



RESEARCH ARTICLE

Combined Analysis of Gut Microbiota and Inflammation Levels Reveals the Key Microorganisms Associated with Diarrhea in Hainan Black Goat Kids

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ABSTRACT

Diarrhea in young animals is frequently associated with gut microbiota dysbiosis, severely impacting their health and causing economic losses. However, the key gut microorganisms associated with diarrhea in Hainan black goat kids remain insufficiently understood. To identify potential microbial indicators linked to diarrhea, this study investigated the relationships among gut microbiota, levels of inflammation, and diarrheal status in healthy (n=10) and diarrheic (n=10) goat kids. The results demonstrated that the gut microbial diversity in diarrheic goat kids was significantly lower compared to healthy goat kids. LefSe and t-test analyses identified three bacterial genera that differed significantly between healthy and diarrheic goat kids. Of these, *Escherichia_Shigella* was enriched in diarrheic goat kids, while *Bacteroides* and *Fournierella* were enriched in healthy goat kids. Serum levels of TNF- α , IL-1 β , and IL-6 were significantly higher in diarrheic goat kids. Correlation analysis revealed that TNF- α were positively correlated with the abundance of *Escherichia_Shigella*, and negatively correlated with *Bacteroides*, and *Fournierella*. Notably, *Bacteroides* and *Fournierella* exhibited a negative correlation with *Escherichia_Shigella*. ROC analysis further evaluated *Escherichia_Shigella*, *Bacteroides*, and *Fournierella* as potentially key microorganisms for predicting the occurrence of diarrhea in goat kids. In conclusion, diarrheic goat kids exhibit gut microbiota dysbiosis and increased inflammatory responses. Targeting the abundance of *Escherichia_Shigella*, *Bacteroides*, and *Fournierella* may offer a promising microbial strategy for the prevention and treatment of diarrhea in Hainan black goat kids, and the applicability of this finding to other breeds warrants further investigation.

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INTRODUCTION

The sustained global growth in goat milk and meat production and consumption underscores the critical role of goats in nutrition, health, and food security (Christopher, 2023). In goat farming, the kid stage is a crucial period for growth and development (Abdelsattar *et al.*, 2023). However, diarrhea in goat kids has long been a major barrier to improving production efficiency. Studies have shown that the incidence of diarrhea in lambs within 30 days of birth is 34.21% and the mortality rate is 15.69% (Mariano *et al.*, 2018), and in goats the incidence of diarrhea is also as high as 36.7%, posing a serious threat to

the economic efficiency of farming (Mahmood *et al.*, 2014). Therefore, investigating the causes of diarrhea in goat kids is of significant importance for promoting the development of the current goat industry and the livestock sector. Diarrhea is commonly associated with gut microbial dysbiosis, primarily manifested as an imbalance between beneficial and pathogenic microorganisms (Li *et al.*, 2021). Studies in pigs have shown that diarrheic pigs exhibit a significant reduction in beneficial gut bacteria compared to healthy pigs (Kong *et al.*, 2021; Wang *et al.*, 2023a). Similar observations have been made in calves, where diarrheic calves display a higher relative abundance of pathogenic bacteria such as *Escherichia_Shigella* and

Streptococcus in their fecal microbiota compared to healthy calves (Gomez *et al.*, 2022). These studies indicate that diarrhea is closely associated with imbalances in the gut microbiota. Therefore, regulating gut microbial dysbiosis is a key strategy for alleviating diarrhea. Hainan black goat is a dominant caprine species in Hainan Province, which distinguishes itself with remarkable characteristics such as adaptability to coarse fodder and superior meat quality (Quan *et al.*, 2019). However, diarrhea has been a significant constraint on the development of the Hainan black goat industry. Currently, there is limited research on the relationship between diarrhea and gut microbiota in Hainan black goat kids, which prompted us to initiate this study.

Diarrhea is frequently associated with elevated host inflammatory levels and impaired immune function (Yue *et al.*, 2011). Studies have demonstrated that serum levels of pro-inflammatory cytokines, such as tumor necrosis factor- α (TNF- α), interleukin-1 beta (IL-1 β), and interleukin-6 (IL-6) are significantly elevated in diarrhea calves compared to healthy counterparts (Albayrak and Kabu, 2006). Conversely, diarrheic calves exhibit significantly lower serum levels of total protein (TP) and albumin (ALB) than healthy calves (Choi *et al.*, 2021). Alterations in the gut microbiota are closely intertwined with inflammatory responses (Dai *et al.*, 2021). In the piglet diarrhea model induced by ETEC infection, the addition of fermented soybean meal can effectively alleviate diarrhea symptoms by modulating the intestinal microbiota and the expression of inflammatory cytokines (Wang *et al.*, 2020). Consequently, a combined analysis of gut microbiota and inflammation levels offers a more effective approach to identify key gut microorganisms that hold potential for mitigating diarrhea.

In the present study, we collected rectal fecal and serum samples from healthy and diarrheic Hainan black goat kids. By comparing differences in the gut microbiome and inflammation levels between the two groups and performing correlation analyses, we identified key gut microbes associated with diarrhea, providing novel insights into the early diagnosis and preventive strategies for Hainan black goat kids diarrhea.

