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Exploring age-related changes in gut bacterial community composition of yak: insights from different age groups

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Abstract

Background The Qinghai-Tibetan Plateau (QTP) offers one of the most extreme environments for yaks (*Bos grunniens*). The yak is an indigenous species, and the wild yak was domesticated on the QTP. The gut microbiota plays a vital role in health and animal performance. However, little is known about the progression of gut microbes in different age developmental stages of domesticated yaks.

Method We used the 16 S rRNA gene sequencing method to explore the progression of the fecal bacterial microbiota of 18 different confined domestic yaks at two developmental stages: 3 to 5 years (GT35) and 6 to 8 years (GT68).

Results We found significant differences in gut bacterial communities between the two age groups. The diversity of the gut bacterial community was significantly lower in the GT35 group, which reached stability with age. Bacteroidetes and Firmicutes were the two dominant phyla between the two age groups. Phylum Firmicutes was significantly higher in the GT68 group, and Proteobacteria, Spirochaetes, Tenericutes, and Actinobacteria were highly abundant in the GT35 age group. Genera *Bacteroides*, *Alloprevotella*, and *Anaerovibrio* were abundant in the GT35 group. The short-chain fatty acid (SCFA) producing bacteria *Rikenellaceae* showed higher abundance in GT35. The core bacterial microbiota of the GT68 age group was dominated by *Ruminococcaceae* and *Rikenellaceae*. The gut bacterial community has a great variation between the groups. Based on the exploration of dynamic changes in the gut bacterial community at different ages, our results illustrate that yaks undergo a process of reaching stability and maturity as they age.

Keywords Gut microbiota, Bacterial diversity, Maturity, Domesticated yaks

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Introduction

The yak (*Bos grunniens*) is a member of the genus Bos and is believed to have diverged from cattle between one and five million years ago. It is hypothesized that the yak is more closely related to bison than to other species within its designated genus [1]. Yaks are generously reproducing and living in a crucial environment of the plateau, domesticated by nomadic people more than 7,300 years ago [2]. Yak is the only domesticated animal among all the other livestock that can face food shortages and the harsh environment of the QTP [3]. Yaks are very ancient bovines and play a very important role in the lives of local herdsmen, and also in the ecological niche of the environment of the plateau [4]. People living on the plateau obtain meat, fuel, fur, and milk from yaks [5]. Yak milk contains caseins, an antihypertensive agent having antihypertensive activity [6]. At an altitudes between 3000 and 5500 m on the Qinghai-Tibet Plateau in China, the yak holds the utmost significance as the primary domesticated ruminant [7]. The domestication of animals played a crucial role in the shift from a hunter-gatherer lifestyle to agricultural settlement, as it offered humans a reliable and consistent supply of food, labor, and hides [8]. The Qinghai-Tibet Plateau (QTP), renowned as the roof ridge of the world, boasts an average altitude of over 4000 m. Due to the challenging climate, hypoxia, and low atmospheric pressure, the majority of plants and animals struggle to survive in this region [8, 9]. However, the Tibetan people conquered this challenging land and built a magnificent civilization, with the indispensable assistance of their domesticated yak. The yak is an Indigenous species, and the process of domesticating wild yak took place on the QTP [10]. Domestic yaks are less domesticated compared to other livestock. The yak holds significant importance as a domesticated ruminant on the QTP.

The gut microbiome is a highly intricate and essential ecosystem found in all mammals. It plays a crucial role in extracting and producing nutrients, particularly when animals consume cellulose-rich plants like the Poaceae family, which is especially significant for cattle species [11, 12]. Ruminants, including yaks, heavily rely on the rumen, a complex microbial ecosystem comprised of anaerobic bacteria, methanogens, fungi, and ciliate protozoa. This ecosystem plays a critical role in efficiently digesting crude fiber, such as cellulose, hemicelluloses, and lignin, while also synthesizing microbial proteins to provide essential energy and proteins for animals [13]. The yak intestine is primarily governed by its rumen, both in terms of physical structure and physiological functions. The rumen of yaks stands out from other intestinal segments due to its significantly high bacterial load of microbiota [14]. The complex rumen microbial community of ruminants assists in their adaptation to high-fiber plants and facilitates the fermentation of nutrients to provide the host with energy in the form of volatile fatty acids (VFAs) for growth [15]. The rumen microbial community of ruminants is influenced by a multitude of factors, including diet, age, genetics, breed, and geographical location [16, 17]. These factors have a direct or indirect influence on the rumen microbiota, which in turn is responsive to environmental variations. As a result, the physiological response of the host can be altered [18].

