Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Associations of the ileal microbiota and metabolome with meat quality, nutritional composition, and VFA transport-related genes in grazing Qilian sheep

Ying Xu
Qinghai University
Liangwei Yao
Qinghai University
Yulu Xia
Qinghai University
Wenhao LI
qhdxlwh@163.com
Qinghai University
Changhui Li
Qinghai University
Jianhua Zhang
Qinghai University
Jianbin Liu
Chinese Academy of Agricultural Sciences
Yongwei Yang
Qinghai Provincial Animal Husbandry General Station
Yongrong Fang
Qinghai Provincial Animal Husbandry General Station

Research Article

Keywords: Qilian sheep, ileum, 16S rRNA, metabolomics

Posted Date: November 17th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11110941/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Associations of the ileal microbiota and metabolome with meat quality, nutritional composition, and VFA transport-related genes in grazing Qilian sheep

Xu Ying[1,3],Yao Liangwei [2,3]，Xia Yulu[1,3]，Li Wenhao [*1,3]，Li Changhui[*3, 4]，Zhang Jianhua[2, 3]，Liu Jianbin [3,5]，Chen Yongwei[6]，Fang Yongrong[6]

Abstract

Background: This study aimed to elucidate the molecular mechanisms underlying the adaptation of polled Qilian sheep to high-altitude environments. Horned Qilian sheep were used as controls to compare differences in muscle quality, ileal microbiota and metabolites, volatile fatty acids (VFAs), and the expression of VFA transporter genes. Results: Polled Qilian sheep exhibited significantly higher contents of crude protein, ash, Mn, and K in muscle tissues, as well as higher Fe and Zn contents in certain muscle tissues. The relative abundances of *Bacteroidetes* and *Rikenellaceae_RC9_gut_group* were significantly increased in the ileum of polled Qilian sheep, accompanied by enhanced carbohydrate, lipid, and protein metabolism. Differential metabolites were mainly enriched in caffeine metabolism and lysine degradation pathways. Although the total ileal VFA concentration was lower in polled than in horned Qilian sheep, the expression levels of the VFA transport-related genes *DRA*, *MCT1*, and *NHE2* were significantly upregulated. Correlation analysis further revealed significant associations among the ileal microbiota, metabolites, VFA transporter genes, meat quality traits, and mineral contents. Conclusion: Polled Qilian sheep may improve nutrient and energy utilization efficiency by reshaping the ileal microbiota and its metabolic functions and enhancing VFA transport and utilization. These coordinated adaptations may represent an important strategy for adaptation to high-altitude environments and provide a theoretical basis for evaluating the nutritional value and promoting the industrial utilization of Qilian sheep.

Key Words：Qilian sheep; ileum; 16S rRNA; metabolomics

Introduction

Qilian sheep are classified as a plateau-type Tibetan sheep and represent one of the ancient indigenous sheep populations in China. They are mainly distributed in Qilian County, Haibei Tibetan Autonomous Prefecture, Qinghai Province, and the surrounding alpine regions. Long-term

natural selection and grazing under plateau conditions have endowed Qilian sheep with strong tolerance to cold, coarse forage, and environmental stress, as well as relatively high disease resistance, enabling them to adapt to the low temperatures, hypoxia, and pronounced seasonal fluctuations in forage availability characteristic of high-altitude environments. Qilian sheep are predominantly raised under natural grazing conditions, and their meat is characterized by compact muscle fibers, favorable toughness and elasticity, white adipose tissue, and a relatively mild mutton odor. In addition, Qilian sheep meat contains various amino acids and vitamins, as well as essential trace elements such as Fe, Zn, and Se, and is characterized by a relatively high protein content and low fat content, indicating considerable nutritional value and potential for further development. With changes in dietary patterns and consumer preferences, the demand for mutton has gradually shifted from quantity toward improved quality and nutritional value. Consequently, meat quality and its underlying mechanisms have received increasing attention. Tenderness, meat color, pH, water loss rate, and cooking yield are important indicators for evaluating mutton quality, which is affected by multiple factors, including breed, age, feeding management, and processing conditions[1]. Meat quality also varies among different skeletal muscles because of differences in muscle fiber composition, metabolic characteristics, and physiological functions. Water loss rate is an important indicator of the water-holding capacity of muscle and is closely associated with tenderness, color, flavor, and palatability. In general, a lower water loss rate indicates a greater water-holding capacity and, consequently, better meat quality[2]. Shear force is widely used as an objective indicator of meat tenderness and is mainly determined by the structural and biochemical properties of myofibrils and connective tissues, while also being affected by processing temperature[3]. Previous studies have reported that the crude protein and crude fat contents of mutton range from approximately 12.8% to 18.6% and from 16% to 37%, respectively[4]. The moisture content of meat is closely related to tenderness, juiciness, and palatability[5], and moisture loss during cooking can result in corresponding increases in the relative proportions of protein and fat[6, 7]. Furthermore, meat is an important dietary source of high-quality protein, lipids, and minerals, and its nutritional composition is therefore essential for assessing its nutritional value[8]. Wang et al.[9] analyzed the mineral profiles of 104 sheep and 24 goats from Inner Mongolia and demonstrated significant differences ($P<0.05$) in the concentrations of Mg, Al, K, Ca, Mn, Fe, Cu, Zn, Rb, Sr, and Ba among animals of different geographical origins, breeds,

and feeding systems, suggesting that mineral composition may reflect differences in the nutritional characteristics and origins of meat($P<0.05$).

In addition to host genetic background and feeding environment, gastrointestinal microorganisms and their metabolites play important roles in nutrient digestion, absorption, and energy metabolism in ruminants. Volatile fatty acids (VFAs) are major metabolites produced by microbial fermentation of carbohydrates and certain nitrogen-containing substrates in the gastrointestinal tract[10]. They contribute to maintaining intestinal acid–base homeostasis, inhibiting the proliferation of potentially pathogenic microorganisms, protecting the intestinal mucosal barrier[11], and providing energy to both the host and gastrointestinal epithelial cells. In ruminants, VFAs can provide approximately 70% of the energy required by the host[12]. Acetate, propionate, and butyrate are primarily derived from carbohydrate fermentation, whereas branched-chain VFAs, including isobutyrate and isovalerate, are mainly generated through the fermentation of branched-chain amino acids and other nitrogenous substrates. Acetate and propionate are among the predominant VFAs in the gastrointestinal tract and account for a substantial proportion of total VFAs[13, 14]. Propionate is mainly produced through pathways such as the succinate pathway, and certain members of the phylum *Bacteroidetes* participate in the conversion of dietary carbohydrates into propionate[14]. After absorption, propionate serves as a major gluconeogenic substrate in the liver of ruminants and therefore plays an important role in maintaining glucose homeostasis. Moreover, ruminal propionate concentrations may participate in the regulation of feed intake. Infusion studies in sheep have shown that propionate administration can reduce dry matter intake, suggesting that propionate may regulate feeding behavior through specific sensing mechanisms[15]. Following their production, VFAs must be absorbed and transported across the gastrointestinal epithelium[16]; and their transport efficiency directly affects the utilization of microbial fermentation products by the host[17]. Transepithelial VFA transport involves both passive diffusion of undissociated VFAs and transporter-mediated processes involving various ion exchangers and monocarboxylate transporters[18]. Monocarboxylate transporter 1 (*MCT1*) and monocarboxylate transporter 4 (*MCT4*) participate in the cotransport of monocarboxylates and H^+ across cell membranes[17, 19]. Borthakur et al.[20, 21] reported that butyrate enhanced *MCT1* promoter activity in colonic epithelial cells and increased *MCT1* expression at both the mRNA and protein levels[22].The normal expression and

transport functions of *MCT1* and *MCT4* also depend on associated accessory proteins[23]. Meanwhile, members of the Na^+/H^+ exchanger (*NHE*) family contribute to the maintenance of intracellular pH and ionic homeostasis by regulating transmembrane Na^+/H^+ exchange[24]。In vitro studies have demonstrated that VFAs promote transmembrane Na^+ absorption and induce NHE expression in gastrointestinal epithelial cells[25], with *NHE* expression varying in response to VFA concentrations[26]。In addition, anion exchangers and the Cl^-/HCO_3^- exchanger down-regulated in adenoma (*DRA*) are involved in gastrointestinal epithelial ion transport and acid–base homeostasis[27,28].Loss of *DRA* function has been reported to reduce intestinal HCO_3^- secretion and may be accompanied by alterations in intestinal mucosal architecture and other epithelial ion transport processes[29]。Therefore, VFA concentrations together with the expression patterns of VFA transport- and ion homeostasis-related proteins may collectively reflect the production, absorption, and host utilization of microbial fermentation products.[30, 31].

Different regions of the gastrointestinal tract possess distinct physicochemical environments and physiological functions and consequently harbor spatially heterogeneous microbial communities. Previous studies have suggested that animals with greater production efficiency may exhibit an enhanced capacity to utilize fermentable carbohydrates, resulting in increased production of short-chain fatty acids and microbial proteins[32]，Undigested nutrients entering the small intestine may also provide substrates for local microbial growth and metabolism, thereby further influencing nutrient digestion and absorption. Along the longitudinal axis of the gastrointestinal tract, substantial differences exist in microbial composition, abundance, and function among intestinal segments. The small intestine is a major site for the digestion and absorption of nutrients, water, and electrolytes. Studies in yaks have shown that the duodenum, jejunum, and ileum exhibit relatively similar microbial community compositions[33], while the duodenal microbiota is significantly associated with energy utilization[34], The ileal microbiota may also contribute to the further fermentation of substrates such as cellulose, starch, and polysaccharides[35]. Compared with the rumen, the abundance and community structure of microorganisms in the hindgut of ruminants differ substantially[36], reflecting the selective effects of distinct gastrointestinal ecological niches on microbial colonization and metabolic functions. As an important segment of the small intestine, the ileum not only participates in the further digestion and absorption of nutrients but also plays a critical role in maintaining the intestinal mucosal

barrier and local immune homeostasis. Its structural and functional integrity may therefore be particularly important for the health and nutritional adaptation of Tibetan sheep living in high-altitude environments. Previous studies have demonstrated that the relative abundances of certain bacterial taxa in the jejunum and ileum tend to decrease compared with those in the duodenum[37]. Meanwhile, changes in digestive enzyme activity and bile acid composition in the terminal ileum contribute to the establishment of a characteristic microbial community within the ileal digesta[38]. These microorganisms can ferment nutrients that escape complete host digestion, generating VFAs and other bioactive metabolites that may further regulate intestinal barrier function, mucosal immunity, and host nutrient metabolism. Accordingly, intestinal development and microbial community structure are closely associated with animal growth, development, and health[39].

Complex bidirectional interactions occur between the intestinal microbiota and the host, and microbial community composition and metabolic function are jointly influenced by environmental conditions and host genetics. Temperature, host species, diet, physical activity, age, and geographical location can all affect intestinal microbial composition and function[40]. However, environmental factors alone cannot fully explain the substantial interindividual variation in microbial communities, and increasing attention has therefore been directed toward the contribution of host genetic background to microbial colonization and function. Previous studies have shown that hosts with different genetic backgrounds can retain distinct intestinal microbial communities even when maintained under similar environmental conditions. For example, different Hydra species maintained under identical laboratory conditions retain species-specific microbial community characteristics, suggesting that the host exerts selective effects on its associated microbiota[41], Similarly, studies of chicken lines selected for high and low body weight have demonstrated that host genetic background significantly affects intestinal microbial composition and abundance, with certain microbial taxa exhibiting measurable heritability and genetic correlations[42]. These findings suggest that host genetic characteristics, intestinal microorganisms, and microbial metabolites may form complex interactive networks that jointly regulate host phenotypes and environmental adaptation.

Qilian sheep have developed strong environmental adaptability during long-term exposure to alpine, hypoxic, and extensive grazing conditions. Phenotypic differences between horned and

polled Qilian sheep maintained under comparable ecological and management conditions provide a valuable model for investigating the relationships among host phenotype, intestinal microbiota, and nutrient metabolism. However, information regarding the meat quality and nutritional characteristics of polled Qilian sheep remains limited, and the coordinated relationships among the ileal microbiota, microbial metabolites, and VFA absorption and transport have not been fully elucidated. In particular, whether the intestinal microbial ecosystem contributes to the high-altitude adaptation of polled Qilian sheep by modulating VFA production, transport, and utilization remains unclear. Therefore, the present study compared horned and polled Qilian sheep maintained under the same natural grazing and management conditions. Meat quality traits, conventional nutritional components, and mineral element profiles were determined in the longissimus dorsi and leg muscles. In addition, 16S rRNA gene sequencing and untargeted metabolomics were employed to characterize differences in the ileal microbial community and metabolite profiles. Ileal VFA concentrations and the expression levels of VFA transport-related genes were also determined. Multi-omics correlation analyses were subsequently performed to explore the potential associations within the "intestinal microbiota–metabolites–VFAs–host nutritional phenotype" axis. This study aimed to elucidate the potential microbial and metabolic mechanisms underlying the adaptation of polled Qilian sheep to high-altitude environments and to provide a theoretical basis for evaluating the nutritional value, conserving the genetic resources, and promoting the industrial utilization of Qilian sheep.