MATERIALS AND METHODS

Animal and sample: The experiment was conducted at a goat breeding farm in Hainan Province, China. The subjects comprised 30 kid goats (Singleton). The kids were ear-tagged at birth and housed separately with their mothers (third parity, with mature reproductive performance) in the same pen for two weeks. During the entire experimental period, both the goat kids and pregnant does were not exposed to any antibiotics or pathogenic bacteria and were allowed to feed and drink freely. The feces of goat kids were classified into four levels: 0, normal (pellet); 1, pasty (clumpy); 2, semiliquid (mushy consistency); and 3, liquid (completely liquid stools). The fecal scores were based on descriptions from Sun *et al.* (Sun *et al.*, 2008). Three experienced farm investigators diagnosed whether the goats had diarrhea based on diarrhea scores of ≥ 2 recorded on two consecutive days. Diarrhea occurred in 10 out of 30 goat kids. Finally, fresh fecal samples were collected from healthy (n=10; 6 males and 4

females) and diarrheic (n=10; 6 males and 4 females) kid goats of the same sex. The samples were collected by inserting a sterile cotton swab into the rectum. On the same day, 2–3 mL blood samples were collected from the jugular vein, allowed to clot at ambient temperature for 30 minutes, and then centrifuged at 3000 rpm for 15 minutes at 4°C to separate the serum. All serum samples were stored at -80°C until further analysis. All selected animals underwent the same immunization procedures and had no other diseases prior to sample collection.

Microbial sample preparation and sequencing:

Genomic DNA was extracted from rectal feces using the Universal DNA Purification Kit (DP214-03). The V3-V4 hypervariable regions of bacterial 16S rRNA genes were amplified using universal primers 341F (5'-CCTAYGGGRBGCASCAG-3') and 806R (5'-GGACTACNNGGGTATCTAAT-3'). Thermal cycling parameters consisted of an initial denaturation at 98°C for 1 minute, followed by 30 cycles of denaturation at 98°C for 10 seconds, annealing at 50°C for 30 seconds, and extension at 72°C for 30 seconds, followed by a final extension at 72°C for 5 minutes. Libraries were prepared using the NEBNext Ultra II DNA Library Prep Kit (NEB, E7645B), validated using Qubit and qPCR, and sequenced on an Illumina NovaSeq 6000 (PE250).

Gut microbiome analysis: Using fastp (v0.23.1), raw reads were quality-filtered to obtain clean tags. Denoising with DADA2 in QIIME2 (v2022.2) generated ASVs, which were taxonomically annotated based on the Silva 138.1 database. A phylogenetic tree was constructed using multiple sequence alignment within QIIME2. Venn diagrams were generated in R using the VennDiagram function. Alpha diversity indices were calculated using QIIME2, while beta diversity was analyzed using weighted Jaccard distance-based Principal Coordinates Analysis (PCoA) in R (v4.0.3). Differential taxa across taxonomic levels were identified with LEfSe and t-test. Spearman correlation analysis was applied to examine associations between dominant microbial species and immune indicators. Functional predictions were performed with PICRUSt (v1.1.4).

Receiver Operating Characteristic (ROC) Analysis:

ROC analysis was conducted on differentially abundant bacterial genera using the pROC package, and the results were visualized using ggplot2 (version 3.6.3). AUC values, ranging from 0.5 to 1, indicate diagnostic value, with higher values signifying greater diagnostic accuracy. AUC values of 0.5-0.7, 0.7-0.9, and >0.9 indicated low, high, and very high diagnostic value, respectively.

Serum biochemical parameter measurements: Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and TP levels were quantified using a Hitachi 3500 automated biochemical analyzer. Cytokine levels for TNF- α , IL-1 β , and IL-6 were determined via enzyme-linked immunosorbent assay (ELISA) kits (Xiamen Lianshuo Biotechnology) according to the manufacturer's protocols.

Statistical analysis: Data were analyzed using GraphPad Prism (v8.0) and SPSS Statistics (v27.0.1). Results are

expressed as the mean \pm standard error of the mean (SEM). Statistical significance was defined as $P < 0.05$.

RESULTS

Sequence analysis of gut microbiota in healthy and diarrheic goat kids: Sequencing generated a total of 2,123,631 raw reads (healthy group: 1,040,619; diarrheic group: 1,083,012). After quality filtering, 2,055,617 high-quality reads were retained for subsequent analysis (healthy group: 1,006,266; diarrheic group: 1,049,351). The GC content of all sequences exceeded 50%, while Q20 and Q30 scores were greater than 98.32% and 94.38%, respectively, indicating high base-calling accuracy (Table 1). The rarefaction curves approached saturation (Fig. 1A), demonstrating adequate sequencing depth and sample homogeneity. Venn analysis revealed 620 and 335 ASVs in healthy and diarrheic goat kids, respectively, with only 208 ASVs shared between groups (27.84% of total ASVs; Fig. 1B), indicating substantial differences in gut bacterial communities between health states.

Analysis of gut microbial diversity in healthy and diarrheic goat kids: Alpha and beta diversity metrics were used to examine differences in microbial communities between healthy and diarrheic groups. Compared to the healthy group, the diarrheic group exhibited significantly lower Chao1, Shannon, and Simpson indices (Fig. 2A). PCoA revealed a near-complete separation of samples from the diarrheic and healthy groups (Fig. 2B).