The improvement of rumen microbiota is closely linked to the structural changes that occur in the rumen as the animal ages, a relationship that has been demonstrated in previous studies on herbivores, particularly in yaks and Tibetan sheep [19, 20]. The establishment of the relationship between the host and rumen microbiota starts at birth with the vertical transmission of microbes from the mother. This process is widely recognized as a crucial pathway for the initial colonization and development of the microbiota in newborns [21]. Previous studies have shown that the rumen microbial communities in newborns undergo a rapid colonization process, primarily by aerobic and facultative anaerobic microbial taxa, shortly after birth. However, as the newborns age, these initial microbial populations are gradually replaced by exclusively anaerobic taxa, typically occurring between 6 and 8 weeks of age [22]. Observations have revealed the presence of cellulolytic bacteria in animals as young as 3–5 days old, and these bacteria subsequently become more prevalent in animals aged 2–3 weeks [23]. The researcher conducted a study on the ruminal microbial communities of three 14-day-old pre-ruminant calves and three 42-day-old pre-ruminant calves. The findings revealed the presence of bacteria and functions commonly observed in adult animals [24]. The rumen bacterial community was found to be influenced by age in a study conducted on bovines from birth to adulthood (2 years old) [25]. Age-related variations in the rumen microbial community were also observed in Holstein cattle ranging from 9 to 120 months of age [26]. Similarly, the rumen microbiota of pre-ruminant calves fed milk replacer was also characterized [24]. The transition of weaning calves from a liquid to a solid diet has been shown to induce rapid changes in their rumen microbiota [27, 28].

The previous studies have focused on rumen microbial communities in calves with a narrow age gap, this has limited understanding of age-related changes in the rumen microbiota [25]. and overlooked potential differences between young and adult animals. Our study not only investigates microbial shifts from juvenile to adult stages but also explores how these shifts may relate to the yak's unique adaptations to high-altitude environments like the Qinghai-Tibetan Plateau. These adaptations include dealing with hypoxia, extreme temperatures, and nutrient-poor forages, all of which likely

influence microbial community composition. Compared to other ruminants, yaks harbor unique microbial signatures that may contribute to their efficiency in energy harvesting, immune modulation, and digestion under these harsh conditions [29]. Therefore, in this study, we aimed to bridge the age gap by including a wider range of ages, from young to adulthood, to investigate the age-dependent changes of specific gut microbiota in confined domesticated yaks. Previous studies primarily concentrated on examining the diet, health, metabolism, reproduction, and productivity aspects of yaks [30–34], there has been little investigation into how gut microbial communities change across different age stages. Most studies either focus on a single age group or fail to consider age as a biological factor that could influence microbial composition. This study aimed to examine the age-related changes in the structure and composition of gut bacteria in domesticated yak across two distinct age groups, ranging from young to adulthood, from 3 to 5 and 6–8 years old. By using the 16 S rRNA gene sequencing technique, we were able to analyze the microbial composition in the gut of domesticated yaks. We hypothesized that there would be significant changes in the gut bacterial community across different developmental stages of domestic yak, especially from young to adulthood. This study will help to understand the valuable insights into the potential variations in gut microbiota between young and adult animals, as well as shed light on the interactions among microbes within different age groups of yaks. It will further help us to investigate the possibility of manipulating microbial communities to improve the productivity of animals.

Materials and methods

Site description

The trial was conducted in Minle County, located in Gansu Province, China, during the summer of 2017. Minle has an elevation of 2309.63 m (7577.53 feet) above sea level. The average yearly temperature in Minle County is 15.43°C (59.77°F), 0.81% higher than the national average in China. Typically, the county receives approximately 41.33 millimeters (1.63 inches) of precipitation annually, with 45.3 rainy days accounting for 12.41% of the year. It is renowned for its expansive grasslands and rugged mountain terrain. These diverse natural landscapes offer yaks an optimal environment, abundant with nutritious vegetation, and the ability to withstand the harsh winter conditions.

Sample collection, experimental design, diets, and management

Eighteen male yaks were randomly selected from two different age groups, i.e., 3–5 years old (GT35), and 6–8 years old (GT68) from Gansu Dehua yak fattening farm (elevation, 2,300 m) in Minle County, China. The selected animals shared the same raising protocol, i.e., the feed roughage Barley straw (10%), concentrate mix (90%), and concentrate mix feed ingredients are given in Table S1. The yaks were provided feed and water in compartments two times, i.e., 7 a.m. and 4 p.m., respectively. The animals used in this study were not genetically related or receiving antibiotic treatment. All the yaks were solely fed a combination of roughage (forage) and concentrate mix, without being allowed to graze. The collection of fresh fecal samples took place right after the animals defecated. The samples were carefully taken from the uppermost sections of the piles to prevent any soil contamination. In total, 18 fecal samples were collected, one from each of the 18 male yaks across the two age groups (GT35 and GT68). The collection was conducted using sterile gloves and sampling tubes to ensure freshness and avoid environmental contamination. The fecal samples were temporarily stored in an icebox and immediately transferred to a laboratory freezer at -80 °C. All samples passed quality control checks, and none were lost, contaminated, or excluded during the process.

Analysis of the nutritional composition of the experimental forages

The nutrient composition of forages was assessed for several parameters, including dry matter (DM), organic matter (OM), ash content, crude protein (CP), and ether extract (EE). These analyses were conducted using the method described by the Association of Analytical Chemists (AOAC) in 1990 [35]. In addition, neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined using the method outlined by Goering and Van Soest in 1970 [36]. To further evaluate the nutritional value of the forages, calculations were made for total carbohydrate (TC), non-fiber carbohydrate (NFC), and digestible dry matter (DDM). TC was calculated as 100 - (CP + EE + Ash), NFC as 100 - (NDF + CP + EE + Ash), and DDM (%) as 88.9–0.779 ADF. Hemicellulose content was derived by subtracting ADF from NDF.