Materials and methods

1.1 Experimental Design and Sample Collection

A total of 12 female Qilian sheep (2 years ± 1 month of age), including horned and polled individuals, were selected for this study. All experimental animals were maintained under traditional local natural grazing conditions without any supplementary feeding. All animals used in this study were obtained from a local farm. Before morning grazing, the sheep were slaughtered and sampled in accordance with the procedures approved by the Animal Ethics Committee. Following slaughter, approximately 500 g each of the longissimus dorsi and foreleg muscles were collected from each animal, separately packaged, transported to the laboratory, and stored at −20 °C until analysis of meat quality, nutritional composition, and mineral elements. Within 10

min after slaughter, the intestinal tract was removed, and ileal contents were collected and immediately preserved in liquid nitrogen for subsequent 16S rRNA gene sequencing and metabolomic analysis. All animal experimental procedures were approved by the Animal Ethics Committee of the Academy of Animal Husbandry and Veterinary Sciences, Qinghai University (Approval No. 2026-QHMKY-010). At the end of the experiment, the sheep were deeply anesthetized prior to euthanasia. General anesthesia was induced with xylazine (0.2 mg/kg body weight, intramuscularly), followed by ketamine (5 mg/kg body weight, intravenously). The depth of anesthesia was assessed by the absence of purposeful movement and loss of responses to external stimuli. After confirming that the animals were unconscious and had reached an adequate depth of anesthesia, euthanasia was performed by intravenous administration of an overdose of sodium pentobarbital (100 mg/kg body weight). Death was confirmed by the absence of spontaneous respiration, heartbeat, and corneal reflex. All procedures were performed by trained personnel to minimize animal pain and distress and in accordance with the approved animal welfare and ethical guidelines.

1.2 Determination of Meat Quality Traits

Cooking yield: Approximately 60 g of each meat sample was cut into a rectangular block and weighed before cooking (M_1). The samples were steamed at 80 °C for 30 min and then cooled to room temperature. Surface moisture was gently removed using filter paper, after which the samples were reweighed (M_2). Three replicates were performed for each sample. Cooking yield was calculated as follows:

Cooking yield (%) = $M_2/M_1 \times 100$

Shear force: Shear force was determined using a meat tenderness meter. Each sample was measured in triplicate, and the mean of the three measurements was recorded as the shear force value of the sample.

Water loss rate: Meat samples were trimmed into circular pieces measuring 5 cm in diameter and 1 cm in thickness and immediately weighed using an analytical balance (M_1). Each sample was then placed between approximately 16 layers of neutral filter paper on both the upper and lower surfaces and subjected to a pressure of 35 kg for 5 min using a water-loss testing apparatus. After compression, the sample was removed and reweighed (M_2). Three replicates were performed

for each sample. The water loss rate was calculated as follows:

Water loss rate (%) = $(M_1 - M_2)/M_1 \times 100$

pH: The pH value of each meat sample was measured using a portable waterproof pH meter (HI9125).

1.3 Determination of Nutritional Composition

The nutritional composition of the meat samples, including ash, crude protein, crude fat, and moisture contents, was determined according to the National Food Safety Standards of China. Ash content was determined using the incineration gravimetric method according to GB 5009.4-2016. Crude protein content was determined using the semi-micro Kjeldahl method according to GB 5009.5-2016. Crude fat content was determined using the Soxhlet extraction method according to GB 5009.6-2016. Moisture content was determined using the direct drying method according to GB 5009.3-2016.

1.4 Determination of Mineral Elements

The mineral element concentrations in the meat samples were determined using inductively coupled plasma–optical emission spectrometry (ICP-OES; Thermo Fisher Scientific, USA).

1.5 16S rRNA Gene Sequencing and Analysis of the Ileal Microbiota

Ileum microbial DNA was extracted by MN NucleoSpin 96 Soil kit (MachereyNagel, Düren, Germany), and the specific experimental procedures were carried out according to the kit guidelines. The concentration and purity of DNA were detected by NanoPhotometer (N60, Germany) to ensure the integrity of the extracted DNA samples for subsequent sequencing. The V3–V4 region of 16S rRNA gene was amplified by PCR to further analyze the community structure of rumen microbiota. The primers were (338F 50-ACTCCTACGGGAGGCAGCAG-30 and 806R 50-GGACTACHVGGGTWTCTAAT-30).Sequencing was performed on the Illumina MiSeq platform (Illumina, San Diego, CA,USA), and the bioinformatics analysis was performed using BMKCloud (www.biocloud.net(accessed on 01 June 2024)). To evaluate the quality of the raw data coming back from sequencing, and paired-end splicing (FLASH v1.2.7) and filtering (Trimmomatic v0.33), chimeras (UCHIME v4.2) were removed to obtain optimized sequences (Tags). The operational taxonomic units (OTU) was obtained using the Usearch software; OTU classification and annotation were analyzed by using Silva (Bacteria) taxonomy database for

further taxonomic analysis, and the community structure at different taxonomic levels (phylum, class, order, family, genus, and species) were obtained; the species diversity was analyzed through Alpha diversity, and the Alpha diversity index Ace, Chao1, Shannon, and Simpson were obtained, and a sample rarefaction curve was drawn. Furthermore, Beta diversity analysis was used to obtain PCoA and boxplots based on multiple distances according to the distance matrix (Anosim). Line discriminant analysis Effect Size (LEfSe) analysis was used to find Biomarkers with statistical differences between groups; the software Metastats was used to conduct t-tests on species abundance data between groups; according to the q value, the species that cause the difference in the composition of the two groups of samples are screened out; 16S gene function analysis was performed through Kyoto Encyclopedia of Genes and Genomes (KEGG) and Clusters of Orthologous Groups of proteins (COG) .volatile fatty acids (VFAs).

1.5.1 Microbial DNA Extraction, Library Preparation, and Sequencing

Microbial genomic DNA was extracted from the ileal contents of polled and horned Qilian sheep using the MN NucleoSpin 96 Soil kit (Omega, Shanghai, China) according to the manufacturer's instructions. The V3-V4 hypervariable regions of the bacterial 16S rR NA gene were amplified by PCR using the universal primers 338F (5-ACTCCTACGGGA GGCAGCA-3′) and 806R (5-GGACTACHVGGGTWTCTAAT-3′). The resulting PCR a mplicons were used to construct paired-end sequencing libraries using a two-step library pr eparation protocol. The prepared libraries were subsequently subjected to paired-end sequen cing on an Illumina MiSeq 2500 platform (Illumina, San Diego, CA, USA).

1.5.2 Quality Control and ASV Inference of Raw Sequencing Data

Raw sequencing data were subjected to quality control and sequence processing. Paired-end reads from each sample were merged based on their overlapping regions using FLASH (version 1.2.7) to generate raw tags. The raw sequences were subsequently quality-filtered using Trimmomatic (version 0.33) to obtain high-quality clean reads. Chimeric sequences were identified and removed using UCHIME (version 4.2), and the remaining high-quality sequences were retained as effective tags. The high-quality effective sequences were clustered using USEARCH (version 10.0) against the Greengenes ribosomal RNA gene database (version 13.8) at a sequence similarity threshold of 97%. Low-abundance sequence clusters representing less than 0.005% of the total sequences were removed. Representative sequences were subsequently

taxonomically annotated and classified against the SILVA 16S rRNA gene database. Taxonomic profiles of the microbial communities were generated at different taxonomic levels, and the relative abundances of microbial taxa in each sample were calculated. Community composition profiles at different taxonomic levels were visualized using R.

1.5.3 Alpha Diversity Analysis

Alpha diversity analysis was performed using Mothur (version 1.30) to evaluate microbial richness and diversity within individual ileal samples from horned and polled Qilian sheep. The Shannon, Simpson, ACE, and Chao1 indices were calculated at a 97% sequence similarity threshold. Rarefaction curves and relative abundance accumulation curves were generated to assess sequencing depth and microbial richness across samples.

1.5.4 Beta Diversity Analysis

Beta diversity analysis was performed using QIIME to evaluate differences in ileal microbial community composition and structure between horned and polled Qilian sheep. Based on the corresponding distance matrices, principal coordinate analysis (PCoA) and non-metric multidimensional scaling (NMDS) were performed to visualize differences in microbial community structure among samples, and analysis of similarities (ANOSIM) was used to assess the statistical significance of between-group differences. Differential abundance analysis was further performed to identify potential microbial biomarkers distinguishing horned and polled Qilian sheep. Linear discriminant analysis effect size (LEfSe) and Metastats analyses were used to identify bacterial taxa that differed significantly between the two groups.

1.5.5 Gene function prediction

The functional profiles of the ileal microbial communities were predicted from 16S rRNA gene sequencing data using PICRUSt. Predicted microbial functions were annotated against the Kyoto Encyclopedia of Genes and Genomes (KEGG) database, and differences in metabolic pathways between horned and polled Qilian sheep were subsequently analyzed. In addition, functional annotation based on the Clusters of Orthologous Groups (COG) database was performed to predict differences in the functional categories of prokaryotic proteins within the ileal microbial communities between the two groups.

1.6 Metabolomic Analysis of Ileal Microbial Metabolites

Untargeted metabolomic profiling of ileal contents from polled and horned Qilian sheep (n = 12) was performed using a liquid chromatography–mass spectrometry (LC–MS) platform. After thawing at room temperature, 100 μL of each sample was mixed with 500 μL of extraction solvent containing an internal standard (1000:2, v/v). The extraction solvent consisted of methanol and acetonitrile (1:1, v/v), with the internal standard at a final concentration of 2 mg/L. The mixture was vortexed for 30 s, sonicated in an ice-water bath for 10 min, and incubated at −20 °C for 1 h. Subsequently, the samples were centrifuged at 12,000 rpm for 15 min at 4 °C. A 500-μL aliquot of the supernatant was transferred to a microcentrifuge tube and dried using a vacuum concentrator. The dried extracts were reconstituted in 150 μL of acetonitrile/water (1:1, v/v), vortexed for 30 s, sonicated in an ice-water bath for 10 min, and centrifuged at 12,000 rpm for 15 min at 4 °C. Finally, 120 μL of the supernatant was transferred to a 2-mL autosampler vial for LC–MS analysis. A pooled quality control (QC) sample was prepared by combining 10 μL aliquots from each individual sample. Metabolomic analysis was performed using a Waters ACQUITY I-Class PLUS ultra-high-performance liquid chromatography system coupled to a Waters Xevo G2-XS QTof high-resolution mass spectrometer. Chromatographic separation was achieved using a Waters ACQUITY UPLC HSS T3 column. Samples were analyzed in both positive (ESI+) and negative (ESI−) electrospray ionization modes. Mobile phase A consisted of water containing 5% acetonitrile and 0.1% formic acid, whereas mobile phase B consisted of acetonitrile containing 0.1% formic acid. The flow rate was set at 0.35 mL/min. The gradient elution program was as follows: 0–0.25 min, 98% A and 2% B; 10.0–13.0 min, 2% A and 98% B; and 13.1–15.0 min, 98% A and 2% B. The ion source temperature and desolvation temperature were set at 150 °C and 500 °C, respectively. The cone gas and desolvation gas flow rates were 50 and 800 L/h, respectively. Raw LC–MS data were acquired using MassLynx (version 4.2) and processed using Progenesis QI software for peak detection, alignment, and metabolite identification. Metabolites were annotated by matching spectral information against online databases, including METLIN, together with an in-house metabolite database. Differential metabolites between polled and horned Qilian sheep were screened using the criteria of fold change (FC)>1, $P<0.05$, and variable importance in projection (VIP)>1. The identified differential metabolites were subsequently subjected to Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway annotation and enrichment analysis.

1.7 Determination of Volatile Fatty Acids in Ileal Contents

Ileal content samples were thawed at room temperature and centrifuged at 15,000 rpm for 15 min at 4 °C. Subsequently, 1 mL of the supernatant was transferred to a 1.5-mL microcentrifuge tube, and 0.2 mL of 25% metaphosphoric acid solution containing 2-ethylbutyric acid (2-EB) as an internal standard was added. The mixture was thoroughly mixed and incubated in an ice bath for at least 30 min, followed by centrifugation at 15,000 rpm for 15 min. The resulting supernatant was collected using a 2-mL syringe and filtered through a 0.22-μm membrane filter. The filtrate was transferred to a clean vial for subsequent gas chromatographic analysis.Volatile fatty acids (VFAs) were quantified using an Agilent 7890B gas chromatograph equipped with a flame ionization detector (FID). Chromatographic separation was performed using an AT-FFAP capillary column (30 m × 0.32 mm × 0.50 μm). The split/splitless (SSL) injector and FID temperatures were both maintained at 250 °C. High-purity nitrogen (99.999%) was used as the carrier gas at a total pressure of 100 kPa, with a split ratio of 5:1. The flow rates of air, H_2, and N_2 were 400, 35, and 40 mL/min, respectively. The injection volume was 1 μL. The oven temperature was initially maintained at 120 °C for 3 min, increased to 180 °C at a rate of 10 °C/min, and then held at 180 °C for 1 min.

1.8 RT-qPCR Analysis of VFA Transport-Related Gene Expression in the Ileum

Total RNA was extracted from ileal tissues of horned and polled Qilian sheep using TRIzol reagent (DP762-T1C; Life Technologies, CA, USA). RNA concentration and purity were determined using a NanoDrop 2000 spectrophotometer (Thermo Scientific, MA, USA), and samples with A260/A280 ratios ranging from 1.8 to 2.1 were used for subsequent analyses. RNA integrity was assessed by electrophoretic analysis (Agilent 2100/LabChip GX) and visualized using an agarose gel imaging system (Ready Agarose, Bio-Rad, Hercules, CA, USA). Complementary DNA (cDNA) was synthesized from total RNA using the HiScript® II Q RT SuperMix for qPCR according to the manufacturer's instructions. Gene-specific primers for AE2, DRA, MCT1, MCT4, NHE1, and NHE2 were designed using Primer 5.0, with β-actin used as the reference gene. Detailed primer sequences are provided in Table 1.The relative expression levels of genes associated with VFA transport in ileal tissues were determined by real-time quantitative

PCR (RT-qPCR) using an Applied Biosystems real-time PCR system. Each reaction was performed in a total volume of 20 μL. The amplification conditions were as follows: initial denaturation at 95 °C for 30 s, followed by 40 cycles of denaturation at 95 °C for 10 s and annealing/extension at 60 °C for 30 s. Melting curve analysis was subsequently performed at 95 °C for 15 s, 60 °C for 60 s, and 95 °C for 15 s. The expression levels of target genes were normalized to β-actin, and relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method.

