDA) was performed for different classification levels (Figures 6 and 7). At the phylum level, Firmicutes was obviously more abundant in L-group than in the C-group, whereas the abundance of Cyanobacteria and Actinobacteria was lower. Additionally, the abundance of the Proteobacteria was significantly increased in E-group in comparison with C-group. At the genus level, Lactobacillus and Sphingomonas levels tended to be higher in the L-group than C-group, whereas the Pantoea, Cutibacterium, Glutamicibacter, Turicibacter, Globicatella, Microbacterium, Facklmania, unidentified_Corynebacteriaceae, Brachybacterium, and Staphylococcus showed the opposite
Moreover, a comparison of the E and C groups displayed a significant increase in the abundance of *Globicatella*, *Acinetobacter*, *Aerococcus*, and *Comamonas* as well as a distinct decrease in the abundance of *Roseburia* and *Megasphaera* (Figures 6B and 7B). Meanwhile, the E-group was significantly enriched for *Paenibacillus*, *Aerococcus*, *Comamonas*, *Acinetobacter*, *Corynebacterium*, *Facklamia*, and *Globicatella* in comparison with EL-group (Figures 6C and 7C).

**DISCUSSION**

In livestock industry, diarrhea is widely prevalent in juvenile animals, which is deemed as a crucial factor resulting in the reduction of global animal productivity (Pepin et al., 2004; Diao et al., 2020). Multiple measures have been performed to prevent diarrhea, but it still occurs from time to time. Recently, role of gut microbiota is revealed in the development of diarrhea (Huang et al., 2020). Therefore, the improvement of the intestinal microbial community structure may contribute to alleviate diarrhea (Yue et al., 2019). The significance of the *Lactobacillus* has been widely acknowledged as a result of its role in gut microbiota, metabolism, immunity, and health maintenance, but few reports have been published on the *Lactobacillus* from yaks inhabiting the Tibet Plateau (Li et al., 2018b; Wang et al., 2020). In this study, we analyzed the influence of *Lactobacillus* isolated from yaks on the gut microbiota and investigate whether it could improve the...
microbial community structure of mice with *E. coli*-induced diarrhea. Our results indicated that the *Lactobacillus* administration alleviated the intestinal microbial community of diarrheal mice colonized by *E. coli*.

Previous studies revealed that mammalian gut microbiota was dynamically varied during development and reached stability at maturity (Poroyko et al., 2011; David et al., 2014, Yang et al., 2020). Diarrheal diseases were widespread in the childhood of animals, which may be closely related to their immature gut microbiota (Wang et al., 2018b). Therefore, probiotics supplementation in the juvenile period of animals may reduce diarrhea by improving the structure in the intestinal microbial community (Wang et al., 2018a). Our study found the phylum *Firmicutes* as the most dominant bacteria in all samples regardless of the treatment. Consistent with previous observations, this phylum was also found to be widely distributed in camels, sheep, goats, and roe deer, indicating its important role in intestinal ecology and function (Li Z. et al., 2014; Zeng et al., 2017; Lei et al., 2018). The *Firmicutes* is responsible for digestion of cellulose and its richness in the gut contributes to meet the energy and nutrition requirements of animals in the growth and development process (Sun et al., 2016). Additionally, *Firmicutes* is mainly composed of gram-positive bacteria including *Lactococcus*, *Bacillus*, and *Lactobacillus* and most of them are perceived as beneficial bacteria, which are conducive to inhibit the proliferation of pathogenic bacteria and improve the intestinal environment (Garneau et al., 2008).

Importantly, our study also found a higher variation in some bacterial phyla and genera of different treatment groups, and this variation may play a crucial role in the intestinal ecosystem and function. *Cyanobacteria* comprises a great quantity of cyanotoxin-producing bacteria, posing a great threat to animal and human health (Carmichael, 1992). Wang et al. (2018b) observed that the proportion of *Actinobacteria* in the gut of diarrheal goat was significantly increased. Moreover, the synergy of *Actinobacteria* with one partner or host can easily be transformed into a pathogenic interaction with another (Miao and Davies, 2010). Mice in the L-group displayed increased

