Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2025.331

RESEARCH ARTICLE

Microbiome Analysis Shows the Adverse Impacts of Diarrhea on the Intestinal Homeostasis in Yaks

Dongjing Wang[1]*, Jiangyong Zeng[1], Hongcai Ma[1], Majeeda Rasheed[2], Zhonghua Su[3], Rania Ali El Hadi Mohamed[4] and Essam H. Ibrahim[5,6]

[1]Institute of Animal Husbandry and Veterinary Medicine, Xizang Academy of Agriculture and Animal Husbandry Sciences, Lhasa, 850009, Xizang, China; [2]Department of life Sciences, Khwaja Fareed University of Engineering and Information Technology, Rahim Yar Khan 64200, Pakistan; [3]Xizang Autonomous Region Animal Disease prevention and Control Center, China; [4]Department of Biology, College of Science, Princess Nourah bint Abdulrahman University, P.O. Box 84428, Riyadh 11671, Saudi Arabia; [5]Biology Department, Faculty of Science, King Khalid University, P.O. Box 9004, Abha 61413, Saudi Arabia; [6]Blood Products Quality Control and Research Department, National Organization for Research and Control of Biologicals, Cairo, Egypt.
*Corresponding author: wdj17808745806@sina.cn

ARTICLE HISTORY (25-782)

Received:	August 06, 2025
Revised:	December 14, 2025
Accepted:	December 18, 2025
Published online:	December 21, 2025

Key words:
Diarrhea
Genera
Gut microbiota
Phyla
Yak

ABSTRACT

The gut microbiota is closely associated with a variety of gastrointestinal diseases. However, there is no clear relationship between gut microbiota and diarrhea in yaks. Here, we investigated the effect of diarrhea on the gut microbiota of yaks. Results showed that diarrhea greatly reduced the diversity of gut fungi but had no significant effect on gut bacteria. Additionally, the relative abundance of specific bacterial and fungal taxa differed significantly between healthy and diarrheal yaks. A total of 20 genera were significantly increased in the gut bacterial community of diarrheic yaks compared to the healthy yaks, while the relative abundance of 2 phyla and 10 genera has significantly decreased. Fungal taxonomic analysis showed that the relative abundance of 1 phylum and 62 genera has significantly increased in the diarrheic yaks compared to the healthy yaks, while the relative abundance of 7 phyla and 123 genera has significantly decreased. In summary, this study demonstrated the negative impact of diarrhea on the gut microbiota homeostasis of yaks. Furthermore, this research contributes to the understanding of the characteristics of gut microbiota in plateau yaks, thus laying the foundation for protection of yak health and relieving diarrhea from the microbial perspective.

To Cite This Article: Wang D, Zeng J, Ma H, Rasheed M, Su Z, Mohamed RAE, Ibrahim EA, 2025. Microbiome analysis shows the adverse impacts of diarrhea on the intestinal homeostasis in yaks. Pak Vet J, 45(4): 1650-1661. http://dx.doi.org/10.29261/pakvetj/2025.331

INTRODUCTION

A host gut harbors approximately 100 trillion microorganisms, and their genetic materials are 150 times more abundant than the genetic materials of the host genome (Bian *et al*., 2025). These microorganisms maintain a complex ecological balance that participates fundamentally in maintaining host homeostasis (Hou *et al*., 2025). For example, the gut microbiome takes part in various biological activities in the host, including nutrient metabolism and the processes of digestion and absorption (Li *et al*., 2025). However, gut microbiota is easily influenced to change by internal and external factors. Common internal influencing elements are age, gender, and genetics (Wang *et al*., 2022). Exercise, diet, and environmental pollutants-microplastics, antibiotics, and heavy metals-are also key driving factors that affect gut microbiota and even disturb gut microbial homeostasis (Zhang *et al*., 2022; Chang *et al*., 2025). The severity of this dysbiosis corresponds closely with many diseases, from minor cramps, diarrhea, and constipation to serious colorectal cancer (Hu *et al*., 2022; Adil *et al.,* 2023; Mehnaz *et al*., 2023).

Diarrhea is a prevalent public health problem in animal husbandry. It seriously threatens animal welfare and causes huge economic losses (Quintero *et al*., 2019). Studies have shown that diarrhea not only decreases productivity but also has a negative impact on milk production and immune function (Liu *et al*., 2024). Moreover, it was known as a main cause of death among newborn animals. It was demonstrated that 39% of deaths occurring within three weeks after birth were due to diarrhea. The causes of diarrhea are very complicated and come from different factors, such as bacteria, viruses, parasites, and fungi. Among these etiological agents, bacterial infections represent the most common and pose serious health risks, particularly in calves. Although many measures have been carried out to prevent diarrhea in calves, obvious improvement has not been achieved so far. Therefore, exploring the causes of diarrhea plays a very significant role in effective therapy and prevention. Recent research increasingly implicates gut microbial dysbiosis as a central mechanism in calf diarrhea (Hui *et al*., 2020; Wang *et al*., 2024).

Yaks are an essential source of meat and make a huge contribution to the economic, social, and cultural development in the Tibetan region (Li *et al*., 2023; Ni *et al*., 2025). Yak meat possesses favorable nutritional characteristics, including highly palatable, high protein content, low fat content and elevated micronutrient density, aligning with contemporary dietary preferences (Wang *et al*., 2022). In contrast with traditional beef, the fat content of yak meat was as low as 3%-5%, and its protein content is higher than beef. Furthermore, it has a high level of essential amino acids and trace elements, which are vital in human nutrition, and can be used to improve conditions such as anemia and immune function (Zhao *et al*., 2025). However, yak populations are susceptible to gastrointestinal and nutritional disorders. This may have contributed to relatively small population of yaks globally. There are about 16.02 million yaks in China, accounting for about 90% of the global yak population, according to statistics. Diarrhea is one of the important diseases causing death in yaks and restricting the development of the yak industry. Although a variety of diarrhea prevention measures against yaks have been implemented, little outcome has been achieved so far. Recently, there has been increasing awareness of the key link between gut microbiota and gastrointestinal diseases. For example, Wang *et al*. (2023) found that diarrhea significantly disturbed the composition and structure of gut microbiota in Beigang pigs. Li *et al*. (2021) also observed gut microbial dysbiosis in diarrheal giraffes, and that diversity and abundance of beneficial bacteria changed greatly. However, no study has been conducted to explore the changes in diarrheal yaks. Therefore, we assume that diarrhea could result in significant changes in the gut microbiota of yaks.

MATERIALS AND METHODS

Sample Collection: A total of 12 yaks, 6 apparently healthy ones and 6 that had diarrhea, were selected for this study. These were nearly 2-year-old yaks. All the yaks belonged to the same commercial feedlot and received an identical diet and habitat. No medication had been given to the diarrheic yaks. Fecal samples were collected from the middle section of the feces using a sterile sampler to minimize surface contamination. Finally, the fecal samples from each yak were placed in cryovials for subsequent analysis.

Analysis of the gut microbiota: The sequencing and analysis of the gut microbiota were conducted based on previous research (Li *et al*., 2021). In short, DNA was extracted from each fecal sample using a commercial kit. After ensuring its quality, PCR amplification was performed using universal primers (338F: ACTCCTACG GGAGGCAGCAG-3 and 806R: GGACTACHVGGGTW TCTAAT). Subsequently, we performed quality control of the PCR amplification products, sequencing library construction, and paired-end sequencing. Microbial diversity was calculated based on the number of OTUs in each sample. Furthermore, differences in microbial composition were visualized using LEfSe and Metastats analysis. Data are expressed as mean ± SD, and the threshold for differential bacterial taxa was set at $P<0.05$.

RESULTS

Data Acquisition and Analysis: We conducted amplicon sequencing on the collected fecal samples, resulting in 800,085 original bacterial sequences (HA=399,827, DA=400,258, ranging from 79,882 to 80,232) and 799,626 original fungal sequences (HA=399,663, DA=399,963, ranging from 79,672 to 80,298) (Table 1, 2). In addition, these raw data were further filtered and 460,377 (HA=229,287, DA=231,090, ranging from 42,980 to 49,638) valid bacterial sequences and 600,879 (HA=287,555, DA=313,324, ranging from 54,692 to 64,260) valid fungal sequences were obtained. These valid sequences were subsequently clustered into 5,425 (HA=3,115, DA=3,287, ranging from 800 to 952) bacterial OTUs and 7,520 (HA=4,815, DA=3,557, ranging from 800 to 1,302) fungal OTUs, based on sequence similarity (Fig. 1A, B, C, G, H, I). Specifically, the gut bacterial community comprised 2,138 individual OTUs in the HA and 2,310 in the DA. Similarly, the gut fungal community exhibited 3,963 individual OTUs in the HA and 2,705 in the DA. Notably, we also identified 977 core bacterial OTUs and 852 core fungal OTUs. The results of rarefaction curve showed that the sequencing depth was adequate (Fig. 1D, E, F, J, K, L).