Table 1 Transport gene primers information

Gene	Primers (5'-3')	Length	Annealing temperature	ID
AE2	F: AAGATCCCTGAGAACGCCGA R: AGCAGAAAGAGGAAGCGCAC	152bp	60℃	XM_027969012.1
DRA	F: TGTGGCGGCTTCCAGAATTT R: CACAGGCTTGTTTGGGAGCA	167bp	60℃	NM_001280717.1
MCT1	F: GGACTGTGTCATCTGGCAGC R: TGGGGTCCAACAAGGTCCAT	134bp	60℃	XM_004002335.4
MCT4	F: ACGGCTCAGCCTTAGTAAACTTC R: AATGGAGTTGTGCGAGTTGGT	144bp	60℃	NC_0402252.1
NHE1	F: GCTTCTTCGTGGTGTCCCTG R: CCATGATGCCTGACAGGTGG	174bp	60℃	XM_004005085.4
NHE2	F: TTCTTTGTCGTGGGGATCGG R: CGTGATTGCCATGATGCCTG	180bp	60℃	XM_027967037.1
β-acting	F: AGCCTTCCTTCCTGGGCATGGA R: GGACAGCACCGTGTTGGCGTAGA	113bp	60℃	NM_001009784

1.9 Statistical Analysis

Experimental data were initially organized using Microsoft Excel 2010 and subsequently analyzed using SPSS Statistics 24.0. Data are presented as the mean ± standard error of the mean (SEM). Differences between horned and polled Qilian sheep were evaluated using independent-samples t-tests, including comparisons of VFA concentrations between the two groups. The relative expression levels of VFA transport-related genes were calculated using the $2^{-\Delta\Delta Ct}$ method. Spearman's rank correlation analysis was performed to assess associations among microbial taxa, metabolites, VFA concentrations, VFA transport-related gene expression, meat quality traits, and mineral element concentrations. Differences and correlations were considered statistically significant at $P < 0.05$.

2Results

2.1 Comparison of Meat Quality Traits Between Horned and Polled Qilian Sheep

As shown in Table 2, significant differences in meat quality traits were observed between polled and horned Qilian sheep. The cooking yield of both muscle types was significantly higher in polled Qilian sheep than in horned Qilian sheep (P<0.05), whereas shear force, water loss rate, and pH were significantly lower in polled Qilian sheep (P<0.05). In addition, both cooking yield and shear force were higher in the hindlimb muscle than in the longissimus dorsi.

Table 2 Comparison of meat quality of different parts of horned and polled Qilian sheep.

Trait	Tissue	Horned	Polled	P-value
Cooking yield/%	Longissimus dorsi	44.577±0.911	54.747±0.412	0.000
	hindlimb muscle	53.567±0.610	54.870±0.122	0.022
Shearing force/N	Longissimus dorsi	35.160±0.975	29.118±0.789	0.001
	hindlimb muscle	35.337±0.095	28.808±0.944	0.000
Water loss /%	Longissimus dorsi	30.103±0.202	29.180±0.295	0.011
	hindlimb muscle	36.293±0.127	29.157±0.415	0.000
pH	Longissimus dorsi	7.190±0.040	6.857±0.050	0.001
	hindlimb muscle	6.333±0.120	6.623±0.071	0.023

Note: $P < 0.05$ indicates a significant difference in meat quality traits between horned and polled Qilian sheep.。

2.2 Comparison of Nutritional Composition Between Horned and Polled Qilian Sheep

As shown in Table 3, differences in the nutritional composition of different muscle tissues were observed between polled and horned Qilian sheep. Ash content in both muscle tissues was significantly higher in polled Qilian sheep than in horned Qilian sheep (P<0.05), with a higher ash content in the longissimus dorsi than in the hindlimb muscle. Crude protein content in both muscle tissues was significantly higher in polled Qilian sheep than in horned Qilian sheep (P<0.05). Crude fat content was also higher in both muscle tissues of polled Qilian sheep than in horned Qilian sheep, with a significant difference observed in the hindlimb muscle (P<0.05). Moisture content was higher in both muscle tissues of polled Qilian sheep than in horned Qilian sheep; however, the differences were not statistically significant (P>0.05).

Table 3 Comparison of nutrients in different parts of horned and polled Qilian sheep.

Trait	Tissue	Horned	Polled	P-value

Ash Content	Longissimus dorsi	5.187±0.717	11.562±0.381	0.000
	Hindlimb muscle	3.378±0.317	7.116±0.769	0.001
Crude fat	Longissimus dorsi	6.221±0.480	8.831±1.940	0.086
	Hindlimb muscle	5.900±0536	10.213±0.671	0.001
Crude protein	hindlimb muscle	22.731±0.290	24.398±0.249	0.002
	Hindlimb muscle	20.364±0.164	23.611±0.240	0.000
Moisture Content	Longissimus dorsi	75.471±0.654	76.671±0.595	0.078
	Hindlimb muscle	73.119±0.047	76.940±0.325	0.014

Note: $P<0.05$ indicates a significant difference in the nutritional composition of muscle tissues between horned and polled Qilian sheep.

2.3 Comparison of Mineral Element Contents Between Horned and Polled Qilian Sheep

As shown in Table 4, differences in mineral element contents were observed in different muscle tissues between horned and polled Qilian sheep. Cr content in the longissimus dorsi was significantly higher in horned Qilian sheep than in polled Qilian sheep ($P<0.05$), whereas the opposite pattern was observed in the hindlimb muscle, with significantly higher Cr content in polled Qilian sheep ($P<0.05$). Fe and Zn contents in the longissimus dorsi were significantly higher in horned Qilian sheep than in polled Qilian sheep ($P<0.05$). Mn and K contents in both muscle tissues were higher in polled Qilian sheep than in horned Qilian sheep. Ca content in the hindlimb muscle was significantly higher in polled Qilian sheep than in horned Qilian sheep ($P<0.05$), while Mg content in the longissimus dorsi was significantly higher in polled Qilian sheep ($P<0.05$). Se content was higher in both muscle tissues of polled Qilian sheep than in horned Qilian sheep, with a significant difference observed in the hindlimb muscle ($P<0.05$).

Table 4 Comparison of trace elements in different parts of horned and polled Qilian sheep.

Trait	Tissue	Horned	Polled	P-value
Cr	Longissimus dorsi	0.160±0.036	0.097±0.012	0.004
	Hindlimb muscle	0.053 ±0.012	0.173±0.006	0.000
Cu	Longissimus dorsi	0.543±0.072	0.480±0.035	0.243
	Hindlimb muscle	0.873±0.015	0.937±0.050	0.105
Fe	Longissimus dorsi	21.380±0.462	16.363±0.482	0.000
	Hindlimb muscle	20.227±0.450	19.763±0.838	0.446
Mn	Longissimus dorsi	0.253±0.029	0.310±0.050	0.164
	Hindlimb muscle	0.243±0.015	0.277±0.021	0.089
Zn	Longissimus dorsi	22.293±0.772	17.010±1.469	0.005
	Hindlimb muscle	22.587±0.673	22.713±0.326	0.784
Ca	Longissimus dorsi	0.862±0.118	1.001±0.004	0.111

K	Hindlimb muscle	0.901±0.021	1.046±0.020	0.001
	Longissimus dorsi	34.326±1.389	35.160±3.564	0.725
	Hindlimb muscle	37.209±0.862	38.740±0.557	0.061
Mg	Longissimus dorsi	2.758±0.034	3.147±0.040	0.000
	Hindlimb muscle	2.864±0.046	2.956±0.063	0.110
Na	Longissimus dorsi	5.407±0.401	4.826±0.128	0.075
	Hindlimb muscle	5.582±0.113	5.869±0.042	0.015
Se	Longissimus dorsi	25.227±0.276	29.183±4.466	0.200
	Hindlimb muscle	24.363±0.284	28.320±1.224	0.005

Note: $P<0.05$ indicates a significant difference in mineral element contents between horned and polled Qilian sheep.

2.4 16S rRNA Gene Sequencing Analysis of the Ileal Microbiota in Qilian Sheep

The ileal contents of polled and horned Qilian sheep (n = 12) were subjected to 16S rRNA gene sequencing. Principal coordinate analysis (PCoA) revealed a clear separation in ileal microbial community structure between the two groups. A total of 10,606 amplicon sequence variants (ASVs) were identified, including 4,874 ASVs in horned Qilian sheep and 4,711 ASVs in polled Qilian sheep (Fig. 1B). The number of ASVs unique to horned Qilian sheep was significantly higher than that in polled Qilian sheep ($P<0.05$).Rarefaction curves were generated to assess whether the sequencing depth was sufficient to capture microbial richness and diversity within the samples. As shown in Fig. 1C, the rarefaction curves gradually approached a plateau at approximately 40,000 reads, indicating that the sequencing depth was sufficient for subsequent microbial community analyses.To further characterize differences in the ileal microbiota between the two groups, alpha diversity was analyzed. The ACE and Chao1 indices were significantly higher in polled Qilian sheep than in horned Qilian sheep ($P<0.05$), whereas no significant differences were observed in the Shannon or Simpson indices ($P>0.05$) (Fig. 1C–F). These results indicate that polled Qilian sheep exhibited greater ileal microbial richness, whereas overall microbial diversity, as reflected by the Shannon and Simpson indices, did not differ significantly between the two groups.

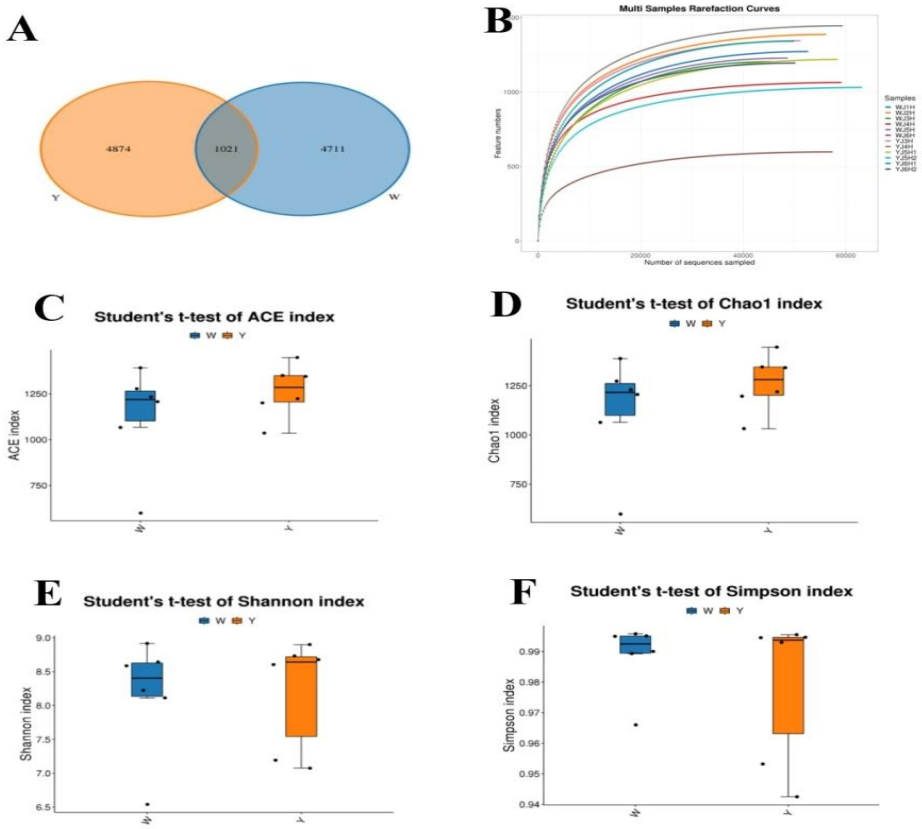


Figure 1. Analysis of microbial diversity in horned and polled Qilian sheep. A, ASV Venn diagram. B, Sample dilution curve. C-F, Microbial diversity indicators.

At the phylum level, *Firmicutes*, *Bacteroidetes*, *Patescibacteria*, *Spirochaetota*, *Verrucomicrobiota*, *Desulfobacterota*, *Cyanobacteria*, *Fibrobacterota*, and *Campylobacterota* were the dominant phyla in the ileal microbiota (Fig. 2A). The relative abundances of *Firmicutes*, *Spirochaetota*, and *Desulfobacterota* were significantly higher in polled Qilian sheep than in horned Qilian sheep ($P<0.05$) (Fig. 2C). In addition, *Patescibacteria* differed significantly between the two groups ($P<0.05$); however, the direction of this difference should be verified against the original data.