Comparative analysis of gut microbial composition in healthy and diarrheic goat kids: Healthy and diarrheic goat kids were examined at different taxonomic levels to characterize the composition of their gut microbial populations. At the phylum level, the top four bacterial phyla in terms of relative abundance in the healthy and diarrheic groups were Firmicutes (46.54% vs. 45.91%), Proteobacteria (23.54% vs. 40.55%), Bacteroidota (26.60% vs. 12.8%), and Actinobacteriota (2.57% vs. 0.64%) (Fig. 3A). At the genus level, the core bacterial genera in the healthy and diarrhea groups, including *Escherichia_Shigella* (16.75% vs. 40.19%), *[Ruminococcus]_gnavus_group* (7.26% vs. 16.91%), *Bacteroides* (25.55% vs. 12.49%), and *Butyrivibrio* (10.68% vs. 9.85%), account for over 60% of the total microbiota in both groups (Fig. 3B). Additionally, Gram-negative bacteria accounted for 58.86% in healthy and 62.6% in diarrheic groups. The proportion of *Escherichia_Shigella* within Gram-negative bacteria was higher in the diarrheic group than in the healthy group (60% vs. 28%) (Fig. 3C, D).

Differentially abundant genera between healthy and diarrheic goat kids: LEfSe analysis (LDA score=4) identified 4 genera with significantly differential abundances. Specifically, *Bacteroides*, *Comamonas*, and *Fournierella* were significantly enriched in healthy goat kids, while *Escherichia_Shigella* was significantly enriched in diarrheic goat kids (Fig. 4A). T-test analysis further identified 5 differentially abundant genera. *Escherichia_Shigella* exhibited the most pronounced

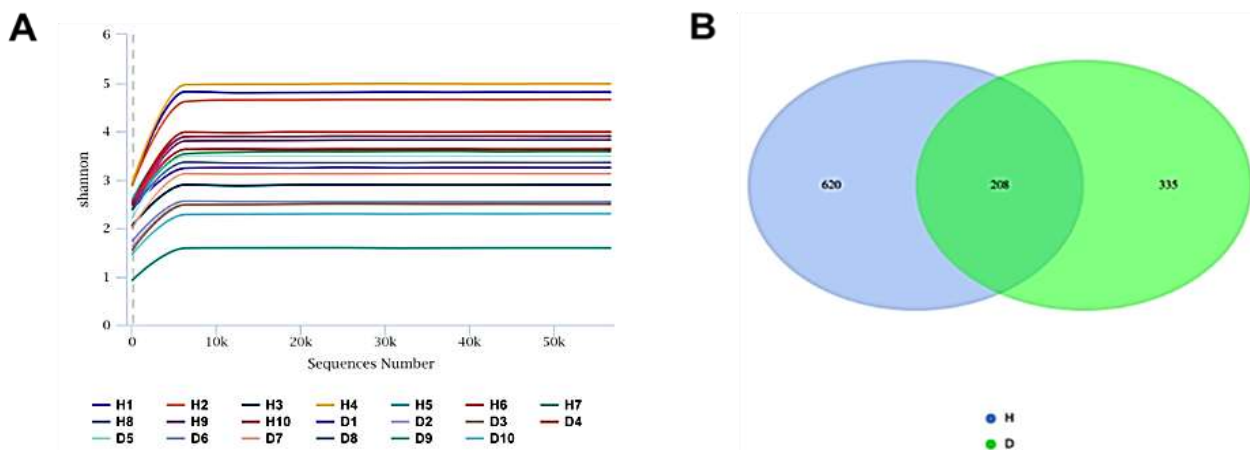


Fig. 1: Comparative sequence analysis of the gut microbiota in healthy (group H) and diarrheic (group D) of goat kids. (A) Rarefaction curves. (B) Venn diagram.

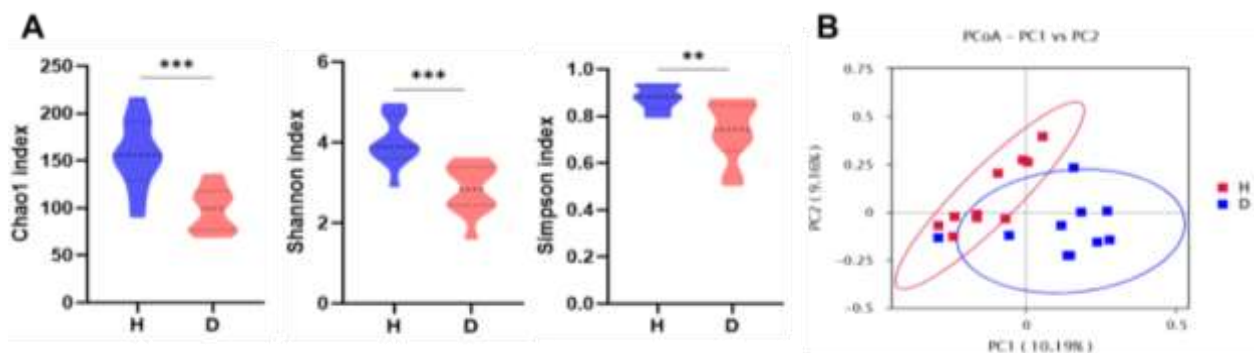


Fig. 2: Comparative analysis of gut microbial diversity in healthy (group H) and diarrheic (group D) goat kids. (A) Alpha diversity indices: Chao1, Shannon, and Simpson (* $P < 0.01$, *** $P < 0.001$). (B) PCoA based on Jaccard distance.