Diarrhea changed the gut microbial diversity of yaks: The Chao1, ACE, Simpson, and Shannon indices for the HA were 898.39, 896.21, 0.99 and 8.58, while these indices for the DA were 908.43, 905.29, 0.99 and 8.52, respectively (Fig. 2A, B, C, D). There are no obvious differences in the Chao1 (898.39±20.85 versus 908.43±28.70, $P>0.05$), ACE (896.21±19.74 versus 905.29±28.07, $P>0.05$), Simpson (0.99±0.0009 versus 0.99±0.0019, $P>0.05$), and Shannon (8.58±0.089 versus 8.52±0.072, $P>0.05$) indices of gut bacterial community between the HA and DA. Moreover, the Chao1, ACE, Shannon, and Simpson indices for the gut fungal community in the HA were 1173.59, 1169.95, 7.84, and 0.98, while those in the DA were 982.23, 976.25, 7.17, and 0.96, respectively (Fig 2E, F, G, H). Comparative analysis demonstrated that the Chao1 (1173.59±34.74 versus 982.23±59.15, $P=0.029$), ACE (1169.95±35.00 versus 976.25±60.61, $P=0.03$), Simpson (0.98±0.0044 versus 0.96±0.0029, $P=0.017$), and Shannon (7.84±0.32 versus 7.17±0.17, $P=0.016$) indices for the HA were significantly higher than those for the DA, indicating that diarrhea significantly reduces the diversity and abundance of gut fungal community. Furthermore, the PCoA plot based on beta diversity showed that the samples from both the HA and DA clustered together in the gut bacterial community, whereas a separation trend was observed in the gut fungal community (Fig. 2I, J, K, L). This suggests that diarrhea has a more pronounced impact on the structure of gut fungal community.

Diarrhea changed the gut bacterial composition of yaks: At the phylum level, the *Firmicutes* (52.52%), *Bacteroidota* (35.83%) and *Spirochaetota* (4.40%) were the three most dominant phyla in the DA (Fig. 3A). Meanwhile, the bacterial phyla in HA were predominated by *Firmicutes* (58.49%), *Bacteroidota* (35.22%) and *Verrucomicrobiota* (2.34%) in descending order. Other phyla such as *Actinobacteriota* (0.34%, 0.41%), *Cyanobacteria* (0.48%, 0.19%), *Desulfobacterota* (0.14%, 0.45%) and *Elusimicrobiota* (0.052%, 0.09%) in HA and DA were found in low concentrations. At the genus level, *UCG_005* (14.10%), *Rikenellaceae_RC9_gut_group* (9.47%) and *unclassified_Lachnospiraceae* (8.71%) were abundantly found in the HA (Fig 3B). Moreover, *UCG_005* (11.69%) was the most dominant bacteria, followed by *Rikenellaceae_RC9_gut_group* (7.70%) and *unclassified_UCG_010* (6.88%) in DA. Moreover, the abundance distribution of dominant fungi between the HA and DA could be visualized by clustered heatmaps (Fig. 3C).

At the phylum level, *Firmicutes* and *Cyanobacteria* were significantly more preponderant in the HA than in the DA (Fig. 4A). Moreover, a comparison of the DA and HA groups indicated a significant reduction in the abundance of 10 (*Prevotellaceae_Ga6A1_group*, *Lachnospiraceae_NK3A20_group*, *Bacteroides*, *unclassified_Prevotellaceae*, *Rikenellaceae_RC9_gut_group*, *Defluviitaleaceae_UCG_011*, *uncultured_Clostridium_sp*, *Atopobium*, *Ruminococcus* and *Dorea*) genus and a significant increase in the abundance of 20 (*Anaerovorax*, *unclassified_Paludibacteraceae*, *uncultured_Bacteroidales_bacterium*,

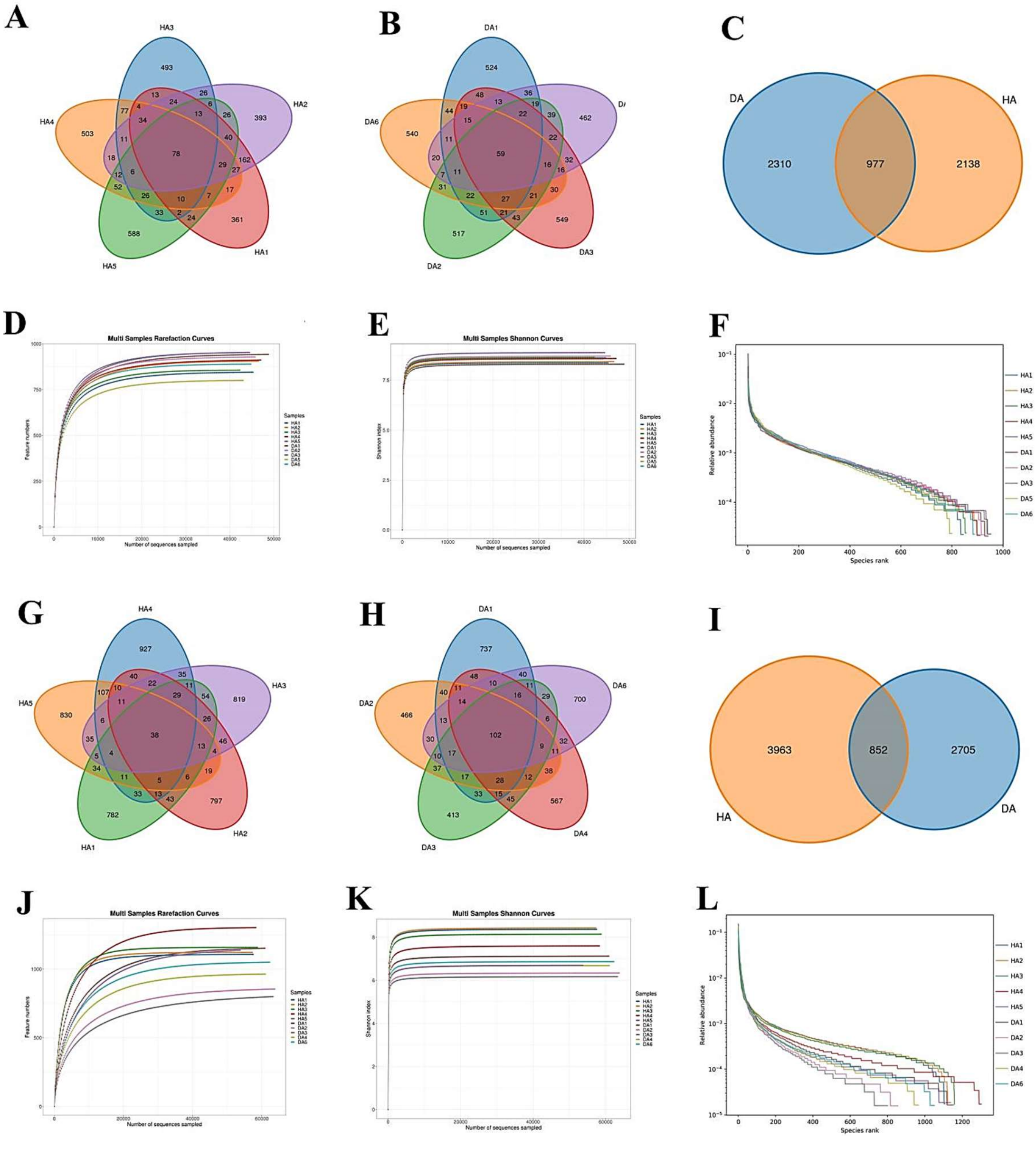

Fig. 1: OTUs count and sequencing depth assessment. A, B, C, G, H, I: Venn diagram. D, E, F, J, K, L: Rarefaction curves.