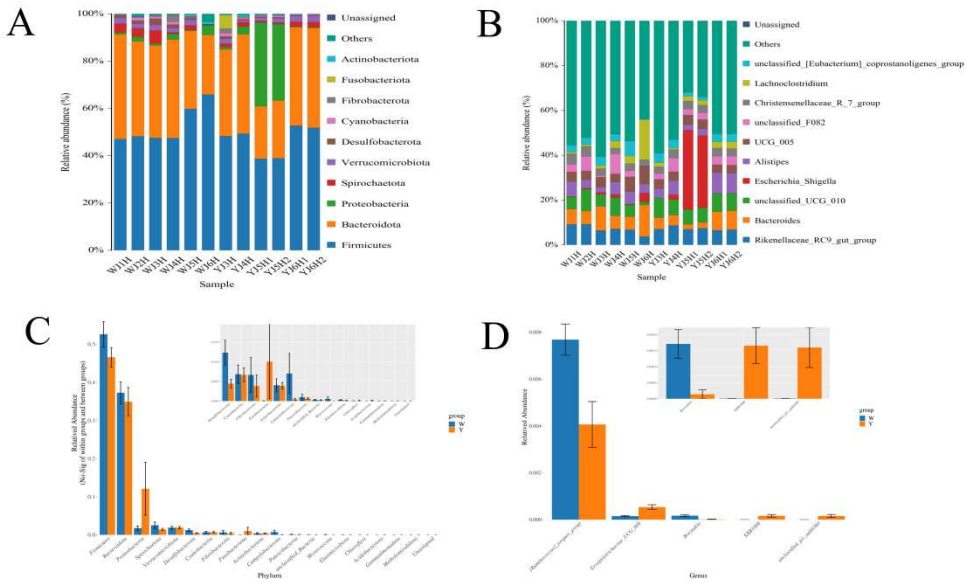


Figure 2. Analysis of microbiological composition of horned and polled Qilian sheep.. A, B Microbial composition at the level of phylum and genus. C, D Analysis of species differences at phylum and genus level

Functional prediction of the ileal microbiota based on KEGG pathways revealed significant differences between horned and polled Qilian sheep. Compared with horned Qilian sheep, polled Qilian sheep exhibited significantly higher predicted abundances of functions associated with Global and overview maps, Signal transduction, Amino acid metabolism, and Energy metabolism. In contrast, functions related to Membrane transport, Nucleotide metabolism, Translation, and Replication and repair were significantly more abundant in horned Qilian sheep than in polled Qilian sheep (Fig. 3A).COG functional analysis further revealed that the predicted abundances of Energy production and conversion, Lipid transport and metabolism, Signal transduction mechanisms, and Cell wall/membrane/envelope biogenesis were significantly higher in polled Qilian sheep than in horned Qilian sheep. In contrast, functions associated with Amino acid transport and metabolism, Carbohydrate transport and metabolism, Translation, ribosomal structure and biogenesis, and Function unknown were more abundant in horned Qilian sheep than in polled Qilian sheep (Fig. 3B).

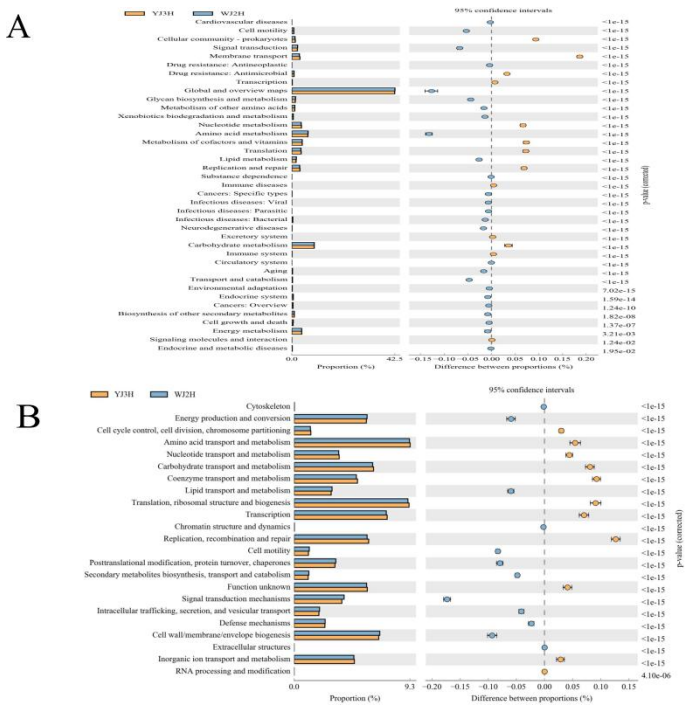


Figure 3. Intestinal microbial function analysis of horned and polled Qilian sheep. A KEGG functional analysis. B COG functional analysis

2.5 Metabolomic Analysis of Ileal Contents

Untargeted metabolomic analysis was performed to characterize differences in the met abolite profiles of ileal contents between polled and horned Qilian sheep. Principal compo nent analysis (PCA) revealed a clear separation between the two groups, while samples wi thin each group showed good clustering, indicating distinct ileal metabolite profiles betwee n horned and polled Qilian sheep (Fig. 4A).Orthogonal projections to latent structures-discr iminant analysis (OPLS-DA) further demonstrated clear discrimination between the two gro ups. The R^2X, R^2Y, and Q^2 values indicated good model performance, with $Q^2 > 0.5$, sug gesting satisfactory predictive ability of the OPLS-DA model (Fig. 4B).Based on the prede fined screening criteria, a total of 408 differential metabolites were identified between poll ed and horned Qilian sheep, of which 323 were upregulated and 85 were downregulated i n polled Qilian sheep (Fig. 4C). Further analysis of the metabolites exhibiting the largest f old changes showed that Cyclocalopin D, 10-Acetoxyoleuropein, dTDP-5-dimethyl-L-lyxose, 24,25-Dihydrolanosterol, 2-Hydroxy-desipramine glucuronide, Oxonol, and Hesperetin were among the metabolites with markedly higher abundances in polled Qilian sheep. In contra st, Aminofurantoin, Arjunolic acid 3-glucoside, Filipin II, Pro Leu Ser, 1-Methylpiperidin-4 -yl butanoate, Nebramine, and Geldanamycin were among the metabolites with higher abun

dances in horned Qilian sheep (Fig. 4D).

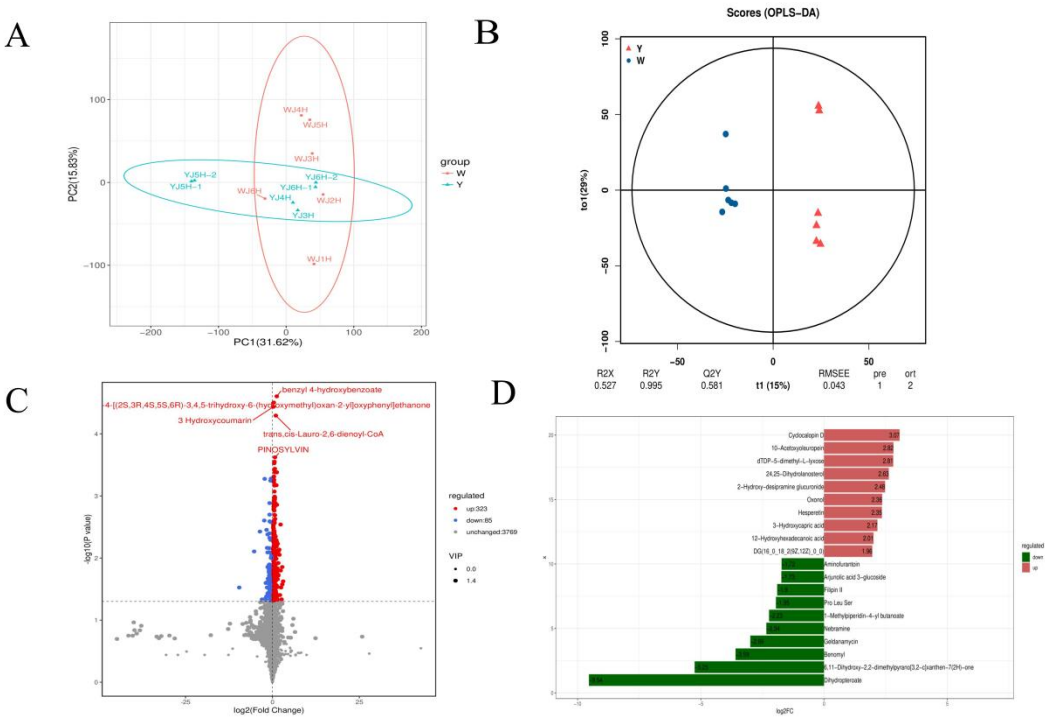


Figure 4. Quality control diagram of rumen microbial metabolome data of horned and polled Qilian sheep. A PCA analysis. B OPLS-DA analysis. C Volcanic maps. D column chart of positive ion difference multiples

KEGG pathway annotation and enrichment analysis of differential metabolites in the ileal contents of polled and horned Qilian sheep are shown in Fig. 5. The differential metabolites were mainly annotated to pathways associated with Lipid metabolism, Metabolism of cofactors and vitamins, Amino acid metabolism, Digestive system, and Nucleotide metabolism. Downregulated differential metabolites were mainly enriched in Efferocytosis and Glycerolipid metabolism. In contrast, the upregulated differential metabolites were enriched in 13 pathways, among which ABC transporters, Antifolate resistance, Axon regeneration, Histidine metabolism, and Prolactin signaling pathway were significantly enriched.

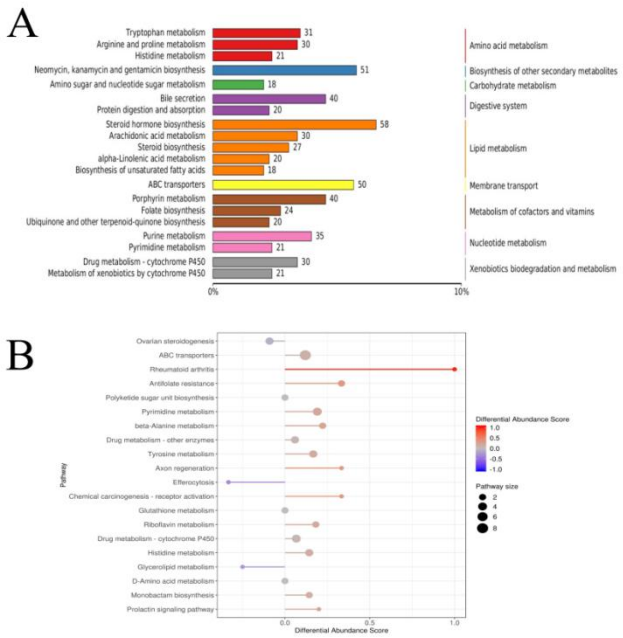


Figure 5. KEGG functional analysis diagram of differential metabolites in rumen microbiomes of horned and polled Qilian sheep. A Differential metabolite KEGG pathway classification annotation. B differential metabolite differential abundance score diagram.

2.6 Ileal VFA Concentrations and Expression of VFA Transport-Related Genes

As shown in Table 5, ileal VFA concentrations differed between horned and polled Qilian sheep. The total VFA concentration in the ileum was significantly higher in horned Qilian sheep than in polled Qilian sheep (P<0.05). In contrast, the concentrations of propionate, isobutyrate, butyrate, isovalerate, valerate, and caproate were higher in polled Qilian sheep than in horned Qilian sheep, with significant differences observed for propionate, isobutyrate, butyrate, and isovalerate (P<0.05). The acetate-to-propionate ratio (A/P) was significantly higher in horned Qilian sheep than in polled Qilian sheep (P<0.05).The relative expression levels of genes associated with ileal VFA transport, including *AE2*, *DRA, MCT1, MCT4, NHE1* and *NHE2* are shown in Fig. 6. The expression levels of *DRA*, *MCT1* and *NHE2* were significantly higher in polled Qilian sheep than in horned Qilian sheep (P<0.05), whereas *AE2* expression was significantly higher in horned Qilian sheep than in polled Qilian sheep (P<0.05).

Table 5 Volatile Fatty Acid Content in the Ileum of horned and polled Qilian sheep.

Trait	Tissue	Horned	Polled	*P*-value
ileum	Acetate/（mmol/L）	2.644±0.426	2.891±0.852	0.053

Propionate/（mmol/L）	0.711±0.256	0.810±0.185	0.046
Isobutyrate /（mmol/L）	0.177±0.460	0.335±0.103	0.006
Butyrate/（mmol/L）	0.202±0.040	0.258±0.163	0.043
Isovalerate/（mmol/L）	0.108±0.041	0.125±0.038	0.046
Valerate/（mmol/L）	0.103±0.043	0.125±0.083	0.059
caproic acid（mmol/L）	0.002±0.001	0.003±0.000	0.057
VFAs/（mmol/L）	3.947±10.557	4.547±1.058	0.000
A/P/（mmol/L）	4.037±1.081	3.577±0.577	0.038

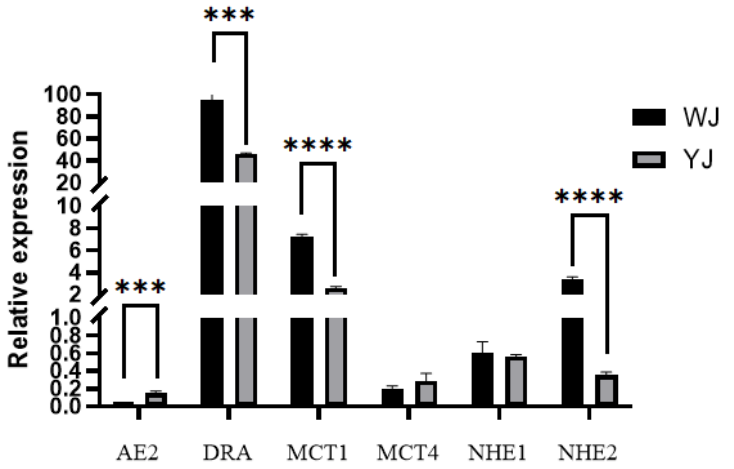


Figure 6 . Ileum VFAs transport gene expression results of horned and polled Qilian sheep.