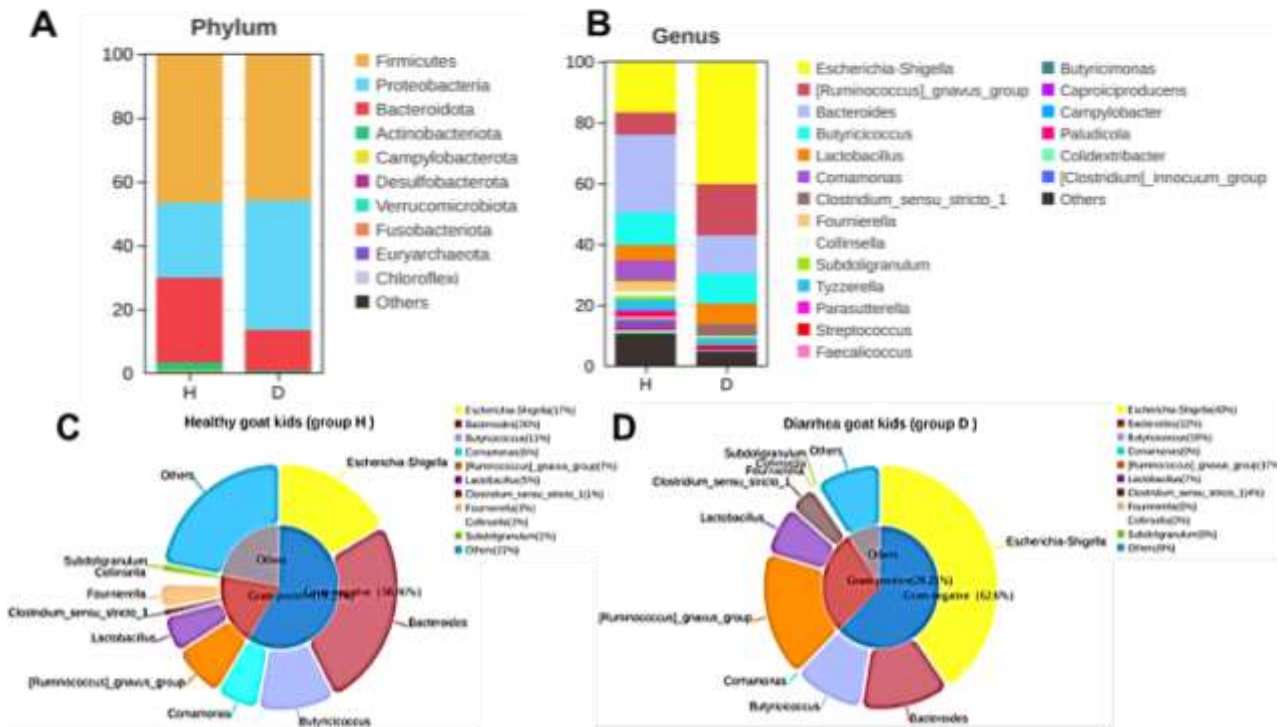


Fig. 3: Distribution of gut microorganisms at the phylum (top 10) and genus levels (top 20) in healthy (group H) and diarrheic (group D) goat kids. (A, B) Differences in microbial community structure and abundance at the phylum and genus levels. (C, D) Relative abundance and distribution of the top 10 genera at the genus level.

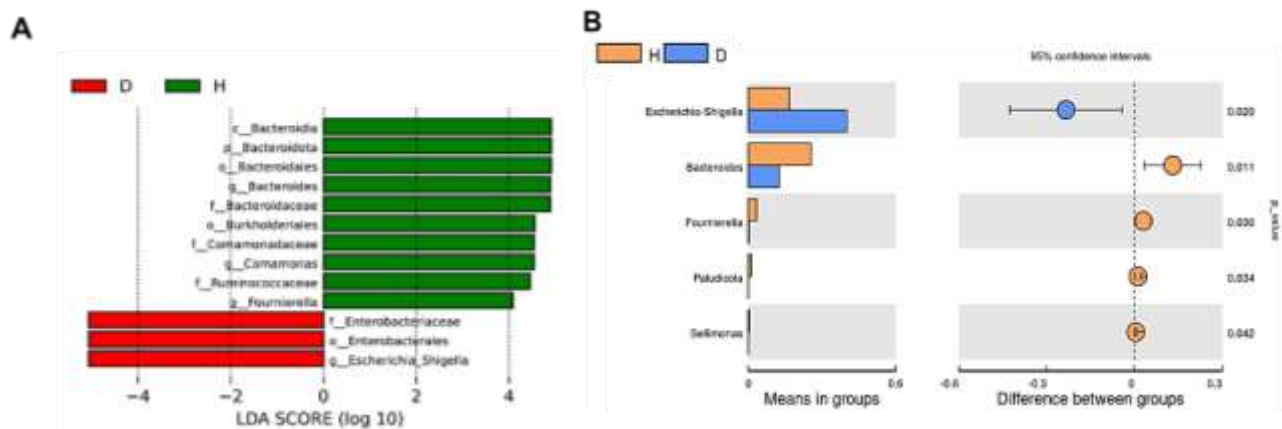


Fig. 4: Differential genera abundance in healthy (group H) and diarrheic (group D) goat kids. (A) Linear Discriminant Analysis (LDA) score histogram. (B) T-test analysis chart showing group comparisons.