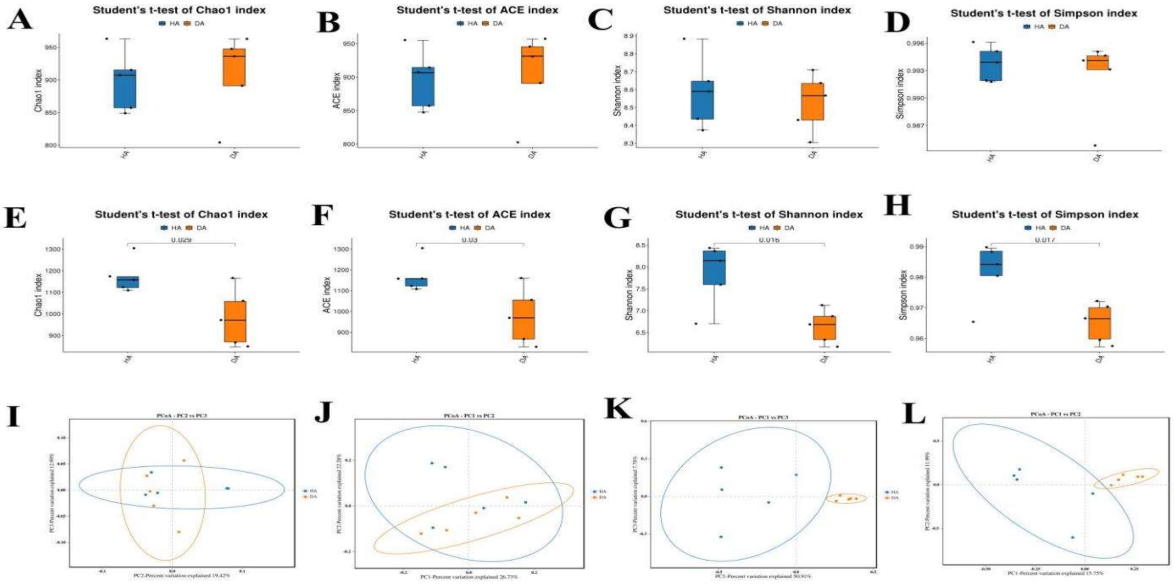

Fig. 2: Analysis of gut microbial diversity. A, E: Chao1. B, F: ACE. C, G: Shannon. D, H: Simpson. I-L: PCoA plot.

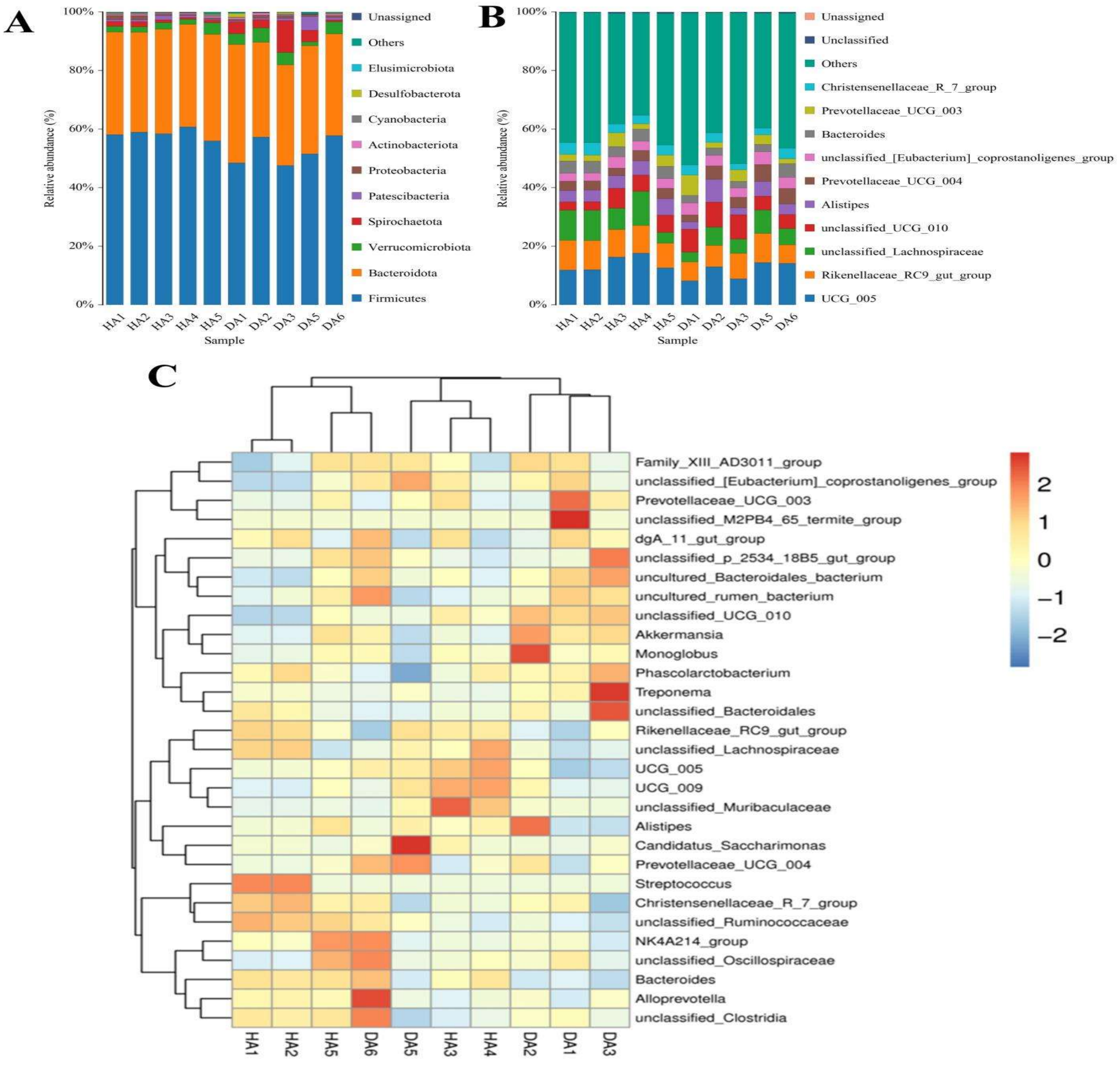

Fig. 3: Composition of dominant bacteria in the gut microbiota of the DA and HA at the phylum and genera levels. A: Phylum level. B: Genus level. C: Cluster heatmap.