2.7 Correlation Analysis Between Ileal Microbiota and Metabolites

Based on the ileal microbiota and metabolomic profiles, correlation analysis was furth er performed to investigate associations between microbial taxa at the phylum level and m etabolite modules Fig. 7. As shown in the correlation heatmap, phylum-level microbial tax a were associated with 10 metabolite modules. Chloroflexi, Acidobacteriota, Nitrospirota, H alanaerobiaeota, Bdellovibrionota, Deinococcota, and Methylomirabilota were strongly positi vely correlated with the MEsalmon module (P<0.001), while Gemmatimonadota was also p ositively correlated with MEsalmon (P<0.05). Fibrobacterota was strongly positively correla ted with MEred (P<0.001). Fusobacteriota was positively correlated with MEturquoise (P<0. 05), whereas Campylobacterota was strongly positively correlated with MEyellow (P<0.001) and positively correlated with MEtan (P<0.05). In addition, significant positive correlation s were observed between Spirochaetota and MEgreenyellow, Planctomycetota and MEblack, Armatimonadota and MEbrown, Cloacimonadota and MEbrown, and Actinobacteriota and MEtan (P<0.05). In contrast, Verrucomicrobiota was negatively correlated with MEyellow (P<0.01), while Fibrobacterota was negatively correlated with MEgreen and MEtan, and C

yanobacteria was negatively correlated with MEtan (P<0.05). Collectively, these results rev ealed extensive associations between ileal microbial taxa and metabolite modules, with ME salmon, MEyellow, and MEred representing potentially important modules associated with variations in the ileal microbial community. Correlation analysis between genus-level micro bial taxa and differential metabolites further revealed a complex association network. Anae rocolumna was positively correlated with Cyclocalopin D and Minaprine (P<0.05), while u ncultured_gir_aah93h0 was positively correlated with Cyclocalopin D, Minaprine, and 1,2-D ihydronaphthalene-1,2-diol (P<0.05). uncultured_Desulfovibrionaceae showed strong positive correlations with Cyclocalopin D and Minaprine (P<0.01), whereas Brenakia was negativel y correlated with Picolinamic acid and (1′ S)-Averantin. These findings indicate close ass ociations between genus-level microbial taxa and ileal metabolites, forming a complex micr obiota–metabolite correlation network. *Erysipelotrichaceae_UCG-009*, Campylobacter, and Elusimicrobium were among the microbial taxa associated with variations in differential m etabolites, providing potential candidates for further investigation of key microbial genera a nd their associated metabolic pathways.

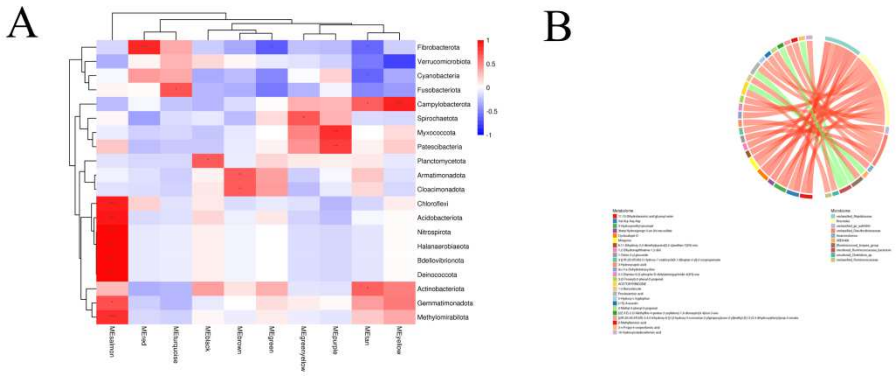


Figure 7. Correlation analysis of ileal microbiomes and metabolites in Qilian sheep. A Microbiomes and metabolites correlation heat map. B Microbiomes and metabolites correlation chord diagram

Note: *P< 0.05, **P< 0.01, ***P< 0.001. The same below.

2.8 Correlation Analysis Among Ileal Microbiota, VFAs, VFA Transport-Related Genes, and Meat Quality Traits

Correlation analysis was performed among the top 10 ileal microbial genera, VFAs, VFA transport-related genes, meat quality traits, nutritional components, and mineral elements. *Erysipelotrichaceae_UCG-009* was significantly or highly significantly positively correlated with the expression levels of *DRA, NHE2*, and *MCT1*, as well as with pH, shear force (SF), water loss rate (CL), and the concentrations of Na, Cr, Fe, and Zn. In contrast, *Erysipelotrichaceae_UCG-009* was significantly or highly significantly negatively correlated with isobutyrate, propionate, and butyrate concentrations, *AE2* expression, cooking yield (CY), crude fat (CF), and crude protein (CP) ($P<0.05$ or $P<0.01$). [Ruminococcus]_torques_group and Atopobium were positively correlated with Ca and negatively correlated with Fe ($P<0.05$). In addition, *[Ruminococcus]_torques_group* was strongly positively correlated with isobutyrate concentration and *AE2* expression, but negatively correlated with the expression levels of DRA, *NHE2*, and *MCT1* ($P<0.05$ or $P<0.01$) (Fig. 8A–C). Spearman's rank correlation analysis was further performed to evaluate the relationships between ileal VFAs and VFA transport-related genes in horned and polled Qilian sheep. As shown in(Fig. 8D), *AE2*, *DRA*, *MCT1*, *MCT4*, *NHE1*, and *NHE2* exhibited extensive correlations with individual VFAs. *AE2* and *DRA* were positively correlated with several VFAs. Strong positive correlations were also observed among several individual VFAs, particularly acetate, butyrate, isovalerate, and valerate. Collectively, these results demonstrate close associations among the ileal microbiota, VFAs, VFA transport-related gene expression, meat quality traits, nutritional composition, and mineral element contents, suggesting coordinated relationships among intestinal microbial composition, fermentation products, epithelial transport-related processes, and host nutritional phenotypes.

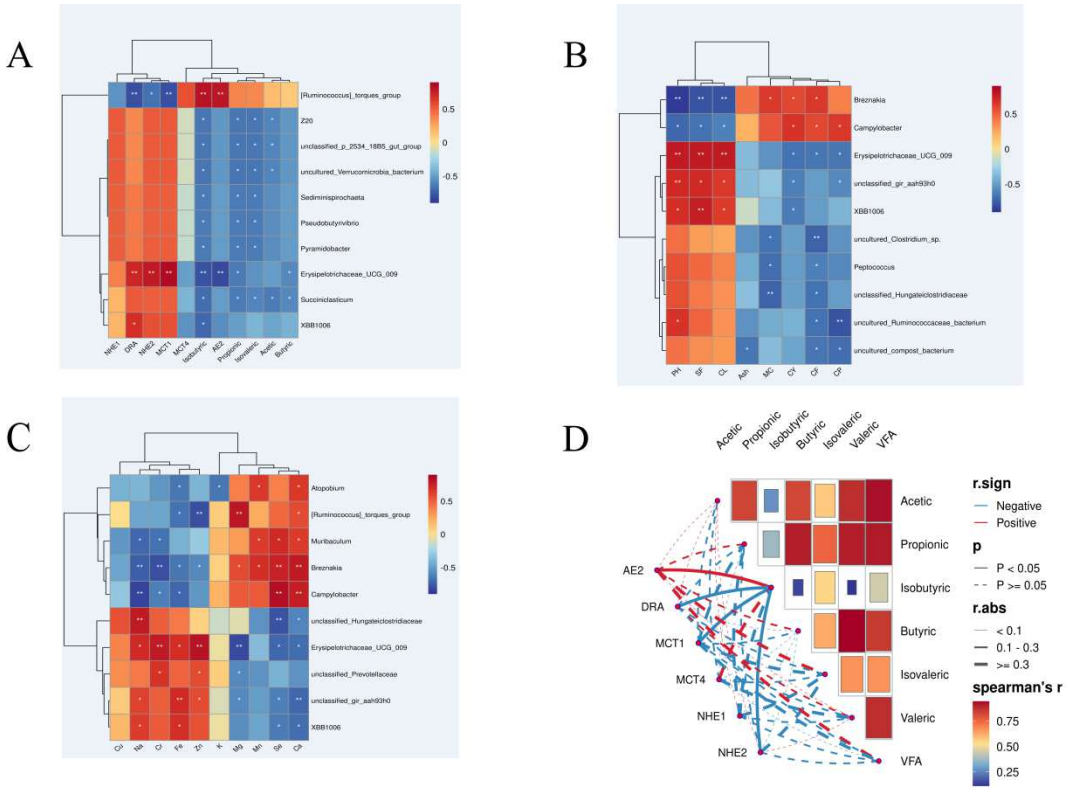


Figure 8. Correlation heatmap between horned and polled Qilian sheep. A Heat map of correlation between ileal microbiomes and VFAs and transport genes. B Correlation heatmap of the ileal microbiome, meat quality traits, and nutritional composition. C Correlation heatmap of the ileal microbiome and trace elements. D Heat map of correlation between ileal VFAs and transport genes

Note: CY, cooking yield; SF, shear force; CL, water loss rate; Ash, ash content; CF, crude fat; CP, crude protein; MC, moisture content.

3Discussion

本The purpose of this study was to analyze the differences of quality, nutrient composition, mineral elements, ileal microbiome, metabolome, VFAs and their transporter gene expression between Qilian sheep and horned Qilian sheep under natural grazing conditions, and to explore the adaptation mechanism of Qilian sheep to plateau based on ileal microbiome and metabolome. Meat quality is the key index to evaluate livestock and poultry products. [43]. Cooked meat rate can well reflect the cooking loss of mutton, that is, the moisture content of meat. [44]. In this study, the cooked meat percentage in different muscle tissues of polled Qilian sheep was significantly higher than that of polled Qilian sheep（P<0.05）, the results showed that the cooking loss of polled

Qilian sheep was relatively low, and the meat quality was the best in polled Qilian sheep. Shear stress is an important index to evaluate meat quality, and there is an interaction between shear stress and tenderness, and tenderness is one of the important factors affecting meat quality [44]，The water loss rate of mutton refers to a kind of water retention ability of mutton, which is related to many eating qualities of mutton, such as flavor, tenderness, color, nutrition and yield during processing[45]，furthermore, the lower the moisture loss rate, the better the water-holding capacity, the better the moisture retention, and the better the meat quality. In the present study, shear force, water loss rate, and pH values in different muscle tissues of polled Qilian sheep were significantly lower than those of horned Qilian sheep（$P<0.05$），indicating that the meat of polled Qilian sheep exhibited greater tenderness. This finding is consistent with the results reported by Carlos et al.[46] in beef, and this phenomenon may be associated with long-term adaptive evolution. Differences in meat quality were also observed among different muscle cuts and between horned and polled Qilian sheep. Overall, meat from horned Qilian sheep exhibited lower shear force, greater tenderness, and better palatability, with the longissimus dorsi showing superior quality to the leg muscle. In contrast, meat from polled Qilian sheep had a higher cooking yield, suggesting a greater yield of cooked meat.

Ash represents the inorganic residue remaining after the volatilization or combustion of organic components and mainly consists of inorganic salts and oxides, particularly oxides of various mineral elements. Ash content can be used as an indicator for evaluating potential contamination of meat and, to some extent, reflects its mineral composition and nutritional value. [47]，In the present study, the ash contents of different muscle tissues from polled Qilian sheep were 11.562 and 7.116, respectively, both of which were significantly higher than those observed in horned Qilian sheep ($P<0.05$). Moreover, the ash content was higher in the longissimus dorsi than in the leg muscle. These findings suggest that meat from polled Qilian sheep may contain higher levels of total minerals, potentially indicating greater nutritional value from the perspective of mineral composition. Protein is an important indicator of the nutritional composition of mutton. Previous studies have shown that mutton is a rich source of high-quality protein[48], and also contains various vitamins and unsaturated fatty acids. Owing to its favorable nutritional profile and palatability, mutton is widely regarded as a nutritionally valuable meat product. In the present study, the crude protein content of meat from polled Qilian sheep was significantly higher than

that from horned Qilian sheep (P<0.05), suggesting that polled Qilian sheep meat may have greater nutritional value, particularly in terms of protein content. The crude fat measured in the present study mainly represents intramuscular fat, which is an important indicator of meat quality and plays a key role in determining meat flavor and palatability[49]，In the present study, the crude fat content in the leg muscle of polled Qilian sheep was significantly higher than that of horned Qilian sheep (P<0.05), suggesting that the higher intramuscular fat content may contribute to improved palatability and tenderness of meat from polled Qilian sheep. However, excessive fat deposition may result in an undesirable greasy mouthfeel; therefore, an appropriate level of intramuscular fat is essential for maintaining desirable sensory characteristics of meat[50]. Intramuscular fat can effectively improve meat tenderness, palatability, and flavor by reducing shear force and enhancing juiciness[51]. Dry matter content is inversely related to the moisture content of mutton and can provide an indirect indication of its juiciness and palatability. It also reflects the overall content of non-water constituents, including organic components and minerals, and can therefore serve as an indicator of the nutritional composition of mutton. In the present study, the moisture content of different muscle tissues from polled Qilian sheep was numerically higher than that of horned Qilian sheep, although the differences were not statistically significant ($P > 0.05$). This tendency may indicate a greater water-holding capacity and lower cooking loss in meat from polled Qilian sheep, which is consistent with the meat quality characteristics described above and is also in agreement with the findings reported by Syed et al[52].