DNA extraction and sequencing

The fecal samples were subjected to thorough homogenization before DNA extraction. Genomic DNA was then extracted from a 1-ml mixture containing 0.211–0.299 mg of feces and VXL buffer using a bead beater (Mini-bead Beater, Bio Spec Products, Bartlesville, UK). DNA extraction was performed using the QIAamp FastDNA Stool Mini Kit (Qiagen, Hilden, Germany). The DNA quantity and quality were assessed using the NanoDrop 2000 (Thermo Scientific, Wilmington, USA). Subsequently, DNA samples were diluted to a concentration of 80ng/µl before PCR amplification. For PCR amplification,

we used the commonly employed universal primer pair 338 F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'), which target the V3-V4 hypervariable region of the 16 S rRNA gene. This primer pair has been extensively validated in previous microbiome studies for its high specificity and broad bacterial coverage [37]. To ensure amplification efficiency and accuracy, we conducted gradient PCRs during preliminary trials to optimize the annealing temperature and cycling conditions. The PCR amplification conditions for the V3-V4 hypervariable region of the 16 S rRNA gene were as follows: an initial denaturation step at 94 °C for 3 min, followed by 30 cycles of denaturation at 94 °C for 40 s, annealing at 56 °C for 60 s, and extension at 72 °C for 60 s. A final extension step was performed at 72 °C for 10 min. The PCR products were visualized using agarose gel electrophoresis to confirm the presence of clear, specific bands of the expected size. The PCR products were purified from the gel using the GeneJET Gel Recovery Kit (Thermo Scientific, USA) following the manufacturer's instructions. The purified amplicons were utilized for library construction, and sequencing was conducted using an Illumina MiSeq system with the MiSeq Reagent Kit v2 2 × 250 bp (Illumina, San Diego, USA).

Bioinformatics and statistical analysis

The barcodes and primer sequences were truncated after the sequencing process. The raw data underwent quality filtering using QIIME (Quantitative Insights into Microbial Ecology, Version 1.9.1) software to obtain clean tags. Low-quality sequences were removed through this process [38]. To obtain valid tags, the clean tags were processed using UCHIME (version 2.4.2) software to remove chimera sequences [39]. To cluster the valid tags into Operational Taxonomic Units (OTUs), Vsearch (version 2.4.2) software was utilized, employing a 97% similarity threshold [40]. The bacterial taxa classification of the OTUs was performed using the representative sequences, which were compared against the Silva database (Version 123) (https://www.arb-silva.de/). The RDP Naive Bayesian classifier algorithm was employed for this classification process [41, 42]. In the QIIME software, a rarefaction curve was constructed to assess the sequencing depth. Additionally, bar graphs at the phylum, family, and genus levels were created using GraphPad Prism version 8.00 for Windows (www.graphpad.com/) to visualize the taxonomic composition. Using QIIME software (Version 1.9.1), alpha diversity indices including Chao1, Shannon, Simpson, and Goods-coverage were calculated. Alpha diversity and relative abundances of bacterial phyla, families, and genera between the two groups were identified through Wilcoxon's ranked test with a false discovery rate (FDR) correction. These indices provide insights into the richness, evenness, and coverage of the microbial community. The β-diversity of bacteria in the GT35 and GT68 was visualized using non-metric multidimensional scaling (NMDS) analysis based on ANOSIM and the Bray-Curtis dissimilarity matrix in the vegan and ggplot2 packages of R4.1.2. These plots provide a visual representation of the variation in microbial community composition among different samples. Significance was affirmed at ($p < 0.05$), and P values were changed using false discovery rate to eliminate false-positive results. Co-occurrence network analysis was constructed based on significant genera ($p < 0.05$) with abundance > 0.001, and R value > 0.4. The Co-occurrence network was visualized in Gephi.

Results

The chemical composition of forage pasture is presented in Table S2. The dry matter contents, ash, crude protein, ether extract, non-fiber carbohydrate, hemicellulose, OM, insoluble dietary fiber such as NDF and ADF contents, TC contents, and digestible dry matter contents of forage were the same for both GT35 and GT68 groups.

Sequencing data and bacterial diversity analysis

A total of 1,542,493 raw reads and 1,443,748 clean, high-quality reads were obtained from 18 fecal samples of confined domestic yaks, with an average of 85,694.06 raw and 80,208.22 clean sequence reads per sample, respectively, after filtering and removal of chimeric sequences. The rarefaction curve for observed species reached a plateau, indicating sufficient sequencing depth to accurately capture the microbial composition of each sample. Good's coverage exceeded 99.1%, and the average read length was 415.05 bp (Figure S1). The OTU analysis revealed 70 common OTUs across both groups, with 1,196 and 1,495 unique OTUs identified in the GT35 and GT68 groups, respectively (Figure S2).