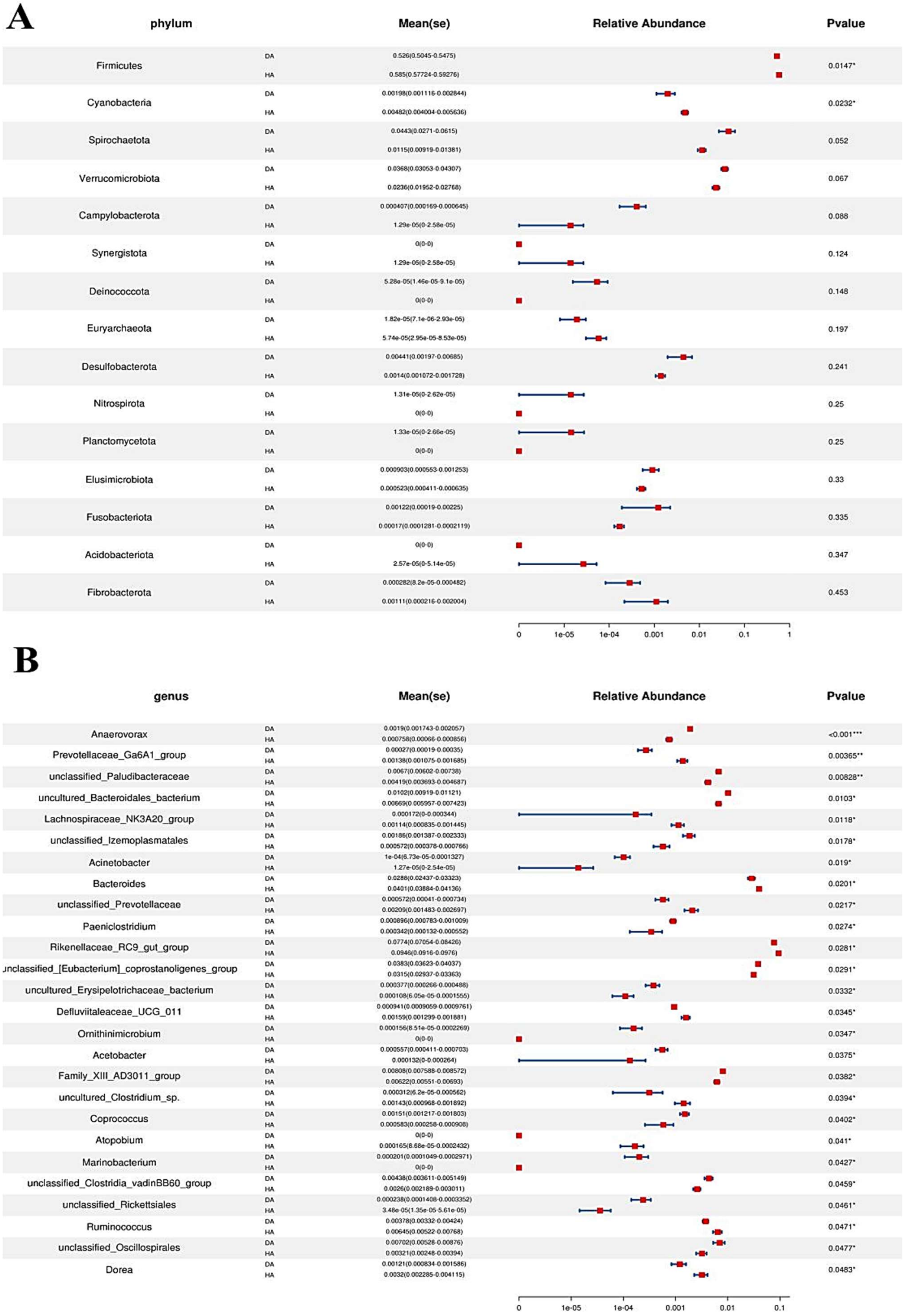

Fig. 4: Statistical analysis of significantly different intestinal bacterial phyla (A) and genera (B) associated with yak diarrhea.

unclassified_Izemoplasmatales, *Acinetobacter*, *Paeniclostridium*, *unclassified_[Eubacterium]_coprostanoligenes_group*, *uncultured_Erysipelotrichaceae_bacterium*, *Ornithinimicrobium*, *Acetobacter*, *Family_XIII_AD3011_group*, *Coprococcus*, *Marinobacterium*, *unclassified_Clostridia_vadinBB60_group*, *unclassified_Rickettsiales*, *unclassified_Oscillospirales*, *Candidatus_Soleaferrea*, *Candidatus_Stoquefichus*, *Fermentimonas*, and *Mailhella*) genus (Fig. 4B). Using LEfSe to further identify the differential bacteria associated with diarrhea (Fig. 5).

Diarrhea changed the gut fungal composition of yaks: At the phylum level, *Ascomycota* (52.62%, 42.11%), *Basidiomycota* (15.64%, 49.05%) and *Mortierellomycota* (3.59%, 2.00%) were the most preponderant in the HA and DA (Fig. 6A). Other phyla such as *Glomeromycota* (3.13%, 0.59%), *Neocallimastigomycota* (1.76%, 0.82%), *Rozellomycota* (1.64%, 0.72%), *Olpidiomycota* (0.23%, 0.079%) and *Mucoromycota* (0.094%, 0.027%) in HA and DA were identified in low ratios. At the genus level, the *Elaphomyces* (13.36%), *Sebacina* (12.74%) and *Russula* were abundantly present in the DA, whereas the dominant genus found in the HA were *Fusarium* (5.85%), *Mortierella* (3.53%) and *Acremonium* (2.80%) (Fig. 6B). The cluster heat map further showed the distribution of dominant fungal abundances between the HA and DA (Fig. 6C).

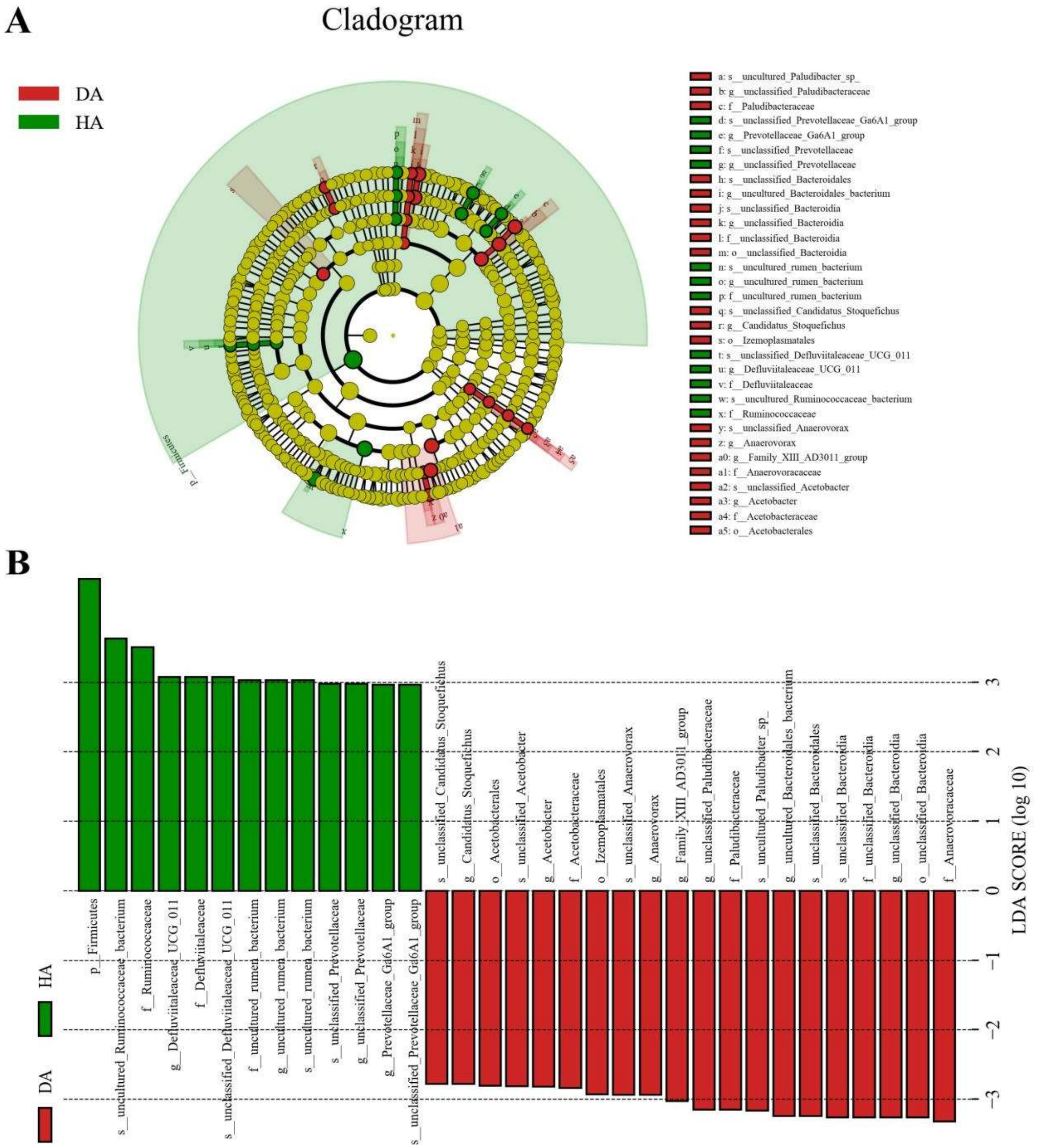

Fig. 5: The microbial taxa that exhibited significant changes during yak diarrhea were analyzed using the LEfSe. A: The evolutionary relationships among various species were examined across different taxonomic levels. Yellow dots represent taxa that did not exhibit significant changes, while green and red dots indicate taxa with significant differences. B: Only bacterial taxa with LDA values greater than 3 are displayed.