Mineral elements are essential nutrients for the human body and play important physiological roles in maintaining fluid and electrolyte homeostasis, acid–base balance, and other biological functions[53]. Potassium, calcium, and magnesium contribute to the regulation of acid–base homeostasis and are involved in various physiological and metabolic processes essential for normal cellular function[54]. In addition, mutton is a valuable dietary source of zinc, an essential trace element involved in the synthesis and function of numerous enzymes and proteins. Adequate zinc intake is particularly important for normal growth and development, immune function, and cognitive development in children, whereas zinc deficiency may impair immune function and increase susceptibility to adverse health outcomes[55]. In the present study, the concentrations of Fe and Zn in the longissimus dorsi of horned Qilian sheep were significantly higher than those in polled Qilian sheep（P<0.05）; The concentrations of Mn and K in different muscle tissues were

higher in polled Qilian sheep than in horned Qilian sheep. In addition, the Ca concentration in the leg muscle and the Mg concentration in the longissimus dorsi were significantly higher in polled Qilian sheep than in horned Qilian sheep（P<0.05）；Selenium (Se) is an essential trace element that is widely distributed in the environment and plays important roles in human health[56]，Hintze et al [56] reported strong associations among Se concentrations in soil, plants, and animal muscle, with beef produced in high-Se areas containing substantially higher Se concentrations than that produced in low-Se areas. Therefore, Se content has been proposed as a potential indicator for distinguishing the geographical origin of animal products. In the present study, Se concentrations in different muscle tissues were higher in polled Qilian sheep than in horned Qilian sheep, with a significant difference observed in the leg muscle (P<0.05). These findings indicate that meat from polled Qilian sheep may provide a relatively greater dietary source of Se and may have potential nutritional value for populations with insufficient Se intake. Moreover, the relatively high Se content may represent a favorable nutritional characteristic of polled Qilian sheep meat.By regulating epithelial transporters and ion channels, the small intestine (duodenum, jejunum and ileum) absorbs most nutrients along the entire intestinal tract[57]. Compared to the jejunum, the ileum possessed higher efficiency of glucose uptake[58]. In the present study, at the phylum level, *Firmicutes*, *Patescibacteria*, *Spirochaetota*, and *Desulfobacterota* were more abundant in the ileum of polled Qilian sheep. *Firmicutes* have been reported to contribute to the degradation of dietary fiber and nutrient utilization, thereby potentially promoting host growth and development[59]. At the genus level, the relative abundance of *Rikenellaceae_RC9_gut_group*, which has been reported to be involved in the degradation of plant-derived polysaccharides [60] was significantly higher in the ileum of polled Qilian sheep than in that of horned Qilian sheep (P<0.05). In addition, *Breznakia* was identified as one of the predominant genera in the ileum of polled Qilian sheep. Further KEGG functional enrichment analysis revealed that pathways related to Global and overview maps, Signal transduction, Amino acid metabolism, and Energy metabolism were significantly enriched in polled Qilian sheep[61]，suggesting a greater functional potential for signal transduction, amino acid metabolism, lipid metabolism, energy metabolism, and environmental adaptation. In contrast, functions associated with Membrane transport, which may facilitate interactions between the gut microbiota and host cells[62], were significantly enriched in the ileal microbiota of horned Qilian sheep；COG functional analysis further revealed

that functional categories including Energy production and conversion, Lipid transport and metabolism, Signal transduction mechanisms, and Cell wall/membrane/envelope biogenesis were significantly enriched in polled Qilian sheep compared with horned Qilian sheep. Among these categories, Cell wall/membrane/envelope biogenesis is involved in the formation and maintenance of bacterial cell walls, cytoplasmic membranes, and outer membrane structures, which are closely associated with bacterial growth and division, adhesion and colonization, as well as tolerance to bile acids and antimicrobial stress[63], enrichment of this functional category generally suggests an enhanced microbial potential for cellular structure biogenesis, environmental stress tolerance, and intestinal colonization, which may further influence microbe–mucosal interactions, intestinal barrier function, and microbial community stability. In comparative studies of core microbial functions in pigs and other livestock species, this category has frequently been identified as an important functional module together with Energy production and conversion and Lipid transport and metabolism[64]. Furthermore, pathway enrichment analysis identified significant enrichment of ABC transporters, Antifolate resistance, Axon regeneration, Histidine metabolism, and the Prolactin signaling pathway.

Metabolomics plays an important role in characterizing the physiological and biochemical status of animals [65]. In the present study, distinct differences in the ileal metabolite profiles were observed between horned and polled Qilian sheep. Metabolites such as Aminofurantoin, Nebramine, and Geldanamycin were more abundant in the ileum of horned Qilian sheep, whereas Cyclocalopin D, 10-Acetoxyoleuropein, dTDP-5-dimethyl-L-lyxose, 24,25-Dihydrolanosterol, 2-Hydroxy-desipramine glucuronide, and Hesperetin exhibited higher levels in the ileum of polled Qilian sheep. 24,25-Dihydrolanosterol is an important sterol intermediate involved in the cholesterol biosynthesis pathway. Its increased abundance may indicate alterations in sterol metabolism in polled Qilian sheep, potentially associated with cholesterol biosynthesis, membrane lipid formation, and the metabolism of bile acid precursors[66], Hesperetin is a plant-derived flavanone that has been reported to possess antioxidant and anti-inflammatory properties and to modulate the gut microbiota. Changes in its abundance may influence the redox status of the intestinal mucosa, intestinal barrier integrity, and host immunometabolism[67]. Further KEGG enrichment analysis of the differential metabolites revealed that the metabolic differences between horned and polled Qilian sheep were mainly associated with pathways related to Lipid metabolism,

Metabolism of cofactors and vitamins, Amino acid metabolism, Digestive system, and Nucleotide metabolism. Among these pathways, alterations in Lipid metabolism may reflect the remodeling of fatty acid, glycerophospholipid, and bile acid metabolism, which may subsequently affect lipid absorption, intestinal mucosal barrier function, and fat deposition[68], The ABC transporters pathway was enriched in the ileum of polled Qilian sheep. ABC transport systems are involved in the transport and uptake of a wide range of substrates by gut microorganisms, including amino acids and other nutrients, thereby potentially facilitating nutrient utilization and microbial growth. [69]. In addition, the Histidine metabolism pathway was significantly enriched in the ileum of polled Qilian sheep, suggesting potential alterations in the utilization of amino acids and the metabolism of histidine-derived metabolites by the gut microbiota. These metabolic changes may be associated with protein metabolism, antioxidant capacity, and intestinal immune homeostasis. [70], In contrast, the Prolactin signaling pathway was significantly enriched in horned Qilian sheep, suggesting a potential association with host endocrine regulation and immunometabolic processes. Collectively, these findings revealed significant differences in ileal metabolite profiles and enriched metabolic pathways between horned and polled Qilian sheep under the same grazing conditions. Compared with horned Qilian sheep, polled Qilian sheep exhibited higher abundances of metabolites associated with energy metabolism and endocrine-related pathways. These metabolic differences may reflect distinct functional characteristics of the ileal microbiota in polled Qilian sheep, particularly with respect to the transmembrane transport of nutrient substrates, amino acid utilization, and the transformation of nitrogen-containing metabolites[71].

After entering the gastrointestinal tract, dietary nutrients are digested and hydrolyzed by various digestive enzymes into absorbable molecules, such as free amino acids, monosaccharides including glucose and fructose, and other small molecules.These digestion products are subsequently transported across the intestinal epithelium into enterocytes via specific membrane transporters, maintaining the intestinal barrier function is crucial for organismic health, and SCFAs produced by gut microbiota play a vital role in this process[72]。In the present study, the total volatile fatty acid (VFA) concentration was significantly higher in polled Qilian sheep than in horned Qilian sheep, suggesting greater microbial fermentation activity and an increased availability of VFAs as energy substrates for the host. The acetate-to-propionate (A/P) ratio is associated with the efficiency of dietary energy utilization, with a lower A/P ratio generally

indicating a more energetically favorable fermentation pattern[73], In the present study, the A/P ratio was significantly lower in polled Qilian sheep than in horned Qilian sheep ($P<0.05$). Together with the higher total VFA concentration, these findings suggest that polled Qilian sheep may exhibit a fermentation pattern conducive to more efficient utilization of dietary substrates and greater VFA production. Butyrate, an important microbial metabolite, has been reported to contribute to improved growth performance and immune function, whereas propionate serves as an important precursor for energy metabolism and plays a critical role in maintaining normal physiological functions[74], In the present study, the concentrations of both butyrate and propionate in the ileum of polled Qilian sheep were significantly higher than those in horned Qilian sheep ($P<0.05$), suggesting enhanced microbial fermentation and a greater availability of fermentation-derived energy substrates in the ileum of polled Qilian sheep. In the gastrointestinal tract, a substantial proportion of butyrate is metabolized by epithelial cells, particularly intestinal epithelial cells, and oxidized to CO_2 or converted into ketone bodies, which are generated during fatty acid oxidation and can serve as alternative energy substrates[75]. Following transporter-mediated uptake across the intestinal epithelium, volatile fatty acids (VFAs) can enter the circulation and subsequently contribute to energy metabolism, growth, and other physiological processes. Their intestinal transport and absorption are mediated by several transport proteins encoded by genes such as *AE2*, *DRA*, *NHE*, and *MCT*. Among these, *AE2* and *DRA* encode anion exchangers that participate in the exchange of VFA anions and HCO_3^- across the epithelial membrane, thereby contributing to VFA absorption and the maintenance of intestinal acid–base and ionic homeostasis. *MCT1* plays a critical role in the transport and absorption of VFAs across the intestinal epithelium[76], whereas DRA functions primarily as a Cl^-/HCO_3^- exchanger and contributes to VFA absorption across the apical membrane of intestinal epithelial cells. NHE are membrane transport proteins that mediate the exchange of extracellular Na^+ and intracellular H^+ and may indirectly facilitate VFA absorption by regulating the transmembrane proton gradient and local pH[77]，Previous studies have demonstrated that *NHE* isoforms, including *NHE1* and *NHE2*, are expressed in the gastrointestinal epithelium, where their activity may influence epithelial VFA absorption. In the present study, the expression levels of the three VFA transport-related genes described above were significantly higher in the ileal epithelium of polled Qilian sheep than in that of horned Qilian sheep ($P<0.05$). These findings suggest that polled Qilian sheep may have a

greater capacity for intestinal VFA transport, absorption, and subsequent utilization under high-altitude conditions. The present study showed that *MCT1* expression in the ileal epithelium of polled Qilian sheep was significantly higher than that in horned Qilian sheep. Heredia et al[78] reported that hypoxia increased the expression of *MCT1* and *MCT4* in human adipocytes. Similar to other glycolysis-related proteins, *MCT4* is upregulated under hypoxic conditions through an HIF-1α-dependent regulatory mechanism, thereby facilitating the efflux of lactate generated by enhanced glycolysis and preventing its intracellular accumulation. Although differences in species, tissue type, and *MCT* isoforms should be considered, these findings suggest that the elevated *MCT1* expression observed in polled Qilian sheep may be associated with metabolic adaptation to the hypoxic environment. Together with the VFA-related findings described above, enhanced intestinal VFA transport and utilization may represent an important metabolic characteristic contributing to the adaptation of Qilian sheep to the harsh, cold, and hypoxic conditions of the Qinghai–Tibetan Plateau.

Collectively, these findings reveal coordinated alterations among the ileal microbiome, metabolome, volatile fatty acids (VFAs), and VFA transport-related genes in Qilian sheep. These results suggest that the ileal microbiota may not only participate in the production and transformation of local metabolites but also contribute to host nutrient utilization by modulating VFA production, transmembrane transport, and intestinal nutrient absorption. Such microbiota–metabolite–host interactions may further be associated with nutrient deposition and the development of meat quality traits in Qilian sheep. In the present study, Anaerocolumna, uncultured_gir_aah93h0, and uncultured_Desulfovibrionaceae showed significant or highly significant positive correlations with Cyclocalopin D and Minaprine. In addition, uncultured_gir_aah93h0 was significantly positively correlated with 1,2-Dihydronaphthalene-1,2-diol. Cyclocalopin D is potentially a secondary metabolite of fungal or plant origin, whereas 1,2-Dihydronaphthalene-1,2-diol is generally associated with the oxidative degradation of aromatic compounds. These correlations suggest potential associations between specific ileal microbial taxa and the transformation of exogenous or aromatic compounds; however, the underlying metabolic relationships require further functional validation[79]. Therefore, the coordinated changes between these microbial taxa and metabolites may reflect their potential involvement in the biotransformation of forage-derived secondary metabolites, fungal

natural products, or exogenous aromatic compounds[80]. Further analysis of the associations between predominant bacterial genera and VFAs, VFA transport-related genes, meat quality traits, nutritional components, and trace elements revealed that *Erysipelotrichaceae_UCG_009* was closely associated with multiple host nutritional and metabolic traits. These findings suggest that *Erysipelotrichaceae_UCG_009* may represent a potentially important microbial taxon linking intestinal microbial metabolism with host nutritional phenotypes [79]. *Erysipelotrichaceae_UCG_009* showed significant or highly significant positive correlations with the expression levels of *DRA*, *NHE2*, and *MCT1*, as well as with pH, shear force, cooking loss, and the concentrations of Na, Cr, Fe, and Zn. In contrast, it was significantly or highly significantly negatively correlated with isobutyrate, propionate, butyrate, *AE2* expression, cooking yield, crude fat content, and crude protein content. Members of the family *Erysipelotrichaceae* have frequently been associated with host lipid metabolism, bile acid metabolism, and inflammatory status. Therefore, variations in the abundance of *Erysipelotrichaceae_UCG_009* may be associated with changes in the production of fatty acid fermentation products, ion transport across the intestinal epithelium, and nutrient absorption[81]. In the present study, *Erysipelotrichaceae_UCG_009* was negatively correlated with multiple VFAs, crude fat, and crude protein, while showing positive correlations with several VFA transport-related genes and trace elements. These associations suggest that *Erysipelotrichaceae_UCG_009* may be more closely associated with the regulation of the intestinal luminal ionic environment and epithelial transport processes. Given that pH, shear force, and cooking loss are closely related to meat quality characteristics, the correlations between this bacterial taxon and these traits further suggest that the ileal microbiota may indirectly contribute to variations in the physicochemical properties of muscle by modulating nutrient absorption, mineral utilization, and host metabolic status [82]. *[Ruminococcus]_torques_group* and *Atopobium* were significantly positively correlated with Ca but negatively correlated with Fe, suggesting that these bacterial taxa may be associated with mineral absorption, complexation, or the regulation of the intestinal microenvironment. In addition, [*Ruminococcus]_torques_group* showed a highly significant positive correlation with isobutyrate and *AE2* expression, whereas it was significantly or highly significantly negatively correlated with the expression levels of *DRA*, *NHE2*, and *MCT1*. Ruminococcus-related bacteria are known for their capacity to degrade complex carbohydrates, and their fermentative activity can generate

metabolites such as acetate, butyrate, and branched-chain fatty acids[83]. These associations suggest that *[Ruminococcus]_torques_group* may be involved in the interplay between microbial carbohydrate fermentation, VFA metabolism, epithelial transport, and mineral utilization in the ileum. Isobutyrate is primarily produced through the microbial degradation of branched-chain amino acids, particularly valine. Therefore, the positive correlation between *[Ruminococcus]_torques_group* and isobutyrate may reflect the potential involvement of this bacterial group in branched-chain amino acid metabolism or protein fermentation. Further correlation network analysis revealed extensive associations between *AE2*, *DRA*, *MCT1*, *MCT4*, *NHE1*, and *NHE2* and different VFAs, suggesting that ileal VFA transport and absorption are not governed by a single transporter but may involve the coordinated participation of monocarboxylate transporters, anion exchangers, and Na^+/H^+ exchangers[84]. The positive correlations of *AE2* and *DRA* with several VFAs further suggest that anion exchange systems may facilitate transepithelial VFA transport by contributing to the maintenance of epithelial acid–base homeostasis and transmembrane ionic gradients[85], taken together, the coordinated variations in multiple VFAs may reflect an overall shift in the utilization and fermentation of carbohydrate- and protein-derived substrates by the ileal microbiota[86]. Collectively, these findings suggest that differences in the ileal microbial ecosystem between horned and polled Qilian sheep may contribute to variations in host energy utilization, mineral homeostasis, and the development of meat quality traits by modulating VFA production, ion and monocarboxylate transport, and nutrient deposition.