Table I: Sequencing information of healthy (group H) and diarrheic (group D) samples.

Sample	RawPE	Combined	Qualified	Avglen(nt)	GC	Q20	Q30
H1	102085	101330	98747	414.94	0.521	0.985	0.9483
H2	105399	104467	101770	417.43	0.5144	0.9859	0.9502
H3	102762	102203	99844	422.83	0.541	0.9869	0.9537
H4	104175	103043	100430	418.86	0.5057	0.9845	0.9472
H5	102743	101839	98822	416.03	0.5106	0.9844	0.9462
H6	105203	104440	101937	417.85	0.5239	0.9866	0.9528
H7	103190	102416	99770	422.5	0.5166	0.9861	0.9509
H8	107685	106941	104115	419.45	0.5325	0.9858	0.9502
H9	103690	102839	100284	416.66	0.5153	0.9832	0.9438
H10	103687	103059	100547	416.22	0.5132	0.9863	0.9519
D1	118078	117267	114254	413.24	0.5336	0.9857	0.951
D2	103301	102655	99879	422.24	0.5388	0.9849	0.948
D3	102908	102231	99546	421.57	0.5382	0.9862	0.9515
D4	102217	101623	99497	412.36	0.5218	0.9876	0.9559
D5	115890	115252	112424	418.59	0.5204	0.9863	0.9522
D6	105551	104837	102400	416.08	0.534	0.9865	0.9513
D7	104359	103651	101255	420.37	0.5104	0.9868	0.9525
D8	115904	115248	112629	416.02	0.5244	0.9871	0.9547
D9	109191	108564	105461	428.75	0.5407	0.9847	0.9457
D10	105613	104906	102006	425.63	0.536	0.9858	0.9507

difference, being significantly increased in the diarrheic group. In contrast, *Bacteroides*, *Fournierella*, *Paludicola* and *Sellimonas* were significantly decreased in the diarrheic group (Fig. 4B). Both analyses consistently showed significant differences in the relative abundances of *Escherichia Shigella*, *Bacteroides*, and *Fournierella* between the healthy and diarrheic groups.

Functional prediction of gut microbiota in healthy and diarrheic goat kids: Microbial functional differences between healthy and diarrheic goat kids were analyzed using PICRUSt metagenomic prediction. Compared to healthy goat kids, diarrheic goat kids exhibited marked downregulation of amino acid metabolism pathways (including arginine, proline, pyruvate, glycine, serine, and threonine metabolism) and associated enzymatic activities. In contrast, transcription factor-related pathways and DNA replication, recombination, and repair protein pathways were significantly upregulated (Fig. 5).

Changes in serum parameters between healthy and diarrheic goat kids: Compared to healthy goat kids, diarrheic goat kids exhibited significantly elevated serum levels of the pro-inflammatory cytokines TNF- α , IL-1 β , and IL-6 (Fig. 6A). Compared to healthy goat kids, diarrheic goat kids showed a trend towards increased serum levels of ALT and AST, along with a trend towards decreased serum levels of TP (Fig. 6B).

Association between serum parameters and gut microbiota: Spearman's correlation analysis revealed associations between gut microbiota and serum parameters (Fig. 7A). *Bacteroides*, *Fournierella*, and *Paludicola* were negatively correlated with TNF- α and IL-1 β . *Comamonas*, *Faecalicoccus*, *Butyricoccus*

Caproiciproducens, and *Caproiciproducens* showed negative correlations with TNF- α . *Colidextribacter* was negatively correlated with IL-1 β and IL-6. *Streptococcus* was negatively correlated with ALT. Notably, *Escherichia Shigella* exhibited a positive correlation with TNF- α , and *Campylobacter* exhibited a positive correlation with AST. The ROC analysis revealed that *Escherichia Shigella*, which was significantly increased in the diarrheic group, exhibited an AUC value >0.81 (Fig. 7B). Conversely, *Bacteroides* exhibited AUC values >0.83, and *Fournierella* exhibited AUC values >0.89 in the diarrhea group (Fig. 7C). It is worth noting that inverse correlations were observed between the relative abundances of *Bacteroides* and *Fournierella* with *Escherichia Shigella* (Fig. 7D).