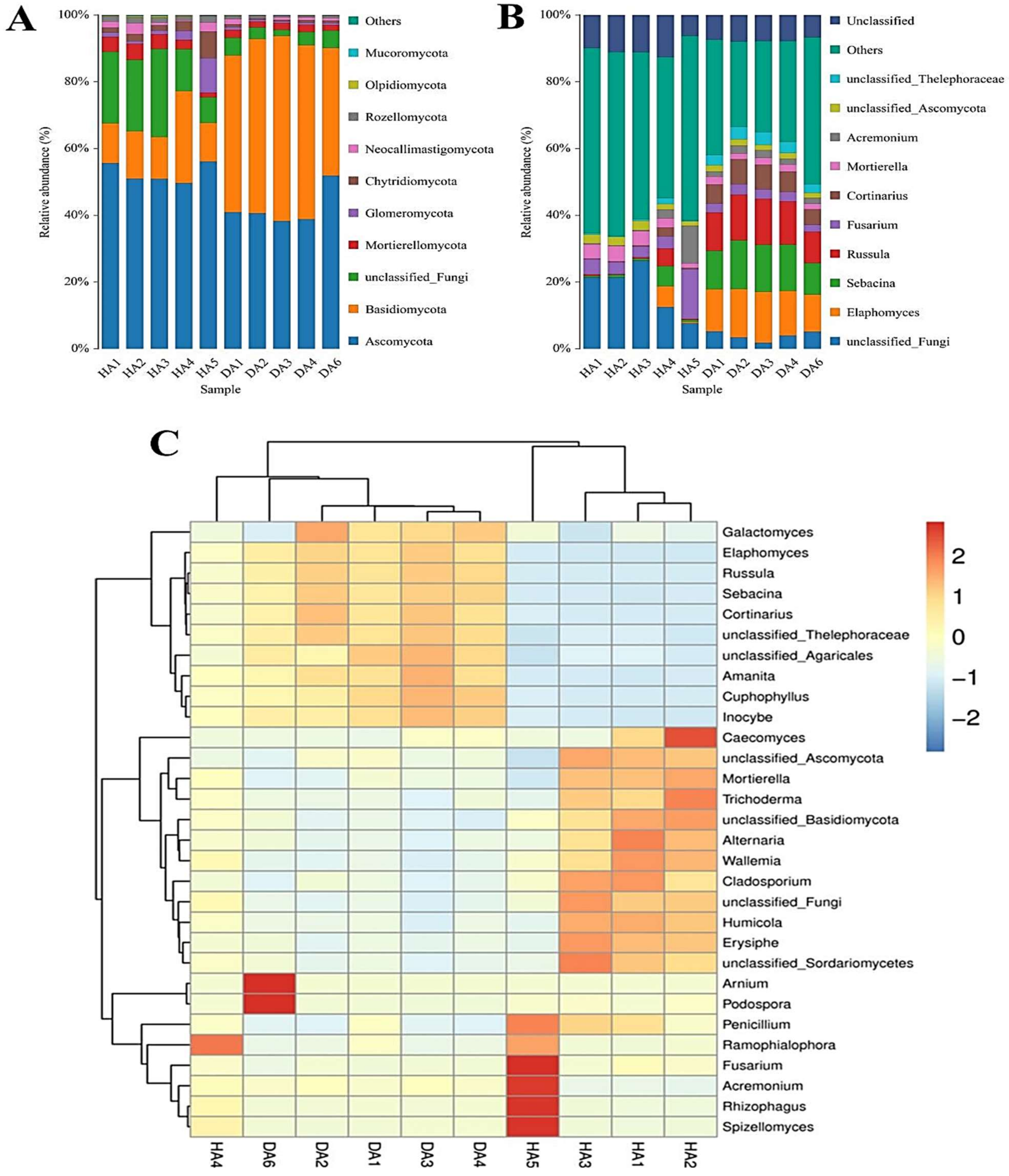

Fig. 6: Composition of dominant fungi in the gut microbiota of the DA and HA at the phylum and genera levels. A: Phylum level. B: Genus level. C: Cluster heatmap.

At the phyla level, the DA showed significantly higher abundance *Basidiomycota*, whereas the HA enriched for *Rozellomycota*, *unclassified_Fungi*, *Ascomycota*, *Mucoromycota*, *Olpidiomycota*, *Mortierellomycota* and *Chytridiomycota* (Fig. 7A). Moreover, 185 genera were identified to be significantly different between HA and DA (Fig. 7B). Among these, the relative abundances of 62 (*Inosperma*, *Russula*, *Cortinarius*, *Sebacina*, *Trichoglossum*, *Cenococcum*, *Hodophilus*, *Inocybe*, *Tuber*, *Pseudocraterellus*, *Iodophanus*, *Amanita*, *Cuphophyllus*, *Laccaria*, *Hygrocybe*, *Genea*, *Thelephora*, *Helvella*, *Keithomyces*, *Tricholoma*, *Pezicula*, etc.) genera dramatically increased, while the relative abundances of 123 (*Wallemia*, *Candida*, *Thermomyces*, *Cephalotrichum*, *Ceratobasidium*, *Lecanicillium*, *Aureobasidiu*, *Stagonospora*, *Penicillium*, *Diutina*, *Olpidium*, *Solicoccozyma*, *Metacordyceps*, *Selenophoma*, *Microscypha*, *Humicola*, *Alpinaria*, *Cladorrhinum*, *Comoclathris*, *Alternaria*, *Phaeosphaeria*, *Phaeococcomyces*, *Papiliotrema*, *Chloridium*, *Vishniacozyma*, *Sonoraphlyctis*, *Erysiphe*, *Ilyonectria*, *Chaetomium*, *Dactylonectria*, *Sarocladium*, *Corneriella*, etc.) genera dramatically reduced during diarrhea. Using LEfSe to further identify the differential bacteria associated with diarrhea (Fig. 8).

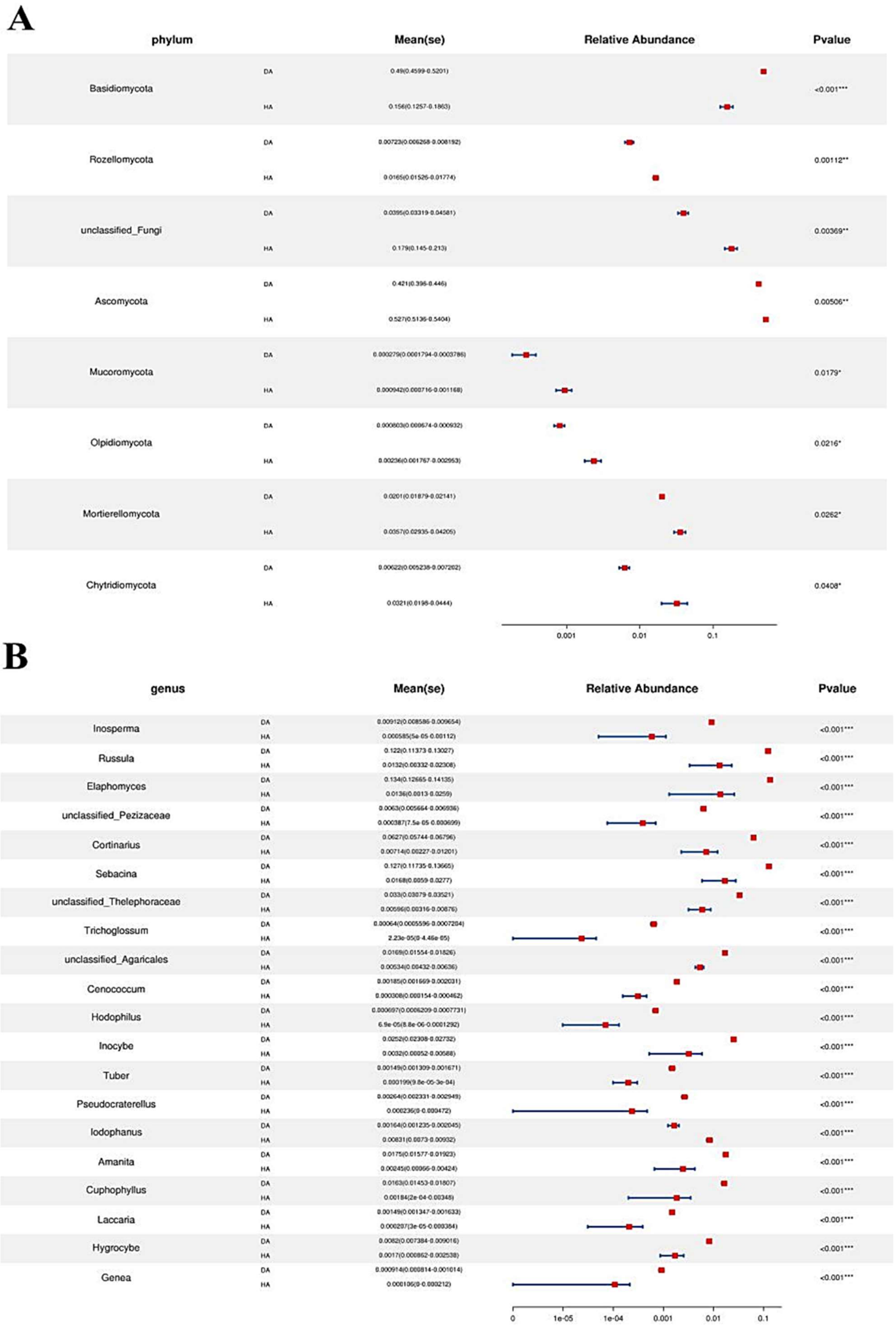

Fig. 7: Statistical analysis of significantly different intestinal fungal phyla (A) and genera (B) associated with yak diarrhea.