Result

This study demonstrated that, under the same grazing conditions, horned and polled Qilian sheep exhibited significant differences in meat quality, nutritional composition, and ileal microbial and metabolic characteristics. Polled Qilian sheep had higher contents of crude protein, crude fat, and several mineral elements, while differences in the ileal microbiota and metabolites were mainly associated with nutrient utilization and lipid, amino acid, and energy metabolism. Polled Qilian sheep also exhibited higher ileal concentrations of total volatile fatty acids (VFAs), propionate, and butyrate, a lower acetate-to-propionate (A/P) ratio, and higher expression levels of several VFA transport-related genes, suggesting greater potential for microbial fermentation and

VFA transport and utilization. Correlation analyses further revealed close associations of predominant bacterial taxa with VFAs, VFA transport-related genes, nutritional components, mineral elements, and meat quality traits. Collectively, horned and polled Qilian sheep exhibited distinct ileal microbial and metabolic profiles, which may contribute to the development of meat quality traits by modulating VFA production and transport as well as nutrient absorption and deposition. These findings provide a theoretical basis for elucidating the potential gut microbial mechanisms underlying differences in meat quality between horned and polled Qilian sheep and for the evaluation and utilization of Qilian sheep germplasm resources.

Declarations

Ethics approval and consent to participate: All studies involving animal were carried out in accordancewith the regulations for the Administration of Affairs Con-cerning Experimental Animal (Ministry of Science and Tech-nology, China; revise in June 2004), and sample collectionprotocols were approved by the Livestock Care Committee of Academy of Animal Science and Veterinary Medicine, Qinghai University (Approval No. 2026-QHMKY-010). Consent to participate was not applicable to this study. Informed consent was obtained from the animal owners for the use of the animals in this study.

Consent for publication: Not Applicable.

Availability of data and materials： The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession numbers can be found below: (Sequence Read Archive (SRA): PRJNA818841 (Microbial sequence)/PRJNA819418 (Transcriptome sequence)).

Competing interests: The authors declare no competing interests.

Funding： Special Program for Science and Technology Commissioners of Qinghai Province (2026-NK-P38).

Authors' Contributions: Conceptualization, data curation, writing—original draft preparation, X.Y.; funding acquisition,supervision, L.W. and L.C.; methodology, formal analysis,Y.L. and X. Y.; investigation, methodology, L.J.; validation, Z.J.; formal analysis, methodology, C.Y. and F.Y. All authorshave read and agreed to the published version of the manuscript.

Acknowledgments: Thanks to all participants for their advice and support of this study.

4References

[1] Z. Tański, M. Stanisław, Z. Bożena, The quality of modified atmosphere packaged meat from lambs slaughtered at 50 and 100 days of age, Asian-Australas J Anim Sci 25(3) (2012) 428-34.
[2] J. Fahey, K. O'Sullivan, J. Crilly, J. F. Mee, The effect of feeding and management practices on calving rate in dairy herds, Anim Reprod Sci 74(3-4) (2002) 133-50.
[3] J. M. Hughes, S. K. Oiseth, P. P. Purslow, R. D. Warner, A structural approach to understanding the interactions between colour, water-holding capacity and tenderness, Meat Sci 98(3) (2014) 520-32.
[4] V. A. Santos, S. R. Silva, J. M. Azevedo, Carcass composition and meat quality of equally mature kids and lambs, J Anim Sci 86(8) (2008) 1943-50.
[5] N. Atti, M. Mahouachi, The effects of diet, slaughter weight and docking on growth, carcass composition and meat quality of fat-tailed Barbarine lambs. A review, Trop Anim Health Prod 43(7) (2011) 1371-8.
[6] A. Kaliniak-Dziura, P. Domaradzki, M. Kowalczyk, M. Florek, P. Skałecki, M. Kędzierska-Matysek, P. Stanek, M. Dmoch, T. Grenda, E. Kowalczuk-Vasilev, Effect of heat treatments on the physicochemical and sensory properties of the longissimus thoracis muscle in unweaned Limousin calves, Meat Sci 192 (2022) 108881.
[7] Y. Wang, X. Tian, X. Liu, J. Xing, C. Guo, Y. Du, H. Zhang, W. Wang, Focusing on intramuscular connective tissue: Effect of cooking time and temperature on physical, textual, and structural properties of yak meat, Meat Sci 184 (2022) 108690.
[8] F. Toldrá, L. Mora, M. Reig, New insights into meat by-product utilization, Meat Sci 120 (2016) 54-59.
[9] Q. Wang, H. Liu, S. Zhao, M. Qie, Y. Bai, J. Zhang, J. Guo, Y. Zhao, Discrimination of mutton from different sources (regions, feeding patterns and species) by mineral elements in Inner Mongolia, China, Meat Sci 174 (2021) 108415.
[10] J. Ma, J. Wang, S. Mahfuz, S. Long, D. Wu, J. Gao, X. Piao, Supplementation of Mixed Organic Acids Improves Growth Performance, Meat Quality, Gut Morphology and Volatile Fatty Acids of Broiler Chicken, Animals (Basel) 11(11) (2021).
[11] V. Kaplan-Shabtai, N. Indugu, M. L. Hennessy, B. Vecchiarelli, J. S. Bender, D. Stefanovski, C. F. De Assis Lage, S. E. Räisänen, A. Melgar, K. Nedelkov, M. E. Fetter, A. Fernandez, A. Spitzer, A. N. Hristov, D. W. Pitta, Using Structural Equation Modeling to Understand Interactions Between Bacterial and Archaeal Populations and Volatile Fatty Acid Proportions in the Rumen, Front Microbiol 12 (2021) 611951.
[12] S. Kumar, N. Indugu, B. Vecchiarelli, D. W. Pitta, Associative patterns among anaerobic fungi, methanogenic archaea, and bacterial communities in response to changes in diet and age in the rumen of dairy cows, Front Microbiol 6 (2015) 781.
[13] A. Koh, F. De Vadder, P. Kovatcheva-Datchary, F. Bäckhed, From Dietary Fiber to Host Physiology: Short-Chain Fatty Acids as Key Bacterial Metabolites, Cell 165(6) (2016) 1332-1345.
[14] E. A. Smith, G. T. Macfarlane, Dissimilatory amino Acid metabolism in human colonic bacteria, Anaerobe 3(5) (1997) 327-37.
[15] V. B. Horincar, G. Parfene, A. K. Tyagi, D. Gottardi, R. Dinică, M. E. Guerzoni, G.

Bahrim, Extraction and characterization of volatile compounds and fatty acids from red and green macroalgae from the Romanian Black Sea in order to obtain valuable bioadditives and biopreservatives, Journal of Applied Phycology 26(1) (2014) 551-559.
[16] S. Xia, W. Yao, B. Zou, Y. Lu, N. Lu, H. Lei, D. Xia, Effects of potassium diformate on the gastric function of weaning piglets, Animal Production Science 56(7) (2015) 1161-1166.
[17] S. Bilk, K. Huhn, K. U. Honscha, H. Pfannkuche, G. Gäbel, Bicarbonate exporting transporters in the ovine ruminal epithelium, J Comp Physiol B 175(5) (2005) 365-74.
[18] K. Dieho, J. van Baal, L. Kruijt, A. Bannink, J. T. Schonewille, D. Carreño, W. H. Hendriks, J. Dijkstra, Effect of supplemental concentrate during the dry period or early lactation on rumen epithelium gene and protein expression in dairy cattle during the transition period, J Dairy Sci 100(9) (2017) 7227-7245.
[19] X. Gao, M. Oba, Characteristics of dairy cows with a greater or lower risk of subacute ruminal acidosis: Volatile fatty acid absorption, rumen digestion, and expression of genes in rumen epithelial cells, J Dairy Sci 99(11) (2016) 8733-8745.
[20] D. Kirat, J. Masuoka, H. Hayashi, H. Iwano, H. Yokota, H. Taniyama, S. Kato, Monocarboxylate transporter 1 (MCT1) plays a direct role in short-chain fatty acids absorption in caprine rumen, J Physiol 576(Pt 2) (2006) 635-47.
[21] A. Borthakur, S. Saksena, R. K. Gill, W. A. Alrefai, K. Ramaswamy, P. K. Dudeja, Regulation of monocarboxylate transporter 1 (MCT1) promoter by butyrate in human intestinal epithelial cells: involvement of NF-kappaB pathway, J Cell Biochem 103(5) (2008) 1452-63.
[22] M. A. Cuff, D. W. Lambert, S. P. Shirazi-Beechey, Substrate-induced regulation of the human colonic monocarboxylate transporter, MCT1, J Physiol 539(Pt 2) (2002) 361-71.
[23] N. J. Philp, J. D. Ochrietor, C. Rudoy, T. Muramatsu, P. J. Linser, Loss of MCT1, MCT3, and MCT4 expression in the retinal pigment epithelium and neural retina of the 5A11/basigin-null mouse, Invest Ophthalmol Vis Sci 44(3) (2003) 1305-11.
[24] C. Graham, I. Gatherar, I. Haslam, M. Glanville, N. L. Simmons, Expression and localization of monocarboxylate transporters and sodium/proton exchangers in bovine rumen epithelium, Am J Physiol Regul Integr Comp Physiol 292(2) (2007) R997-1007.
[25] C. Felippe Gonçalves-de-Albuquerque, A. Ribeiro Silva, C. Ignácio da Silva, H. Caire Castro-Faria-Neto, P. Burth, Na/K Pump and Beyond: Na/K-ATPase as a Modulator of Apoptosis and Autophagy, Molecules 22(4) (2017) 578.
[26] J. E. Langridge-Smith, Interaction between sodium and chloride transport in bovine tracheal epithelium, J Physiol 376 (1986) 299-319.
[27] W. A. Alrefai, X. Wen, W. Jiang, J. P. Katz, K. A. Steinbrecher, M. B. Cohen, I. R. Williams, P. K. Dudeja, G. D. Wu, Molecular cloning and promoter analysis of downregulated in adenoma (DRA), Am J Physiol Gastrointest Liver Physiol 293(5) (2007) G923-34.
[28] J. R. Aschenbach, G. B. Penner, F. Stumpff, G. Gäbel, Ruminant Nutrition Symposium: Role of fermentation acid absorption in the regulation of ruminal pH, J Anim Sci 89(4) (2011) 1092-107.
[29] S. Mäkelä, J. Kere, C. Holmberg, P. Höglund, SLC26A3 mutations in congenital chloride diarrhea, Hum Mutat 20(6) (2002) 425-38.
[30] C. Talbot, C. Lytle, Segregation of Na/H exchanger-3 and Cl/HCO3 exchanger SLC26A3 (DRA) in rodent cecum and colon, Am J Physiol Gastrointest Liver Physiol 299(2) (2010)

G358-67.
[31] K. Fuwa, S. Hosono, N. Nagano, S. Munakata, R. Fukamachi, T. Okada, S. Takahashi, S. Takahashi, N. Sato, T. Nakayama, Japanese neonate with congenital chloride diarrhea caused by SLC26A3 mutation, Pediatr Int 57(1) (2015) e11-3.
[32] R. L. t. Baldwin, B. W. Jesse, Developmental changes in glucose and butyrate metabolism by isolated sheep ruminal cells, J Nutr 122(5) (1992) 1149-53.
[33] W. Zhao, Y. Wang, S. Liu, J. Huang, Z. Zhai, C. He, J. Ding, J. Wang, H. Wang, W. Fan, J. Zhao, H. Meng, The dynamic distribution of porcine microbiota across different ages and gastrointestinal tract segments, PLoS One 10(2) (2015) e0117441.
[34] W. Yan, C. Sun, J. Yuan, N. Yang, Gut metagenomic analysis reveals prominent roles of Lactobacillus and cecal microbiota in chicken feed efficiency, Sci Rep 7 (2017) 45308.
[35] J. Wang, H. Fan, Y. Han, J. Zhao, Z. Zhou, Characterization of the microbial communities along the gastrointestinal tract of sheep by 454 pyrosequencing analysis, Asian-Australas J Anim Sci 30(1) (2017) 100-110.
[36] D. R. Davies, M. K. Theodorou, M. I. Lawrence, A. P. Trinci, Distribution of anaerobic fungi in the digestive tract of cattle and their survival in faeces, J Gen Microbiol 139 Pt 6 (1993) 1395-400.
[37] S. Mao, M. Zhang, J. Liu, W. Zhu, Characterising the bacterial microbiota across the gastrointestinal tracts of dairy cattle: membership and potential function, Sci Rep 5 (2015) 16116.
[38] R. Lan, Y. Li, Q. Chang, Z. Zhao, Dietary chitosan oligosaccharides alleviate heat stress–induced intestinal oxidative stress and inflammatory response in yellow-feather broilers, Poultry Science 99(12) (2020) 6745-6752.
[39] P. W. van der Wielen, D. A. Keuzenkamp, L. J. Lipman, F. van Knapen, S. Biesterveld, Spatial and temporal variation of the intestinal bacterial community in commercially raised broiler chickens during growth, Microb Ecol 44(3) (2002) 286-93.
[40] L. Lin, F. Xie, D. Sun, J. Liu, W. Zhu, S. Mao, Ruminal microbiome-host crosstalk stimulates the development of the ruminal epithelium in a lamb model, Microbiome 7(1) (2019) 83.
[41] S. Fraune, T. C. Bosch, Long-term maintenance of species-specific bacterial microbiota in the basal metazoan Hydra, Proc Natl Acad Sci U S A 104(32) (2007) 13146-51.
[42] H. Meng, Y. Zhang, L. Zhao, W. Zhao, C. He, C. F. Honaker, Z. Zhai, Z. Sun, P. B. Siegel, Body weight selection affects quantitative genetic correlated responses in gut microbiota, PLoS One 9(3) (2014) e89862.
[43] R. D. Warner, T. L. Wheeler, M. Ha, X. Li, A. E. Bekhit, J. Morton, R. Vaskoska, F. R. Dunshea, R. Liu, P. Purslow, W. Zhang, Meat tenderness: advances in biology, biochemistry, molecular mechanisms and new technologies, Meat Sci 185 (2022) 108657.
[44] L. Majdoub-Mathlouthi, B. Saïd, A. Say, K. Kraiem, Effect of concentrate level and slaughter body weight on growth performances, carcass traits and meat quality of Barbarine lambs fed oat hay based diet, Meat Sci 93(3) (2013) 557-63.
[45] A. K. Rai, M. Singh, T. More, Cutaneous water loss and respiration rates of various breeds of sheep at high ambient temperatures, Trop Anim Health Prod 11(1) (1979) 51-6.
[46] C. Álvarez, L. Koolman, M. Whelan, A. Moloney, Effect of Pre-Slaughter Practises and Early Post-Mortem Interventions on Sheep Meat Tenderness and Its Impact on Microbial