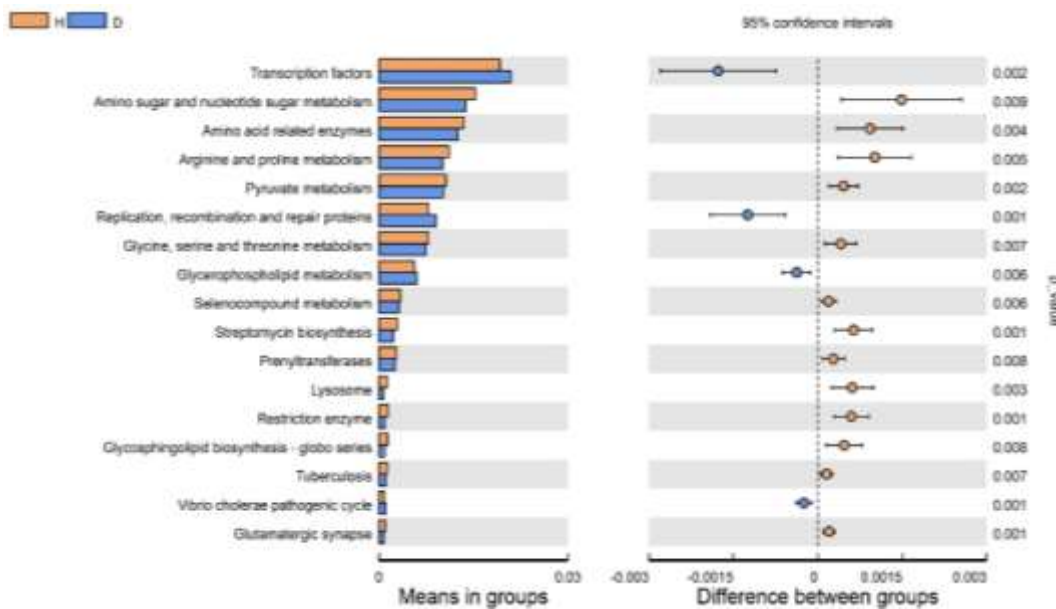


Fig. 5: Differential functional pathways of fecal microbiota in healthy (group H) and diarrheic (group D) goat kids predicted via PICRUSt. Pathway enrichment differences were analyzed using a t-test (P<0.01).

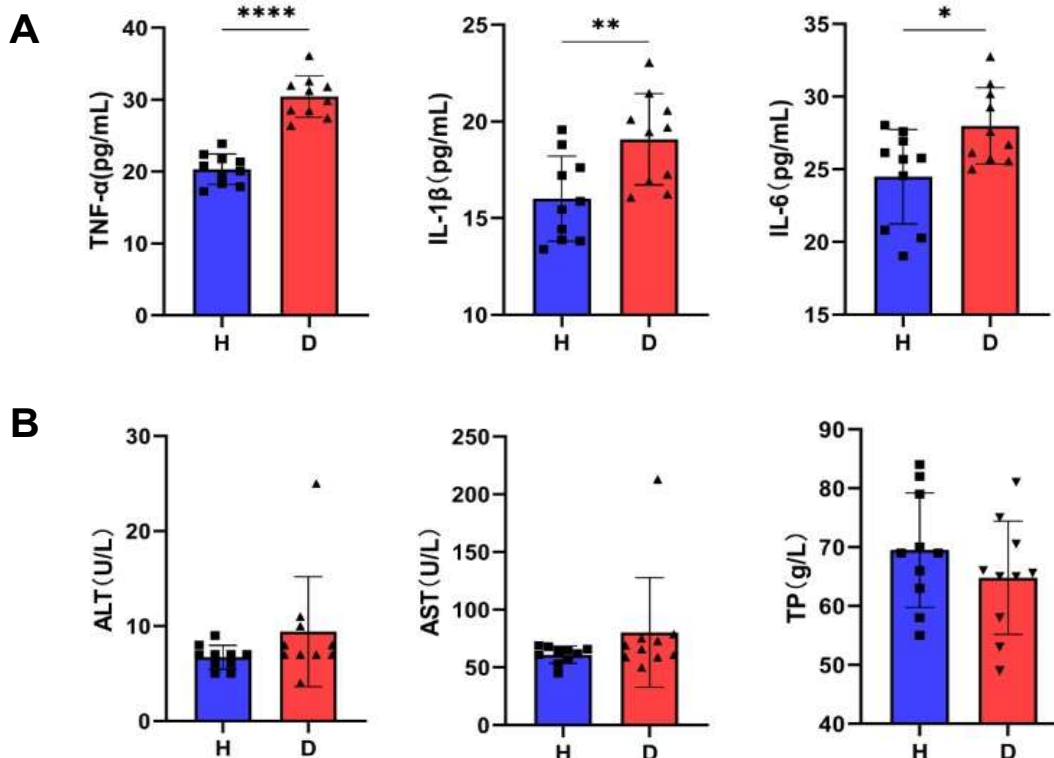


Fig. 6: Changes in immune indicators in healthy (group H) and diarrheic (group D) goat kids. (A) Levels of TNF- α , IL-1 β , and IL-6. (B) Serum levels of ALT, AST, and TP (* P<0.05, ** P<0.01, **** P<0.0001).