Alpha diversity

The alpha diversity of the groups was measured using the Abundance-based Coverage Estimator (ACE), Chao1 index, Shannon index, and Simpson index. ACE and Chao1 index values for group, GT35 were 814.62 ± 102.46 and 798.68 ± 108.71, while GT68 were 1114.27 ± 149.93 and 1107.56 ± 157.11, respectively. The Shannon and Simpson index values for group, GT35, were 7.07 ± 0.56 and 0.97 ± 0.01, while GT68 were 7.71 ± 0.73 and 0.98 ± 0.01, respectively. ACE and Chao1 indices showed significant variations ($p < 0.05$) between the two different age groups (Fig. 1A). The Chao1 index was significantly lower in the GT35 group compared to the GT68 group, while the ACE index was also significantly lower in the GT35 group compared to the GT68 group. The Shannon and Simpson indices did not show significant variations

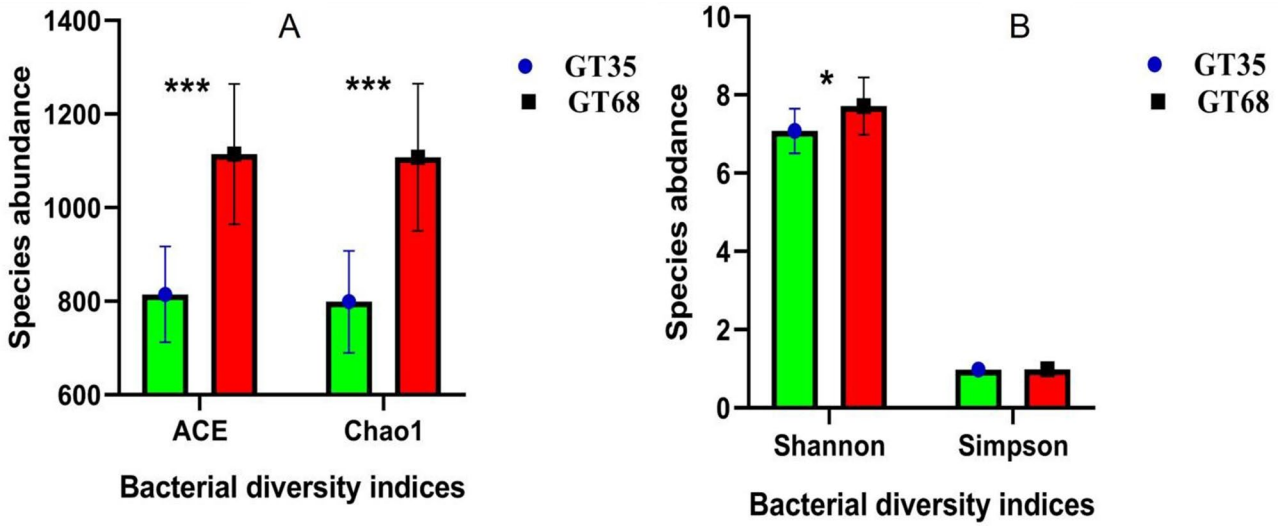

Fig. 1 (**A**) ACE and Chao1 indices of bacterial diversity, Chao1 and ACE indices were significantly lower in the GT35 group compared to GT68 group, (**B**) Shannon and Simpson indices of bacterial diversity of GT35 and GT68 yak groups, Simpson indices did not show a significant difference among groups ($p = 0.44$), ($p < 0.0001$) = ***, ($p < 0.001$) = **, ($p < 0.01$) = *

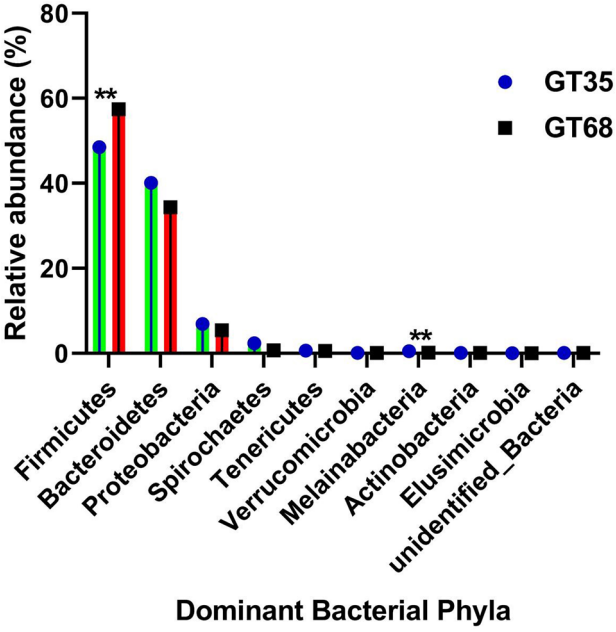

Fig. 2 Major bacterial phyla found in GT35 and GT68 yak groups, ($p < 0.0001$) = ***, ($p < 0.001$) = **, ($p < 0.01$) = *

($p < 0.05$) between the GT35 and GT68 yak groups as presented in Fig. 1B.

Beta diversity

Non-metric multidimensional scaling (NMDS) analysis revealed that the GT35 and GT68 formed distinct bacterial clusters in the ordination space (Figure S3), with significant differences at the taxonomic level ($p = 0.001$). However, the bacterial communities of GT35 were more scattered as compared to the GT68 group, indicating the dissimilarity of the taxonomy between the two groups.