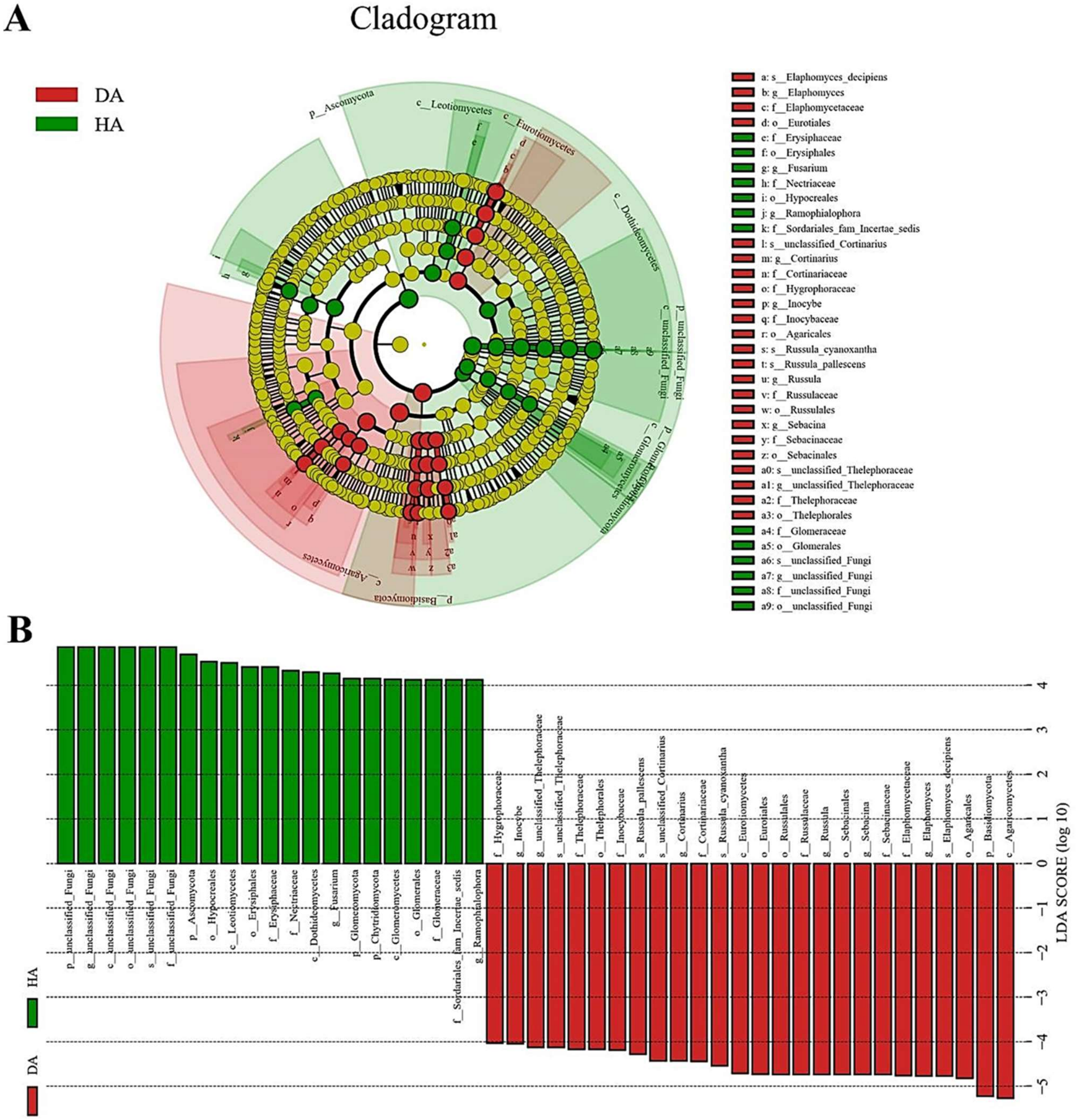

Fig. 8: The microbial taxa that exhibited significant changes during yak diarrhea were analyzed using the LEfSe. A: The evolutionary relationships among various species were examined across different taxonomic levels. Yellow dots represent taxa that did not exhibit significant changes, while green and red dots indicate taxa with significant differences. B: Only taxa with LDA values greater than 3 are displayed.

Correlation Network Analysis: In the gut bacterial community, *Dorea showed* a positive association with *Clostridium_sensu_stricto_1* (0.79), *unclassified_Bacteroidales_RF16_group* (0.78), *uncultured_Parabacteroides_sp* (0.76) and *unclassified_Lachnospiraceae* (0.72) but negatively related to *unclassified_Oscillospirales* (-0.89), *Candidatus_Soleaferrea* (-0.75), *unclassified_F082* (-0.73) and *Akkermansia* (-0.70) (Fig. 9A). *Ruminococcus* was inversely related to *unclassified_p_2534_18B5_gut_group* (-0.73). *Candidatus_Soleaferrea* was positively associated with *Akkermansia* (0.95), *unclassified_UCG_010* (0.87), *Monoglobus* (0.87) and *unclassified_Oscillospirales* (0.75).

In the gut fungal community, *Aspergillus showed* a positive association with *Wallemia* (0.96), *unclassified_Basidiomycota* (0.95), *unclassified_Leotiomycetes* (0.95), *unclassified_Leotiomycetes* (0.95) and *Alternaria* (0.93) but negatively correlated with *Elaphomyces* (-0.98), *Cortinarius* (-0.96), *Inosperma* (-0.96), *Russula* (-0.95) and *Hygrocybe* (-0.93) (Fig. 9B). *Inosperma* was positively associated with *Cortinarius* (0.98) *Elaphomyces* (0.97), *Sebacina* (0.96), *Amanita* (0.95), *Hygrocybe* (0.95), and *Alternaria* (-0.95) but negatively correlated with *unclassified_Basidiomycota* (-0.93) and *unclassified_Fungi* d(-0.96). *Hygrocybe* was positively related to *Cuphophyllus* (0.98), *Amanita* (0.97), *Elaphomyces* (0.95), *Sebacina* (0.95), *Cortinarius* (0.95), *Inocybe* (0.92), but negatively correlated with *unclassified_Basidiomycota* (-0.95), *unclassified_Fungi* (-0.93) and *Alternaria* (-0.93).

DISCUSSION

The rapid economic development and increasing population size have accelerated the growth of the animal husbandry industry, particularly the beef cattle sector, effectively addressing nutritional needs. Statistically, China's beef cattle output is projected to reach 50.99 million heads in 2024, ranking third globally. Moreover, China's annual beef cattle consumption exceeds 10 million tons, with both production and consumption on the rise. Yaks, an essential breed of beef cattle, serve as a vital source of meat protein for local residents (Xiong *et al*., 2022). However, owing to the limited pasture resources and the extreme, changeable climate of the Qingzang Plateau, yaks are extremely susceptible to diarrhea (Luo *et al*., 2024; Zhang *et al*., 2024). Diarrhea is a main cause of yak deaths, which seriously affects the development of the yak industry. Presently, although many methods have been taken to prevent diarrhea in yaks, the effect has been minimal. Recently, studies on diarrheal animals such as horses, sheep, and pigs found that the intestinal microorganisms were significantly changed (Zhou *et al*., 2023; Zhu *et al*., 2024). Yet, up to now, no studies have indicated the connection between yak diarrhea and gut microbiota. Here, we discussed the variation of gut microbiota in diarrheal yaks.

Previous studies indicated that some diseases, such as diarrhea, colitis, and intestinal cancer, could sharply change the diversity of gut microbiota and further cause gut microbial imbalance. For example, Ma *et al*. (2020) showed that diarrhea significantly reduces the gut microbial diversity of rats. Wang *et al*. also demonstrated that diarrhea had a great influence on the diversity of gut microbiota in weaned piglets (Wang *et al*., 2023). Similarly, the present study also found that diarrhea can sharply reduce the diversity of intestinal fungi in yaks. Some studies have confirmed that a reduction in gut microbial diversity threatens host health from many perspectives. For example, several chronic diseases, including obesity, mastitis, and Alzheimer's disease, are closely related to low gut microbial diversity (Qiu *et al*., 2024). Moreover, the reduction of gut microbiota may weaken the competitive inhibition of pathogenic bacteria and further increase the incidence of infections (Yao *et al*., 2025). Also, the reduction of diversity in gut microbiota may lead to an increase in pro-inflammatory factor release and a decrease in anti-inflammatory factors, further inducing systemic inflammation (Yang *et al*., 2021). More seriously, gut microbial imbalance can destroy the intestinal barrier and further disrupt immune homeostasis, which may increase the susceptibility of the host to other diseases.