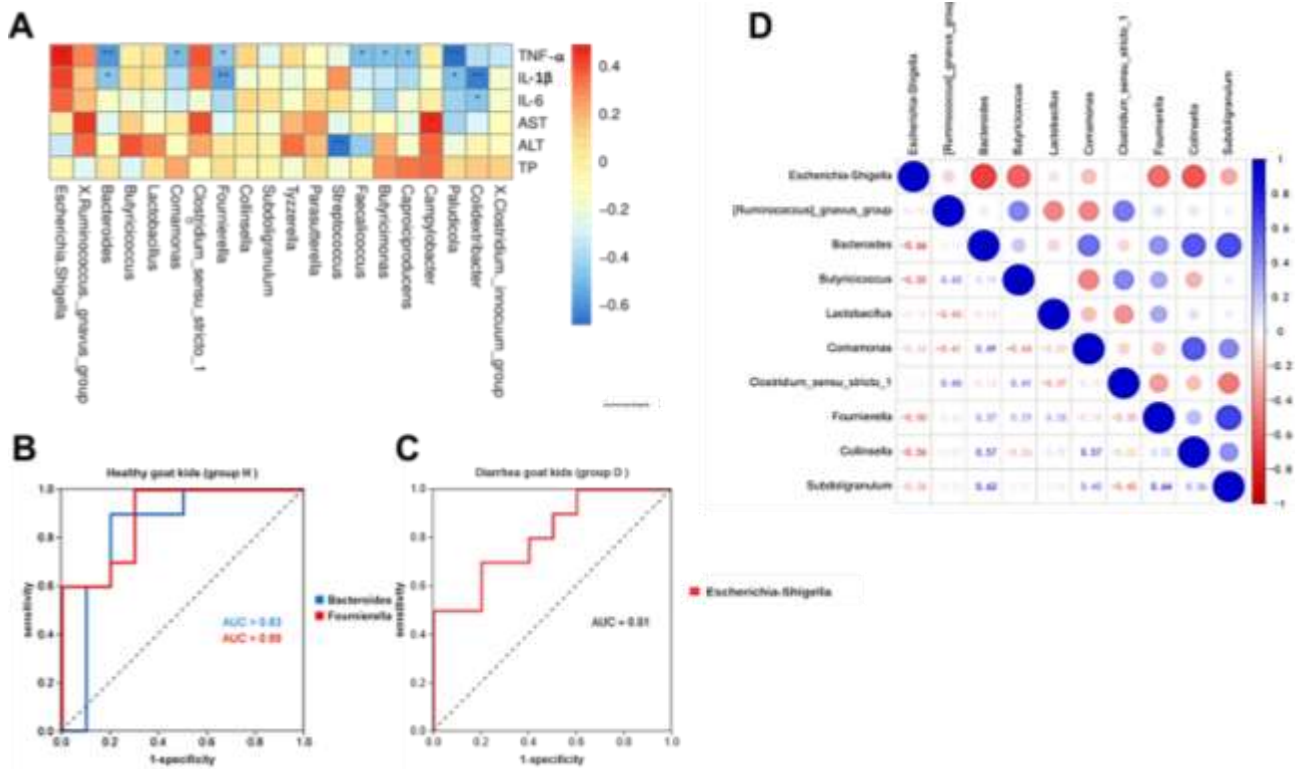


Fig. 7: Association between serum immune markers and gut microbiota in healthy (group H) and diarrhea (group D) groups. (A) Spearman correlation analysis assesses relationships between gut microbiota and immune markers, with red and blue are used to represent positive and negative correlations, respectively. (B, C) ROC analysis identify key gut microbiota associated with diarrhea. (D) The relationships between the abundances of different bacterial genera were assessed using Spearman's correlation coefficient.

DISCUSSION

The gut microbiota can significantly impact overall health through digestion, absorption, immune response, and infection prevention (Alqahtani *et al.*, 2024). Disturbances in the gut microbiota disrupt immune homeostasis, promoting inflammatory responses and is a key factor in the development of diarrhea (Wang *et al.*, 2018). As a core economic category in global ruminant livestock farming, goats are currently facing a persistent production crisis due to diarrhea in goat kids. However, the specific gut microbiota associated with diarrhea in Hainan black goat kids is not yet fully understood. Therefore, we conducted an investigation into the gut microbiota of diarrheic Hainan black goat kids.

Compared to mucosal immunity, serum immunity better reflects the overall health status of the body. Diarrhea can induce an enhanced systemic inflammatory response, and both gut microbiota and serum inflammatory factors play important roles in the pathogenesis of diarrhea (Zhang *et al.*, 2017). Dysregulation of the gut microbiota can lead to increased levels of inflammation in the host, where TNF- α , IL-1 β , and IL-6 are considered key mediators of the inflammatory response (Hernandez-Rodriguez *et al.*, 2004). Our findings indicate that the levels of the inflammatory cytokines in diarrheic goat kids are significantly higher than those in healthy goat kids. This is consistent with the findings of Beheshtipour *et al.* in their study on diarrheic cattle (Beheshtipour and Raeeszadeh, 2020). The elevated level of inflammation can impair gut barrier function through mechanisms such as increased cellular permeability and disruption of tight junctions (Anbazhagan *et al.*, 2018; Kaminsky *et al.*, 2021). Studies

have shown that ALT and AST levels can serve as key indicators for assessing systemic inflammatory responses (Kerner *et al.*, 2005). Compared to healthy goat kids, diarrheic goat kids exhibited a rising trend in the levels of ALT and AST. In contrast, TP is a key indicator of the passive immune status in calves (Todd *et al.*, 2010), showed a decreasing trend in diarrheic goat kids. Collectively, our results demonstrate a significant enhancement of the inflammatory response in diarrheic goat kids, this may be related to the imbalance of gut microbiota.