Dominant gut bacterial communities

Relative abundance of bacterial phyla

At the phylum level, there were 24 bacterial phyla identified in both GT35 and GT68 groups. The most dominant bacterial phyla in GT35 were Bacteroidetes (40.09% ± 0.09), Firmicutes (48.48% ± 0.07), Spirochaetes (2.35% ± 0.02) and Proteobacteria (6.88 ± 0.04), whereas in GT68, the dominant phyla were Firmicutes (57.42% ± 0.03), Bacteroidetes (34.33% ± 0.08), and Proteobacteria (5.45% ± 0.09) (Fig. 2). Firmicutes in GT68 were significantly higher ($p < 0.001$) than in GT35, which may indicate an age-related shift towards improved fermentation and nutrient absorption, as Firmicutes are often associated with energy harvest from complex carbohydrates. However, the relative abundances of Bacteroidetes and Proteobacteria were numerically higher in GT35 compared to GT68, but the differences were not statistically significant ($p > 0.05$). The observed changes in these phyla were relatively modest and fell within the expected range of biological variation. Spirochaetes, Tenericutes, Verrucomicrobia, and Actinobacteria were less abundant phyla; the relative abundance of these phyla was higher ($p > 0.05$) in GT35 than in GT68, while the relative abundance of Elusimicrobia was higher ($p > 0.05$) in GT68 than in GT35. Melainabacteria relative abundance was also significantly higher in GT35 than in GT68, significantly higher ($p < 0.001$). Fibrobacteres and Lentisphaerae were also less abundant phyla and were higher in GT68 than in GT35 ($p > 0.05$).

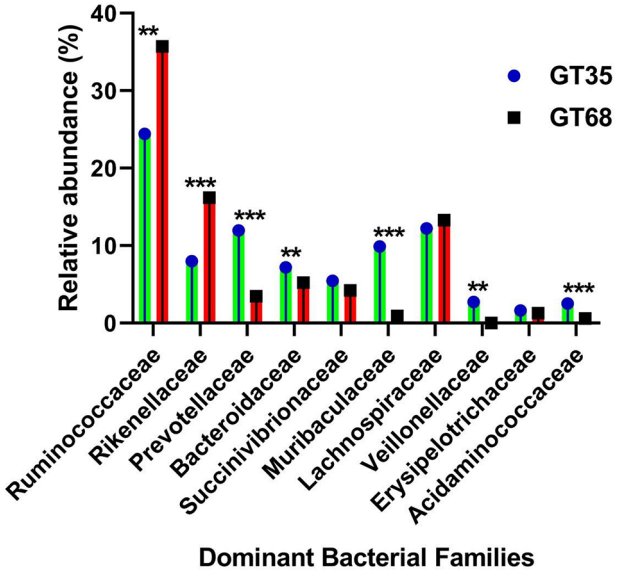

Fig. 3 Major bacterial families found in GT35 and GT68 yak groups, ($p < 0.0001$) = ***, ($p < 0.001$) = **, ($p < 0.01$) = *

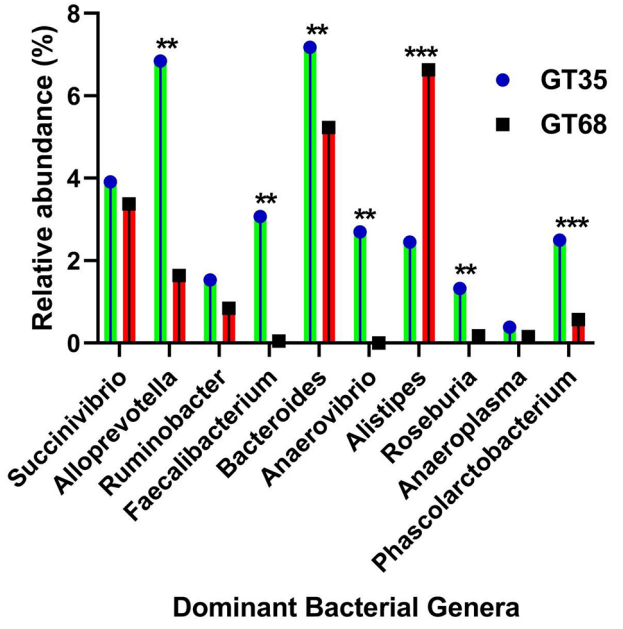

Fig. 4 Major bacterial genera found in GT35 and GT68 yak groups, ($p < 0.0001$) = ***, ($p < 0.001$) = **, ($p < 0.01$) = *