This study found significant changes in some bacteria and fungi, which could be important for the development of diarrhea. However, dominant bacterial and fungal phyla in yak rumen microbiota did not change with different health statuses. This is very important because the dominant taxa identified in yaks are also abundant in other ruminants, indicating that these are vital for the intestinal ecosystem and function. However, we identified two bacterial phyla (down-regulated: *Firmicutes*, *Cyanobacteria*) and eight fungal phyla (up-regulated: *Basidiomycota*; down-regulated: *Rozellomycota*, *unclassified_Fungi*, *Ascomycota*, *Mucoromycota*, *Olpidiomycota*, *Mortierellomycota*, *Chytridiomycota*) whose relative abundances changed significantly due to diarrhea. Previous studies have mentioned that members of *Firmicutes* are mainly responsible for carbohydrate degradation and are highly essential for animal growth (Sun *et al*., 2023). *Firmicutes* comprises a large number of beneficial bacteria that play a crucial role in host immunity, metabolism, digestion, and maintenance of gut microbial homeostasis (Jordan *et al*., 2023). On the other hand, members of *Chytridiomycota* are mainly responsible for cellulose and chitin decomposition. Previous studies have also mentioned that weight loss is one of the symptoms of diarrhea in yaks. Under hypoxic conditions of the plateau, yaks need more energy to maintain their growth. But a significantly decreased abundance of bacteria and fungi involved in material digestion may affect yak growth negatively. Thus, changes in these bacterial and fungal phyla can be associated with the observed weight loss in yaks.

Notably, we also observed significant changes in several beneficial bacteria during episodes of diarrhea, including *Dorea*, *Defluviitaleaceae*, *Ruminococcus*, *Rikenellaceae*, *Bacteroides*, *Lachnospiraceae*, and *Prevotellaceae*. These changes are essential for maintaining gut health and function. *Dorea* participates in dietary fiber fermentation and plays a crucial role in adjusting gut microbial homeostasis and intestinal barrier function. *Defluviitaleaceae*, which is widely distributed in the mammalian intestine, has significant medical and ecological functions, with its abundance being regulated by dietary factors. Previous studies have indicated that *Defluviitaleaceae* can prevent urinary tract infections, potentially by inhibiting the colonization of pathogenic bacteria. *Ruminococcus* is capable of degrading cellulose and assisting the host in digesting resistant starch and high-fiber foods, thereby maintaining intestinal barrier stability (Walls *et al*., 2023). Additionally, *Ruminococcus* is regarded as a core player in intestinal metabolism, contributing to the reduction of risks associated with allergies and colorectal cancer (Nie *et al*., 2025). *Rikenellaceae*, prevalent in the mammalian intestine, particularly in the cecum and colon, plays a vital role in maintaining intestinal homeostasis (Liu *et al*., 2024). *Bacteroides* has been shown to sustain gut homeostasis by degrading starch and polysaccharides (Brown *et al*., 2023). Moreover, several studies on *Bacteroides* have indicated its role in alleviating colitis (Wang *et al*., 2024). *Lachnospiraceae* efficiently degrade complex polysaccharides and collaborate with lactic acid bacteria to maintain intestinal flora balance (Yang *et al*., 2025). *Prevotellaceae* possesses the ability to digest proteins and complex carbohydrates (Cuevas-Sierra *et al*., 2020). Notably, several bacteria that significantly decrease during diarrhea, including *Lachnospiraceae*, *Defluviitaleaceae*, *Dorea*, *Ruminococcus*, *Rikenellaceae*, *Bacteroides*, and *Prevotellaceae*, have been shown to promote or produce short-chain fatty acids (SCFAs). Previous studies have reported that SCFAs promote the proliferation of colon epithelial cells, enhance tight junction protein expression, and reduce the risk of leaky gut (Chen *et al*., 2023; Yu *et al*., 2024). Meanwhile, SCFAs play a crucial role in keeping intestinal environments acidic and preventing pathogen colonization (Zhan *et al*., 2022). Recent studies emphasized their pivotal role in protection against cardiovascular disease, cholesterol reduction, maintenance of intestinal flora balance, and anti-inflammation activities (Abdo *et al*., 2023). This finding agrees with earlier observations by Li *et al.* (2021), who demonstrated distinct decrease in SCFA-producing bacteria abundance in diarrheal giraffes. Conversely, potential pathogens including *Acinetobacter* and *Paeniclostridium* were significantly enhanced in diarrheal animals, consistent with secondary bacterial overgrowth. *Acinetobacter* is one of the core conditional pathogens linked to several hospital infections due to multidrug resistance, outbreak, and high mortality rates (Zarrilli *et al*., 2018). Also, it may affect the blood, respiratory tract, and urinary tract of infected individuals (Hong *et al*., 2023). *Paeniclostridium* can induce systemic toxic shock syndrome, resulting in multiple organ failure among infected individuals; interestingly, its mortality rate is nearly 100% among such infected individuals during delivery (Gonzalez-Astudillo *et al*., 2023). The hemorrhagic toxin produced by *Paeniclostridium* causes specific binding to colonic epithelial receptors, inducing hemorrhagic lesions of the intestine (Nyaoke *et al*., 2020). Previous studies have reported that even though the gut fungal community accounts for about 0.1% of the total gut microbial community, it is indeed essential for host health (Mahtab *et al*., 2021). Accordingly, in this study, we also found significant variation in gut fungi within the yak diarrhea group. These evidence indicated that alterations of gut microbiota might be critical factors that cause diarrhea.

Conclusions: Altogether, this research examines the changes of gut microbiota in diarrheal yaks. Results indicated that the composition and structure of gut bacteria and fungi have undergone significant changes, with the alterations in gut fungi being particularly evident. This research represents a fulfillment in understanding the characteristics and changes of yak gut microbiota during diarrhea and demonstrated that gut microbiota may play an important role in diarrhea. In addition, this study provides a foundation for further research on the prevention and control of diarrhea based on gut microbiota.

Authors contribution: DW and JZ provided the research idea. HM and ZS contributed reagents, materials, and analysis tools. MR, RAEM and EAI revised the manuscript. All authors participated in writing and reviewing the manuscript.

Acknowledgements: This research was funded by the Tibet Autonomous Region Academy of Agricultural and Animal Husbandry Sciences Animal Husbandry and Veterinary Research Institute Coordination Project (XZNKY-XMSTC-2025-003), Agricultural and Animal Husbandry Science and Technology Innovation Project - Plateau Livestock and Poultry Disease Prevention and Control and New Drug Research and Development Innovation Team (XZNKY-2025-CXGC-T20), Key R&D and transformation projects in Tibet Autonomous Region (XZ202301ZY00016N-01).

This research was funded by Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2026R890), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia. The authors extend their appreciation to the Deanship of Research and Graduate Studies at King Khalid University for funding this work through Small Research Project under grant number (R. G. P. 1/234/46).

Conflict of interest: The authors declare that they have no competing interests.

REFERENCES

Abdo A, Zhang C, Al-Dalali S, *et al*., 2023. Marine chitosan-oligosaccharide ameliorated plasma cholesterol in hypercholesterolemic hamsters by modifying the gut microflora, bile acids, and short-chain fatty acids. Nutrients 15: 2923.

Adil M, Hassan QU, Naseer A, *et al.,* 2023. Unmasking bacterial defenders: cutting-edge antimicrobial resistance detection innovations. Continental Vet J 3(2): 55-70

Bian S, Zhu S, Lu J, *et al*., 2025. Targeting gut microbiota in non-alcoholic fatty liver disease (NAFLD): Pathogenesis and therapeutic insights: A review. Int J Biol Macromol. 330:147995.

Brown H, DeVeaux A, Juliano B, *et al*., 2023. Bogh13a(sus) from bacteroides ovatus represents a novel alpha-amylase used for bacteroides starch breakdown in the human gut. Cell Mol Life Sci 80: 232.

Chang R, Zhao W, Zhou B, *et al*., 2025. Environmental fluoride exposure and bone metabolism: molecular pathways and health implications. J Environ Sci 11.010.

Chen X, Qiao T, Mao Z, *et al*., 2023. Caffeic acid improves intestinal barrier functions by regulating colonic bacteria and tight junction protein expression and alleviating inflammation in weaning piglets. Anim Biotechnol 34: 3693-9.

Cuevas-Sierra A, Riezu-Boj J, Guruceaga E, *et al*., 2020. Sex-specific associations between gut prevotellaceae and host genetics on adiposity. Microorganisms 8:938.

Gonzalez-Astudillo V, Asin-Ros J, Moore J, *et al*., 2023. Paeniclostridium sordellii-associated peripartum metritis in goats. Vet Pathol 60: 69-74.