Status, Foods 11(2) (2022).
[47] B. Jiang, Y. Zhou, T. Wang, F. Li, Nutritive value and ruminal degradation of seven Chinese herbs as forage for Tan sheep, Bioengineered 11(1) (2020) 1159-1169.
[48] H. Hamed Hammad Mohammed, G. Jin, M. Ma, I. Khalifa, R. Shukat, A. E. Elkhedir, Q. Zeng, A. E. Noman, Comparative characterization of proximate nutritional compositions, microbial quality and safety of camel meat in relation to mutton, beef, and chicken, LWT 118 (2020) 108714.
[49] Y. Liu, R. Li, Y. Ying, Y. Zhang, Y. Huang, H. Wu, K. Lin, Non-genetic factors affecting the meat quality and flavor of Inner Mongolian lambs: A review, Front Vet Sci 9 (2022) 1067880.
[50] B. Navidshad, M. Royan, Effect of Dietary Fat on Gene Expression in Poultry, A Review, Crit Rev Eukaryot Gene Expr 26(4) (2016) 333-341.
[51] Z. Lu, J. Li, C. Yuan, B. Xi, B. Yang, X. Meng, T. Guo, Y. Yue, Y. Gao, J. Liu, X. Sun, Evaluation of Mutton Quality Characteristics of Dongxiang Tribute Sheep Based on Membership Function and Gas Chromatography and Ion Mobility Spectrometry, Front Nutr 9 (2022) 852399.
[52] S. M. Rafiq, B. C. Ghosh, Effect of potato incorporation on the physico-chemical, textural and sensory properties of processed cheese, Journal of Food Measurement and Characterization 11(2) (2017) 776-780.
[53] Y. Zhao, D. Wang, S. Yang, Effect of organic and conventional rearing system on the mineral content of pork, Meat Sci 118 (2016) 103-7.
[54] G. Genchi, G. Lauria, A. Catalano, M. S. Sinicropi, A. Carocci, Biological Activity of Selenium and Its Impact on Human Health, Int J Mol Sci 24(3) (2023).
[55] M. López Alonso, J. L. Benedito, M. Miranda, C. Castillo, J. Hernández, R. F. Shore, Cattle as biomonitors of soil arsenic, copper, and zinc concentrations in Galicia (NW Spain), Arch Environ Contam Toxicol 43(1) (2002) 103-8.
[56] J. M. Wilkinson, J. Hill, C. J. Phillips, The accumulation of potentially-toxic metals by grazing ruminants, Proc Nutr Soc 62(2) (2003) 267-77.
[57] M. Khosravi, B. Dastar, O. Ashayerizadeh, F. Khajali, Effects of fermented dried brewer's grain on performance, carcass characteristics, morphology of ileum and blood parameters in broiler chickens, Poult Sci 104(8) (2025) 105284.
[58] J. Herrmann, B. Schröder, S. Klinger, A. Thorenz, A. C. Werner, H. Abel, G. Breves, Segmental diversity of electrogenic glucose transport characteristics in the small intestines of weaned pigs, Comp Biochem Physiol A Mol Integr Physiol 163(1) (2012) 161-9.
[59] X. Lin, J. Wang, Q. Hou, Y. Wang, Z. Hu, K. Shi, Z. Yan, Z. Wang, Effect of hay supplementation timing on rumen microbiota in suckling calves, Microbiologyopen 7(1) (2018).
[60] B. Peng, S. Huang, T. Liu, A. Geng, Bacterial xylose isomerases from the mammal gut Bacteroidetes cluster function in Saccharomyces cerevisiae for effective xylose fermentation, Microb Cell Fact 14 (2015) 70.
[61] Q. Fan, X. Cui, Z. Wang, S. Chang, M. Wanapat, T. Yan, F. Hou, Rumen Microbiota of Tibetan Sheep (Ovis aries) Adaptation to Extremely Cold Season on the Qinghai-Tibetan Plateau, Front Vet Sci 8 (2021) 673822.
[62] H. Konishi, M. Fujiya, Y. Kohgo, Host-microbe interactions via membrane transport

systems, Environ Microbiol 17(4) (2015) 931-7.
[63] V. R. Velagapudi, R. Hezaveh, C. S. Reigstad, P. Gopalacharyulu, L. Yetukuri, S. Islam, J. Felin, R. Perkins, J. Borén, M. Oresic, F. Bäckhed, The gut microbiota modulates host energy and lipid metabolism in mice, J Lipid Res 51(5) (2010) 1101-12.
[64] M. Martínez-Álvaro, A. Zubiri-Gaitán, P. Hernández, M. Greenacre, A. Ferrer, A. Blasco, Comprehensive functional core microbiome comparison in genetically obese and lean hosts under the same environment, Commun Biol 4(1) (2021) 1246.
[65] M. Y. Xue, H. Z. Sun, X. H. Wu, J. X. Liu, L. L. Guan, Multi-omics reveals that the rumen microbiome and its metabolome together with the host metabolome contribute to individualized dairy cow performance, Microbiome 8(1) (2020) 64.
[66] L. Chen, M. Y. Ma, M. Sun, L. Y. Jiang, X. T. Zhao, X. X. Fang, S. Man Lam, G. H. Shui, J. Luo, X. J. Shi, B. L. Song, Endogenous sterol intermediates of the mevalonate pathway regulate HMGCR degradation and SREBP-2 processing, J Lipid Res 60(10) (2019) 1765-1775.
[67] X. Ran, G. Hu, W. Guo, K. Li, X. Wang, J. Liu, S. Fu, Hesperetin regulates the intestinal flora and inhibits the TLR4/NF-κB signaling axis to protect the blood-milk barrier and prevent mastitis, Life Sci 342 (2024) 122533.
[68] Y. Sha, X. Guo, Y. He, W. Li, X. Liu, S. Zhao, J. Hu, J. Wang, S. Li, Z. Zhao, Z. Hao, Synergistic Responses of Tibetan Sheep Rumen Microbiota, Metabolites, and the Host to the Plateau Environment, Int J Mol Sci 24(19) (2023).
[69] A. L. Davidson, E. Dassa, C. Orelle, J. Chen, Structure, function, and evolution of bacterial ATP-binding cassette systems, Microbiol Mol Biol Rev 72(2) (2008) 317-64, table of contents.
[70] J. Moro, D. Tomé, P. Schmidely, T. C. Demersay, D. Azzout-Marniche, Histidine: A Systematic Review on Metabolism and Physiological Effects in Human and Different Animal Species, Nutrients 12(5) (2020).
[71] A. Góralczyk-Bińkowska, D. Szmajda-Krygier, E. Kozłowska, The Microbiota-Gut-Brain Axis in Psychiatric Disorders, Int J Mol Sci 23(19) (2022).
[72] B. Thulasinathan, K. N. Suvilesh, S. Maram, E. Grossmann, Y. Ghouri, E. P. Teixeiro, J. Chan, J. T. Kaif, S. Rachagani, The impact of gut microbial short-chain fatty acids on colorectal cancer development and prevention, Gut Microbes 17(1) (2025) 2483780.
[73] H. Liu, T. Xu, S. Xu, L. Ma, X. Han, X. Wang, X. Zhang, L. Hu, N. Zhao, Y. Chen, L. Pi, X. Zhao, Effect of dietary concentrate to forage ratio on growth performance, rumen fermentation and bacterial diversity of Tibetan sheep under barn feeding on the Qinghai-Tibetan plateau, PeerJ 7 (2019) e7462.
[74] J. Zhao, P. Liu, Y. Wu, P. Guo, L. Liu, N. Ma, C. Levesque, Y. Chen, J. Zhao, J. Zhang, X. Ma, Dietary Fiber Increases Butyrate-Producing Bacteria and Improves the Growth Performance of Weaned Piglets, J Agric Food Chem 66(30) (2018) 7995-8004.
[75] J. D. McGarry, D. W. Foster, Regulation of hepatic fatty acid oxidation and ketone body production, Annu Rev Biochem 49 (1980) 395-420.
[76] M. Tafaj, H. Steingass, W. Drochner, Influence of hay particle size at different concentrate and feeding levels on digestive processes and feed intake in ruminants. 2. Passage, digestibility and feed intake, Arch Tierernahr 54(3) (2001) 243-59.
[77] S. Galić, H. P. Schneider, A. Bröer, J. W. Deitmer, S. Bröer, The loop between helix 4

and helix 5 in the monocarboxylate transporter MCT1 is important for substrate selection and protein stability, Biochem J 376(Pt 2) (2003) 413-22.
[78] C. R. Benton, Y. Yoshida, J. Lally, X. X. Han, H. Hatta, A. Bonen, PGC-1alpha increases skeletal muscle lactate uptake by increasing the expression of MCT1 but not MCT2 or MCT4, Physiol Genomics 35(1) (2008) 45-54.
[79] S. R. Schaus, G. Vasconcelos Pereira, A. S. Luis, E. Madlambayan, N. Terrapon, M. P. Ostrowski, C. Jin, B. Henrissat, G. C. Hansson, E. C. Martens, Ruminococcus torques is a keystone degrader of intestinal mucin glycoprotein, releasing oligosaccharides used by Bacteroides thetaiotaomicron, mBio 15(8) (2024) e0003924.
[80] N. O. Kaakoush, Insights into the Role of Erysipelotrichaceae in the Human Host, Front Cell Infect Microbiol 5 (2015) 84.
[81] X. Song, C. Jin, R. Wu, Y. Wang, X. Wang, Gut microbiota and metabolites in lipid metabolism and intramuscular fat deposition: mechanisms and implications for meat quality, J Anim Sci Biotechnol 16(1) (2025) 147.
[82] D. J. Morrison, T. Preston, Formation of short chain fatty acids by the gut microbiota and their impact on human metabolism, Gut Microbes 7(3) (2016) 189-200.
[83] T. J. Hackmann, New biochemical pathways for forming short-chain fatty acids during fermentation in rumen bacteria, JDS Commun 5(3) (2024) 230-235.
[84] G. den Besten, K. van Eunen, A. K. Groen, K. Venema, D. J. Reijngoud, B. M. Bakker, The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism, J Lipid Res 54(9) (2013) 2325-40.
[85] H. Shen, Z. Xu, Z. Shen, Z. Lu, The Regulation of Ruminal Short-Chain Fatty Acids on the Functions of Rumen Barriers, Front Physiol 10 (2019) 1305.
[86] H. Shen, Z. Xu, Z. Shen, Z. Lu, Corrigendum: The Regulation of Ruminal Short-Chain Fatty Acids on the Functions of Rumen Barriers, Front Physiol 12 (2021) 770061.

Authors and Affiliations

Xu Ying[1,3]• Yao Liangwei [2,3]•Xia Yulu[1,3]• Li Wenhao [*1,3]• Li Changhui[*3, 4]• Zhang Jianhua[2, 3]• Liu Jianbin [3,5]• Chen Yongwei[6]• Fang Yongrong[6]

Corresponding author: Li Wenhao and Li Changhui

First author: Xu Ying

✉Li Wenhao [*1,3]

qhdxlwh@163.com

✉Li Changhui[*3, 4]

1990990010@qhu.edu.cn

Xu Ying[1,3]

18298402040@163.com

Yao Liangwei [2,3]

yaolwe@163.com

Xia Yulu[1,3]

970293662@qq.com

Zhang Jianhua[2,3]

qhdx_zjh@163.com

Liu Jianbin [3,5]

liujianbin@caas.cn

Chen Yongwei[6]
15809718176@163.com
Fang Yongrong[6]
17725270995@163.com
[1] Academy of Animal and Veterinary Sciences, Qinghai University, Xining 810016, China；
[2] College of Forestry and Grassland, Qinghai University, Xining 810016, China；
[3] Qilian Tibetan Sheep Industrial Development Research Institute of Qinghai Province, Qilian 810400, China；
[4] College of Agriculture and Animal Husbandry, Qinghai University, Xining 810016, China；
[5] Lanzhou Institute of Husbandry and Pharmaceutical Sciences, Chinese Academy of Agricultural Sciences, Lanzhou 730070, China；
[6] Qinghai Provincial Animal Husbandry General Station, Xining 810016, China