Relative abundance of bacterial families

A total of 129 families were identified in the GT35 and GT68 age groups. The most abundant families in GT35 were *Ruminococcaceae* (24.40%), *Prevotellaceae* (11.95%), *Lachnospiraceae* (12.21%), *Muribaculaceae* (9.89%), *Bacteroidaceae* (7.17%), *Succinivibrionaceae* (5.43%), *Rikenellaceae* (7.99%), *Acidaminococcaceae* (2.50%), *Tannerellaceae* (2.45%), Veillonellaceae (2.72%), and *Spirochaetaceae* (2.35%), which accounted for 89.06% of the total fecal microbiota, while in group GT68 were *Ruminococcaceae* (35.71%), *Succinivibrionaceae* (4.22%), *Prevotellaceae* (3.44%), *Lachnospiraceae* (13.28%), *Muribaculaceae* (0.9%), *Rikenellaceae* (16.19%), *Bacteroidaceae* (5.23%), *Spirochaetaceae* (0.68%), *Erysipelotrichaceae* (1.28%), *Veillonellaceae* (0.00%), accounting for 80.93% between two age groups (Fig. 3). *Ruminococcaceae* and *Rikenellaceae* were significantly higher ($p < 0.001$) in GT68 than in the GT35 group, whereas *Prevotellaceae* and *Bacteroidaceae* were significantly higher ($p < 0.001$) in GT35 than in GT68. *Succinivibrionaceae*, *Lachnospiraceae*, *Spirochaetaceae*, *Erysipelotrichaceae*, *Anaeroplasmataceae*, *Tannerellaceae*, *Acidaminococcaceae*, and *Paludibacteraceae* did not differ ($p > 0.05$) between groups with low abundances.

Overall, there were 228 bacterial genera identified in both the GT35 and GT68 groups. Bacterial genera with a relative abundance of less than 0.05 were grouped as "others." The top 30 dominant genera were observed in both groups. Among the top 30 genera, *Bacteroides*, *Alloprevotella*, *Anaerovibrio*, *Roseburia*, *Blautia*, *Tyzzerella*, *Turicibacter*, and *Sutterella* had significantly higher relative abundances in the GT35 group compared to GT68 ($p < 0.001$). On the other hand, the relative abundances of *Ruminobacter*, *Faecalibacterium*, *Parabacteroides*, and *Succinivibrio* were numerically higher in GT35, but the differences were not statistically significant ($p > 0.05$) (Fig. 4). Great variation was observed among individual animals in the prokaryotic community composition at the phylum, family, and genus levels. Linear discriminant analysis effect size (LEfSe), including LDA, was conducted to examine the differential microbial communities between the GT35 and GT68 age groups. The GT35 group samples contained Bacteroidetes, *Bacteroidaceae*, and *Bacteroides_uniformis*, while the GT68 group harbored *Romboutsia* and *Agathobacter* as biomarker taxa (Fig. 5A and B).

Co-occurrence network analysis

The co-occurrence network was constructed based on significant genera ($p < 0.05$), Spearman's, having abundance more than 0.001, and R-value ($R < 0.04$) to get insight into potential mutualistic interactions of bacteria within each group. The co-occurrence network of the GT68 group displayed that bacterial community complexity was at its peak in the GT68 group, as evidenced by the high number of nodes and edges. The total number of nodes and edges in the GT68 group were 50 and 417, and the numbers of positive and negative interactions were 235 and 182, respectively, while the co-occurrence network of the GT35 group, displayed that, the total number of nodes and edges in GT35 group were 47 and 415, and some positive and negative interactions were 203 and 212, respectively (Table 1), (Figure S4A and S4B).

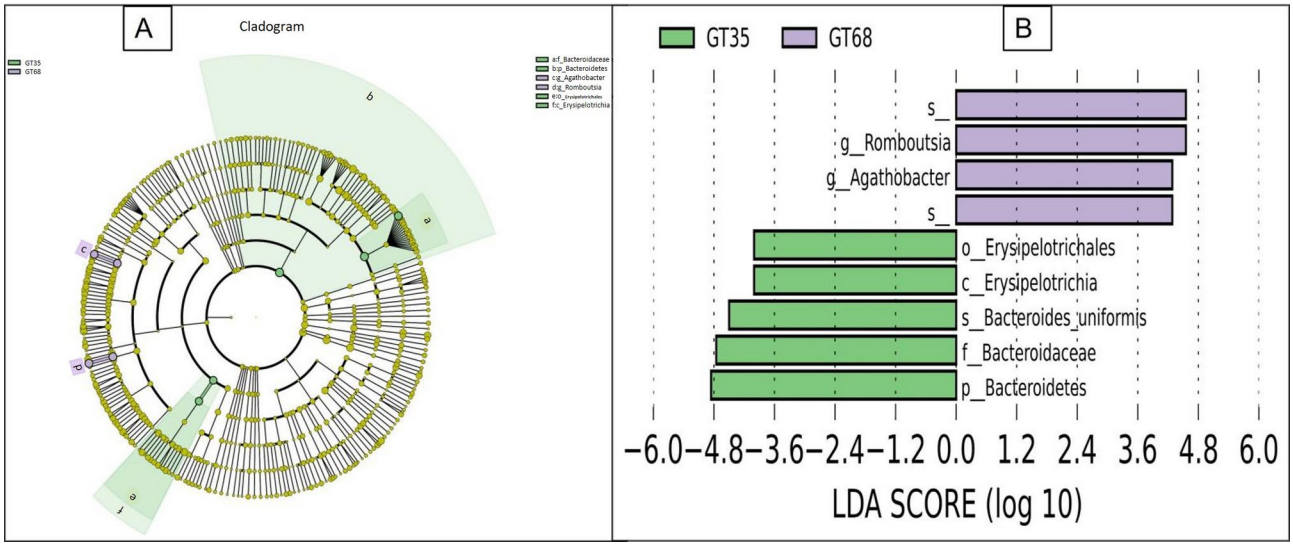

Fig. 5 Cladogram showing differential bacterial taxa (**A**), linear discriminant analysis (LDA) effect size (LEfSe) indicating biomarker taxa (**B**), in GT35 and GT68