Compared to healthy goat kids, those with diarrhea showed a significant reduction in both the diversity and abundance of their gut microbiota. This indicates the occurrence of gut microbiota dysbiosis in diarrheic goat kids. At the phylum level, we found that Firmicutes, Proteobacteria, and Bacteroidota were the dominant phyla in both healthy and diarrheic goat kids, accounting for over 95% of the total abundance. This observation aligns with the findings of Wang *et al.* in goat kids (Wang *et al.*, 2023b). Firmicutes plays a crucial role in the degradation and digestion of animal fibers and cellulose, facilitating the digestion and absorption of nutrients (Sun *et al.*, 2023). Bacteroidota, considered a beneficial bacterium in the gut, not only promotes carbohydrate digestion and absorption but also regulates the host's immune system (Clausen *et al.*, 2024). Proteobacteria is regarded as a microbial signature of gut microbiota dysbiosis. It consists of many Gram-negative bacteria, such as *Escherichia coli*, *Campylobacter concisus*, and *enterohepatic Helicobacter* are strongly linked to the onset of inflammatory bowel disease (Mukhopadhyaya *et al.*, 2012). Our findings indicated that the relative abundance of Proteobacteria was increased in

Hainan black goat kids compared to healthy Hainan black goat kids, while the relative abundance of Bacteroidota was markedly reduced. The increased abundance of Proteobacteria in the gut may increase the susceptibility of diarrheic Hainan black goat kids to pathogenic infection.

At the genus level, the gut microbiota of diarrheic goat kids exhibited significant changes compared to healthy goat kids. Three key bacterial genera were identified through combined statistical tests and correlation analysis. Among them, *Escherichia Shigella* exhibited the largest difference in relative abundance between the two groups and was significantly positively correlated with TNF- α . Furthermore, *Escherichia Shigella* also exhibited significant enrichment in the gut of diarrheic calves (Li *et al.*, 2023). In-depth mechanistic studies have shown that *Escherichia Shigella* can cause gut microbiota dysbiosis by altering the gut environment, promoting the colonization of anaerobic microorganisms, and reducing the abundance of beneficial bacteria (Ma *et al.*, 2022). However, fecal samples primarily reflect the microbial composition of the distal gut, and there can be significant differences in microbial composition among different parts of the intestine (Jiang *et al.*, 2020). Therefore, further research is needed to determine whether targeting *Escherichia Shigella* can serve as a strategy for regulating intestinal homeostasis.

In addition, *Bacteroides* was the most significantly different genus in the healthy group and was significantly negatively correlated with TNF- α and IL-1 β . Our findings align with those reported by Ren *et al.*, who confirmed that a low abundance of *Bacteroides* is a critical microbial marker for inducing diarrhea in weaned piglets (Ren *et al.*, 2022). *Bacteroides* primarily regulates the levels of pro-inflammatory cytokines and maintains gut immune homeostasis by producing short-chain fatty acids (SCFAs) (Cheng *et al.*, 2022). Notably, recent studies have demonstrated that oral administration of *Bacteroides fragilis* to mice is effective in preventing and alleviating diarrhea (Cheng *et al.*, 2025). Overall, *Bacteroides*, as an important probiotic for maintaining gut health, deserves further in-depth investigation for its potential application in preventing and treating diarrhea. *Fournierella*, as one of the differentially abundant genera, was significantly negatively correlated with TNF- α and IL-1 β . Its relative abundance was also significantly reduced in diarrheic calves (Cui *et al.*, 2023). In addition, Liu *et al.* found that *Fournierella* has immunomodulatory capabilities and has been shown to play a role in maintaining gut homeostasis in chickens (Liu *et al.*, 2023). Collectively, our findings reveal an imbalanced gut microbiota in Hainan black goat kids, is strongly associated with the onset of diarrhea. Moreover, ROC analysis and Spearman correlation analysis results further revealed that *Escherichia Shigella*, *Bacteroides*, and *Fournierella* might be crucial bacterial genera for diagnosing diarrhea in Hainan black goat kids. In summary, our results suggest that augmenting the abundance of *Bacteroides* and *Fournierella* could exert a potential intervention effect on diarrhea by suppressing the proliferation of *Escherichia-Shigella*.

This study provides important insights for the early diagnosis and prevention strategies of diarrhea in intensively reared, confined goat kids in tropical regions. However, its applicability to other rearing systems (such as

outdoor grazing) or different breeds of goat kids requires further investigation. Further, key microbial strains associated with diarrhea and their potential applications warrant in-depth research.

Conclusions: Diarrhea in goat kids is closely associated with elevated systemic inflammation levels and gut microbiota dysbiosis. *Escherichia Shigella*, *Bacteroides*, and *Fournierella* may serve as key gut microbiota for the dynamic monitoring of diarrhea in Hainan black goat kids. Modulating the abundance of these bacterial genera could represent a potential strategy for managing diarrhea in Hainan black goat kids, and the applicability of this study to other breeds requires further investigation.

Ethics approval: All animal procedures and sampling methods used in this study were approved by the Hainan University Ethics Committee. Approval number: HNUAUCC-2025-00332.

Data availability statement: Data Availability Statement: All raw sequence data are deposited in the NCBI Sequence Read Archive and the BioSample database (PRJNA1253364).

Competing interests: The authors declare no competing interests.

Authors contribution: LBL designed the experiments. LBL, QL, KPL, XX, XLC, and GZ collected samples. QL, and KPL performed the experiments. QL, XX, and XLC performed analysis. QL and KPL drafted the manuscript. LBL revised the manuscript. All authors reviewed and approved the final version of the manuscript.

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