![Figure 7](image-url)
Firmicutes and decreased Cyanobacteria and Actinobacteria abundance when compared to C-group, indicating a possible reduction in disease risk through Lactobacillus supplementation. Moreover, Lactobacillus and Sphingomonas were enriched in mice treated with Lactobacillus, whereas Pantoea, Cutibacterium, Glutamicibacter, Turicibacter, Globicatella, Microbacterium, Facklamia, unidentified Corynebacteriaceae, Brachybacterium, and Staphylococcus were reduced in the control group. Previously, Lactobacillus had improved the intestinal mucosal immunity and interacted with intestinal epithelial cells against entero-invasive E. coli (Johnson-Henry et al., 2007; Wang et al., 2019; Dong et al., 2019). Studies have also reported that supplementing diet with Lactobacillus daily can prevent non-alcoholic fatty liver disease by ameliorating the intestinal environment and attenuating inflammation in obese mice (Zhang et al., 2020a; Zhang et al., 2020b). Aside from improving immunity and regulating gut microbiota, Lactobacillus supplementation enhanced digestive enzyme activity and intestinal antioxidant ability benefiting the host (Chen F. et al., 2020; Zhang et al., 2020). Sphingomonas can degrade multiple organic matter, displaying the great application potential in environmental protection and industrial production (Leys et al., 2004). Pantoea, a gram-negative pathogenic bacterium, is associated with disease in plants, humans, and rarely in domestic animals (Silva-Rojas et al., 2012). Henker et al. (2020) indicated that Pantoea could induce fibrinonecrotic placentitis and abortions in mare. Zaccone et al. (2020) reported that Pantoea was closely related to bacteremia in humans. Cutibacterium was considered as skin flora contaminant, which can result in pericarditis with serious complications (Fakhri et al., 2020). Moreover, Cutibacterium was also closely related to multiple postoperative complications, including persistent postoperative pain, chronic inflammation, and endoprostheses involving bacterial biofilms because it ubiquitously colonizes the skin and resides in various other locations in the human body (Patel et al., 2009; Achermann et al., 2013; Hudek et al., 2021). Turicibacter is a pro-inflammatory bacterium whose level rises during enteritis (Bretin et al., 2018). Globicatella was previously reported to associate with meningitis and bacteremia (Lau et al., 2006; Seegmuller et al., 2007). Furthermore, Globicatella was also observed in the purulent joint and lung infections in calves and sheep (Vandamme et al., 2001). Microbacterium, a novel bacterial pathogen, was also closely related to bacteremia (Hodgkin et al., 2000; Lau et al., 2002). Facklamia may be relevant to invasive disease such as meningitis and septicemia (Hughes, 2014; Parvataneni et al., 2015). Corynebacteriaceae can lead to endocarditis (Prada et al., 1994). Brachybacterium can cause bloodstream infection (Tamai et al., 2018). Most pathogenic Staphylococcus can produce coagulase, staphylolysin, enterotoxin, and toxic shock syndrome toxin1, resulting in fever, emesis, diarrhea, acute gastroenteritis, and even shock (Gemeinder et al., 2020; White et al., 2020). Moreover, Staphylococcus can invade the host through multiple ways and cause local and systemic infections as well as various invasive diseases such as pneumonia, meningitis, blood poisoning, and septic pyemia (Chen H. A. et al., 2020; Ranzani et al., 2020). Remarkably, E. coli infection significantly increased Acinetobacter, Aerococcus, and Comamonas levels and decreased Roseburia and Megaphaera content as compared to control group. Acinetobacter, a common opportunistic pathogen, is widely colonized in the digestive tract, skin, respiratory tract, and genitourinary tract, which can cause bacteremia, pneumonia, endocarditis, as well as urinary and skin infections (Go et al., 1994; Livermore and Woodford, 2006; Lima et al., 2020). Aerococcus can result in endocarditis and urinary tract infections (Yabes et al., 2018; Sous et al., 2019). Comamonas may be closely related to bacteremia (Liu et al., 2020a; Palacio et al., 2020). Roseburia, a butyrate-producing bacterium, can utilize succrose, cellobiose, galactose, and glycogen (Duncan et al., 2002). Megaphaera was previously reported to produce short-chain fatty acids (SCFAs), displaying a positive regulatory effect to physiological functioning of gut and intestinal permeability (Kim et al., 2002). Conversely, Lactobacillus supplementation significantly reduced Paenibacillus, Aerococcus, Comamonas, and Corynebacterium levels in the ileum of mice induced by E. coli. Although reports of Paenibacillus infections are exceedingly rare, the infection may result in meningitis in some cases (Hunt et al., 2020). Corynebacterium, an acclimatized pathogen, can cause respiratory disease (Samies et al., 1986). This study conveyed a message that Lactobacillus supplementation resulted in an increase in beneficial bacteria and decreased pathogenic bacteria, whereas E. coli infection increased the ratio of harmful and beneficial bacteria. Additionally, Lactobacillus administration effectively ameliorated the microbial community structure of mice induced by E. coli and decreased the proportion of pathogenic bacteria.

CONCLUSION

In summary, this study revealed that the gut microbiota in diarrheal mice induced by E. coli undergoes striking changes, characterized by an increased proportion of harmful bacteria. Conversely, Lactobacillus administration not only improves the microbial community structure of normal mice but also alleviates E. coli-induced diarrhea by mediating gut microbiota. These results expand our understanding of the potential benefits of Lactobacillus from yaks and convey an important message that Lactobacillus may be one of effective methods to attenuate diarrhea in yaks. Importantly, these findings also enriched the knowledge of disease prevention and control system in yaks. However, several limitations in this study need to be noticed, such as individual variation, experimental environment, and small sample size.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/, PRJNA665922.
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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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