Table 1 Topological features of co-occurrence network analysis among GT35 and GT68 at the genera level

S. No	Network attributes	GT35	GT68
1	Nodes	47	50
2	Edges	415	417
3	Average degree	17.95744681	18.68
4	Average_path_length	1.623496762	1.679183673
5	Network diameter	1	1
6	Clustering coefficient	0.553858206	0.533623473
7	Density	0.390379278	0.391408163
8	Heterogeneity	0.260409674	0.292813353
9	Centralization	0.196577243	0.231020408
10	Positive correlation	48.91%	56.35%
11	Negative correlation	51.09%	43.65%

Discussion

Although the yak plays a significant role and is growing in economic importance, our understanding of its microbiota composition has been relatively limited. The predominant factor influencing the composition of the gut microbiome is age variations. Although other factors such as geographical location, the mode of yak husbandry, diet, and hormonal status also play an important role. Age has a significant impact on the composition of the gut microbiota of animals [43]. It is therefore important to examine the impact of age on gut bacterial diversity is crucial in enhancing our comprehension of age-related alterations. In this study, we characterized the gut bacterial community in confined domestic yaks at two different developmental stages from young to adulthood. Yaks, having adapted to high altitudes, rough forage, and extreme environmental conditions, may have developed a distinct gut microbiota to aid in their adaptation. By adopting a young age strategy, the gut microbiota of yaks experienced significant transformations, leading to the establishment of a mature and stable core microbiota over time. We also discovered age-specific functional bacteria in two different age groups.

In our study, we hypothesized that the observed variations in bacterial composition among the animals could be attributed to age-related physiological changes, considering that all the animals were fed the same diet within the same domestic compartment. The composition of gut microbiota is recognized to vary across different stages of development. The microbial community in the rumen of yaks undergoes a series of changes and developments as they progress through different stages of life from birth until they reach 12 years old. This process reflects a maturation of the microbial ecosystem within the rumen [44]. Research conducted on goats has shown that there are noticeable variations in gut microbial diversity as the animals age [43], which is consistent with our results. We recorded that the GT68 group of yaks exhibited significantly higher bacterial diversity compared to the GT35 group. We also hypothesized that an increase in the diversity of the gut microbiota could be linked to the presence of bacterial genera that play a crucial role in the breakdown of complex polysaccharides. Furthermore, we speculated that this enrichment of polysaccharide metabolizing genera may become more pronounced and stable as individuals age.

The core microbiota discovered in our study revealed Bacteroidetes and Firmicutes as the dominant phyla present, which is consistent with the previous reports on cattle [45]. A study reported that the microbial population in the rumen shows increased stability and maturity within the initial 6 weeks following birth [46]. However, we observed significant variations in the core microbiota

between the age groups we examined. The same results were observed in animals from the 6-month and 2-year groups receiving the same diet, indicating that the developmental changes in the rumen microbiota are independent of diet [25]. Other studies have also shown that the bacterial community composition undergoes continuous changes and evolves as individuals grow older [26, 47, 48]. In this study, a high abundance of Actinobacteria in the feces of young yaks (GT35) could assist in the decomposition of various organic substances like cellulose, lignin, and chitin found in their diet [49]. The GT35 group was found to have a high abundance of Spirochetes and Tenericutes phyla. The phylum Spirochetes is known for its ability to ferment plant polymers such as pectin, xylan, and arabinogalactan [50], while phylum Tenericutes can degrade lignin [51]. The GT68 group exhibited a higher abundance of Elusimicrobia, but this difference was not statistically significant compared to GT35. The phylum Elusimicrobia is crucial in the fermentation of sugar and aiding in nitrogen metabolism, which is necessary for optimal animal productivity [52] These phyla are thought to be involved in the adaptation of the confined domestic yak to different substrates for energy generation. In the GT35 group, we observed a high abundance of Proteobacteria compared to the GT68 group. The presence of the Proteobacteria phylum is crucial for maintaining an anaerobic environment in the rumen. This is achieved by reducing the redox potential, which promotes the colonization of strict anaerobes [53]. This particular group of organisms may be connected to boosting the productivity of domestic yaks by supplying them with additional energy.

During our study on the development stages of domesticated yaks, we observed the presence of certain bacterial genera that were specific to different stages. These genera, including *Bacteroides*, *Alloprevotella*, and *Anaerovibrio*, play critical roles in carbohydrate metabolism, fiber degradation, and short-chain fatty acid (SCFA) production, which are essential for ruminant energy balance and digestive efficiency. *Alloprevotella* is known for its proficiency in fermenting plant fiber to produce acetic acid and succinic acid, which contribute to energy supply and digestive health in younger ruminants [54]. The higher abundance of these genera in the GT35 group suggests their role in supporting the fiber utilization and digestive efficiency typical of younger yaks. This trend mirrors findings in cattle and goats, where the abundance of Bacteroides, Alloprevotella, and similar taxa has been linked to improved metabolic functions and dietary adaptation [55].