Hong Y, Lin X, Zhang C, *et al*., 2023. Initial indicators for the prognosis of *Acinetobacter baumannii* bacteremia in children. BMC Infect Dis 23: 640.

Hou J, Wu P, Cai J, *et al*., 2025. Gut microbiota dysbiosis amplifies thiram hepatotoxicity via a mitochondrial-autophagy-apoptosis nexus orchestrated by the gut-liver axis. Cell Signal 136:112104.

Hu Y, Chen Z, Xu C, *et al*., 2022. Disturbances of the gut microbiota and microbiota-derived metabolites in inflammatory bowel disease. Nutrients 14.

Hui H, Wu Y, Zheng T, *et al*., 2020. Bacterial characteristics in intestinal contents of antibiotic-associated diarrhea mice treated with qiweibaizhu powder. Med Sci Monitor 26: e921771.

Jordan C, Brown R, Larkinson M, *et al*., 2023. Symbiotic firmicutes establish mutualism with the host via innate tolerance and resistance to control systemic immunity. Cell Host Microbe 31: 1433-49.

Li A, Liu B, Li F, *et al*., 2021. Integrated bacterial and fungal diversity analysis reveals the gut microbial alterations in diarrheic giraffes. Front Microbiol 12: 712092.

Li W, Zeng W, Zhang Y, *et al*., 2023. A comparative metabolomics analysis of domestic yak (bos grunniens) milk with human breast milk. Front Vet Sci 10: 1207950.

Liu M, Fu J, Liu Y, *et al*., 2024. Pectin from comfrey roots alleviate dss-induced ulcerative colitis in mice through modulating the intestinal barrier. Int J Biol Macromol 282: 137016.

Liu X, Yang J, Yan Y, *et al*., 2024. Effects of yeast peptides on the growth, antioxidant and anti-inflammatory capacities, immune function, and diarrhea status of suckling calves. Front Vet Sci 11: 1454839.

Li Y, Liu K, Li Y, *et al*., 2025. The gut microbiota-derived metabolites regulate bone extracellular matrix homeostasis: Mechanisms and therapeutic implications. Cell Signal 138:112245.

Luo M, Han Y, Chen Y, *et al*., 2024. Unveiling the role of gut microbiota in curcumin metabolism using antibiotic-treated mice. Food Chem 460:140706.

Luo R, Dan W, Luo Z, *et al*., 2024. Alterations in the diversity and composition of the fecal microbiota of domestic yaks (bos grunniens) with pasture alteration-induced diarrhea. BMC Vet Res 20: 355.

Ma Z, Wang H, Ma X, *et al*., 2020. Modulation of gut microbiota and intestinal barrier function during alleviation of antibiotic-associated diarrhea with rhizoma zingiber officinale (ginger) extract. Food Funct 11: 10839-51.

Mehnaz S, Sajid MS, Aslam MA, *et al.,* 2023. Culicoides: A neglected parasite of prime importance in selected areas of Pakistan. Continental Vet J 3(2):86-93.

Mahtab N, Zhou L, Zhang F, *et al*., 2021. Seasonal variations in the gut fungal communities of hooded crane (grus monacha) at wintering and stopover sites in china. Animals 11: 941.

Ni M, Peng L, Zhang Y, *et al*., 2025. Comparative lipidomics analysis of human colostrum, mature milk and yak mature milk. Food Chem 476: 143396.

Nie H, Zhao M, Wu T, *et al*., 2025. Elevated mevalonolactone from ruminococcus torques contributes to metabolically unhealthy obesity development. J Biol Chem 301: 110281.

Nyaoke A, Navarro M, Fresneda K, *et al*., 2020. Paeniclostridium (clostridium) sordellii-associated enterocolitis in 7 horses. J Vet Diagn Invest 32: 239-45.

Qiu M, Li S, Xiao Y, *et al*., 2022. Pathogenic and metagenomic evaluations reveal the correlations of porcine epidemic diarrhea virus, porcine kobuvirus and porcine astroviruses with neonatal piglet diarrhea. Microb Pathog 170: 105703.

Qiu M, Ye C, Zhao X, *et al*., 2024. Succinate exacerbates mastitis in mice via extracellular vesicles derived from the gut microbiota: a potential new mechanism for mastitis. J Nanobiotechnology 22:712.

Quintero B, Corredor F, Salas S, *et al*., 2019. High prevalence of persistently infected animals from bovine viral diarrhea in colombian cattle. BMC Vet Res 15: 23.

Sun R, Niu H, Sun M, *et al*., 2022. Effects of bacillus subtilis natto jlcc513 on gut microbiota and intestinal barrier function in obese rats. J Appl Microbiol 133: 3634-44.

Sun Y, Zhang S, Nie Q, *et al*., 2023. Gut firmicutes: relationship with dietary fiber and role in host homeostasis. Crit Rev Food Sci Nutr 63: 12073-88.

Walls L, Otoupal P, Ledesma-Amaro R, *et al*., 2023. Bioconversion of cellulose into bisabolene using ruminococcus flavefaciens and rhodosporidium toruloides. Bioresour Technol 368:128216.

Wang C, Guo H, Bai J, *et al*., 2024. The roles of different bacteroides uniformis strains in alleviating dss-induced ulcerative colitis and related functional genes. Food Funct 15: 3327-39.

Wang C, Wei S, Chen N, *et al*., 2022. Characteristics of gut microbiota in pigs with different breeds, growth periods and genders. Microb Biotechnol 15: 793-804.

Wang D, Zeng J, Wujin C, *et al*., 2024. Lactobacillus reuteri derived from horse alleviates escherichia coli-induced diarrhea by modulating gut microbiota. Microb Pathog 188: 106541.

Wang M, Zheng H, Wang S, *et al*., 2023. Comparative analysis of changes in diarrhea and gut microbiota in beigang pigs. Microb Pathog 185:106441.

Wang Q, Dong K, Wu Y, *et al*., 2022. Exploring the formation mechanism of off-flavor of irradiated yak meat based on metabolomics. Food Chem X 16: 100494.

Xiong L, Pei J, Wang X, *et al*., 2022. Lipidomics and transcriptome reveal the effects of feeding systems on fatty acids in yak's meat. Foods 11:2582.

Yang J, Ren H, Cao J, *et al*., 2025. Gut commensal lachnospiraceae bacteria contribute to anti-colitis effects of lactiplantibacillus plantarum exopolysaccharides. Int J Biol Macromol 309: 142815.

Yang R, Jia Q, Mehmood S, *et al*., 2021. Genistein ameliorates inflammation and insulin resistance through mediation of gut microbiota composition in type 2 diabetic mice. Eur J Nutr 60:2155-68.

Yao K, Zheng L, Chen W, *et al*., 2025. Characteristics, pathogenic and therapeutic role of gut microbiota in immunoglobulin a nephropathy. Front Immunol 16:1438683.

Yu Y, Zi Y, Fu R, *et al*., 2024. Effects of dietary energy levels on microorganisms and short-chain fatty acids of rumen and tight junction proteins in honghe yellow cattle. Front Microbiol 15: 1335818.

Zarrilli R, Bagattini M, Esposito E, *et al*., 2018. Acinetobacter infections in neonates. Curr Infect Dis Rep 20: 48.

Zhan Z, Tang H, Zhang Y, *et al*., 2022. Potential of gut-derived short-chain fatty acids to control enteric pathogens. Front Microbiol 13:976406.

Zhang J, Shi B, Lu S, *et al*., 2024. Metagenomic analysis for exploring the potential of lactobacillus yoelii fyl1 to mitigate bacterial diarrhea and changes in the gut microbiota of juvenile yaks. Microb Pathog 186: 106496.

Zhang L, Liu Y, Sun Y, *et al*., 2022. Combined physical exercise and diet: regulation of gut microbiota to prevent and treat of metabolic disease: a review. Nutrients 14:4774.

Zhao G, Zhang Y, Wang K, *et al*., 2025. Yak (bos grunniens) meat peptides: effects on immunity, hypoxia tolerance, and antioxidant capacity in hypotonic hypoxia mice. Food Sci Nutr 13: e70522.

Zhou H, Yu B, Sun J, *et al*., 2023. Gut microbiota absence and transplantation affect diarrhea: an investigation in the germ-free piglet model. Anim Biotechnol 34: 3971-7.

Zhu J, Sun Y, Ma L, *et al*., 2024. Comparative analysis of fecal microbiota between diarrhea and non-diarrhea piglets reveals biomarkers of gut microbiota associated with diarrhea. Anim Nutr 19: 401-10.