Bacteroides ferment various sugar derivatives found in plant material to generate energy, while also offering protection against infections [56]. The specific roles and functions of *Anaerovibrio* bacteria are still being studied, but their ability to thrive in anaerobic conditions suggests their importance in various ecosystems and biogeochemical processes. The presence of these particular genera is likely crucial for the overall growth, health, and development of young confined domestic yaks. The genus *Rikenellaceae*, abundant in the GT35 group, is recognized for its ability to produce propionate, succinate, butyrate, and acetate. These compounds play a key role as an energy source for the cells lining the rumen and also contribute to the regulation of rumen function [57]. Firmicutes were found to be the most abundant phylum in both the GT35 and GT68 age groups. However, it was significantly more abundant in the GT68 group compared to the GT35 age group. This suggests that Firmicutes is well-adapted to a diverse range of environmental conditions. The *Ruminococcaceae* displayed high abundance in GT68, indicating their importance in this age group. *Ruminococcaceae* play a significant role in breaking down cellulose and hemicellulose in the rumen of animals [58], while Verrucomicrobia play a crucial role in the degradation of plant fibers, as they possess a wide variety of enzymes that are capable of breaking down glycoside bonds [59]. In our study, *Ruminococcaceae_UCG-005* was identified as a biomarker for GT68, likely reflecting its involvement in enhanced fiber degradation, a key feature of the older group's digestive efficiency. There is consistency between our study and previous research, as it has been observed that the abundance of *Rikenellaceae* and *Lachnospiraceae* increases when there is a higher intake of roughage in the diet [60]. This trend is also seen in our study, particularly in the older age group, GT68. On the other hand, *Prevotellaceae_UCG-003*, which was enriched in GT35, could be involved in fermentative metabolism and energy utilization, characteristics that might be more prominent in younger yaks due to differences in their dietary adaptation and fiber utilization. Overall, we assumed that these genera play a role in aiding the digestion of a high-fiber diet and promoting the health of yaks. This discovery adds new insights to our understanding of how the gut bacterial community varies with age, as it identifies key taxa that are influential in specific age groups.

The observed differences in bacterial abundance between the two age groups, despite receiving the same diet, suggest that the gut bacterial diversity undergoes developmental changes between the ages of 3 and 8 years that are not influenced by diet. The gut microbiota of confined domestic yaks has developed the ability to degrade the tough and fibrous components of their diet, which primarily consist of roughage (forage) and a mixture of concentrated feed. It is worth mentioning that the age range covered in this study is quite wide, and it is possible that conducting a more focused analysis on smaller age groups could provide more nuanced insights

into the variations in gut microbiota during different stages of development.

Conclusion

The gut microbiota composition in confined domestic yaks stabilizes with age, reflecting a maturation process that enhances digestive efficiency and metabolic stability. With age, microbial diversity increases, particularly in mature yaks, which supports improved digestive functions and energy extraction from feed. The core phyla across age groups were Bacteroidetes and Firmicutes, known for their roles in fiber degradation and energy metabolism. In the GT35 group, genera such as *Bacteroides*, *Alloprevotella*, and *Anaerovibrio* were abundant, supporting early-stage fermentation and fiber breakdown, key processes for energy extraction and growth in younger yaks. In GT68, *Rikenellaceae* became more prevalent, likely contributing to improved fiber degradation and SCFA production, which are vital for metabolic stability in mature yaks. Several other genera also exhibited age-related differences, which contribute to the overall maturation of the gut microbiota. This study provides valuable insights into the age-related shifts in gut microbiota composition and their implications for digestive health in yaks. Future studies should explore the functional dynamics of these microbial communities in greater detail, particularly concerning ruminant health and productivity.

Abbreviations

QTP	Qinghai-Tibetan Plateau
NDF	Neutral detergent fiber
ADF	Acid detergent fiber
DM	Dry matter
OM	Organic matter
CP	Crude protein
EE	Ether extract
OTUs	Operational taxonomic units
DNA	Deoxyribonucleic acid
LEfSe	Linear discriminant analysis Effect Size
LDA	Linear discriminant analysis
NMDS	Non-metric multidimensional scaling
PCoA	Principal coordinate analysis
PCR	Polymerase chain reaction
rRNA	Ribosomal ribonucleic acid
VFAs	Volatile fatty acids

Supplementary Information

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Supplementary Material 1

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Author contributions

TS and LD: conceptualization. TS: methodology, investigation, and writing—original draft preparation. TS: software. IM: validation. XG: formal analysis. GA and AD: resources. XG: data curation. LD and XG: writing, review, and editing. SS and AD: visualization. LD: supervision, project administration, and funding acquisition.

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Data availability

The datasets presented in this study can be found in online repositories. The name of the repository/repositories and accession number can be found at https://www.ncbi.nlm.nih.gov/sra/PRJNA1200281 (accessed on 24 December 2024).

Declarations

Ethics approval and consent to participate

This study did not require ethical approval because it involved only the collection of plant samples for feeding and non-invasive fecal sample collection from animals, without causing harm or distress. However, the study protocol was reviewed and acknowledged by the Ethics Committee of the College of Ecology, Lanzhou University, Lanzhou 730000, PR China. Informed consent was obtained from the owners of the domesticated yaks before sample collection.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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