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Exploring the impact of rumen microbiome on ovine flavor-related compounds and comparing flavor profiles between Tibetan sheep and Small-tail Han sheep

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Abstract

The characteristic 'mutton flavor', primarily attributed to branched-chain fatty acids (BCFAs), is influenced by various factors including rumen microbes. This study aims to elucidate the disparities in meat flavor compounds and their underlying regulatory mechanisms mediated by rumen microbes between two important sheep breeds on the Qinghai-Tibetan Plateau. We used LC-MS/MS to analyze BCFAs and rumen short-chain fatty acids (SCFAs), along with metagenomic sequencing to characterize the rumen microbiome. Compared to Tibetan sheep, Small Tail Han sheep exhibited significantly higher concentrations of BCFAs, including 4-ethyloctanoic acid (EOA) and 4-methyloctanoic acid (MOA), as well as SCFAs such as pentanoate, glutarate, and propionate. In contrast, acetate levels were inversely correlated with these fatty acids. Metagenomics revealed a predominance of Bacteroidota (formerly Bacteroidetes) and Bacillota (formerly Firmicutes) in sheep. Furthermore, random forest and LEfSe analyses identified seven bacterial biomarkers, including *Lactobacillus*, *Ligilactobacillus*, *Blautia*, *Anaerovibrio*, *Selenomonas*, *Phocaeicola*, *Sodaliphilus*. Functional analysis indicated differences in carbohydrate degradation capabilities of two breeds. Likewise, strong positive correlations of propionate with MOA, and glutarate with EOA were observed, respectively. The findings are expected to provide critical insights into the potential for modulating meat flavor through nutritional strategies targeting rumen microbes.

Keywords Mutton flavor, Rumen microbiome, Branched-chain fatty acids, Tail adipose, The Qinghai-Tibetan plateau area

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Introduction

Sheep meat is not universally appreciated by all consumers. Unlike pork, beef, and chicken, mutton possesses distinctive odors and flavors often referred to as the 'mutton flavor', which some may find unappealing. The 'mutton flavor' has been identified to be a complex odor composed of a series of compounds, such as, volatile organic compounds and branched-chain fatty acids (BCFAs). Therein three BCFAs, 4-methyloctanoic acid (MOA), 4-ethyloctanoic acid (EOA) and 4-methylnonanoic acid (MNA), are the main characteristic flavor compounds in sheep and goat [1–3]. The few studies indicated that the mutton flavor depends on diet, temperature, breed, sex and age [4–7]. Our previous study has indicated the differentiated effects of pasture-grazing and concentrate-feeding on flavor character of fat in sheep [8]. Meanwhile, the content of flavor related compounds accumulated with age in sheep [9]. The differentiated BCFAs also have been detecting between meat of F1 hybrid sheep between Australian white sheep and native Small-tail Han Sheep (ST) [10]. Additionally, short-chain fatty acids (SCFAs) related rumen microbes have been involved in the generation of BCFAs. SCFAs produced by rumen microbial fermentation are absorbed by the rumen and transported to organs such as muscles. A portion of SCFAs is employed to synthesize BCFAs. Recent literature highlights the significance of the microbiome in shaping the metabolic profiles of ruminants, including sheep, where specific microbial taxa can influence SCFAs production [11, 12]. Except for diet, breed is another dominant factor driving the shift in microbial communities of gastrointestinal tract, and our previous research has demonstrated the differential diversity of gastrointestinal microbiota among ST, the big-tailed Hulun Buir sheep, and the short-tailed Steppe sheep with the same diet, in plain terrain [13].

Tibetan sheep (TS) is a meat sheep breed that can adapt to the special environmental conditions of the Qinghai-Tibetan Plateau area (QTPA) of China and is also the main sheep breed in the QTPA. Due to the high reproductive rate and strong adaptability, ST has been introduced to various regions, including the QTPA. While local residents assert that the meat of TS has a reduced 'mutton flavor' based on their tasting experiences, there is a lack of scientific data or biomarkers to substantiate this claim. Although both TS and ST are important contributors to animal husbandry in QTPA, little research about the difference in meat flavor and the related regulatory modes between them.

Consequently, the primary objective of this study was to compare the mutton flavor-related BCFAs between two sheep breeds, and further explore the potential relevance of rumen microbes, SCFAs and mutton BCFAs in TS and ST by multi-omics data. It can lead to practical applications in animal husbandry, and guide the design of targeted dietary interventions that optimize the mutton flavor.

Methods

Animals and samples collection

Twelve 12-month-old female sheep with similar health status and weights were obtained from one farm on the QTPA (Qinghai Provincial Sheep Breeding and Promotion Center), which is at an altitude of about 3200 m. Wherein Small-tail Han Sheep (ST) and Tibetan Sheep (TS) was each of 6 sheep, which all were growing up through grazing. Fasting for 24 h before slaughter, all ewes were anesthetized and killed with an injection of sodium pentobarbital (> 30 mg/kg), followed by supplemental doses as needed. The injection of sodium pentobarbital is among the most widely endorsed techniques for animal euthanasia, as recognized by the AVMA guidelines [14]. Also, several reports have utilized sodium pentobarbital in experiments involving sheep [15, 16]. Hot carcass obtained after slaughtered when separation of feet, skin, head, lungs and trachea, heart, liver, spleen, and gastrointestinal tract. The base of the tail adipose tissues and rumen contents were sampled into sterile tubes and immediately cryopreserved in liquid nitrogen, then transported to the laboratory for preservation at -80 °C until analysis.

Measurement of adipose flavor compounds and rumen short-chain fatty acids

The tail adipose tissues and rumen contents were processed by the same sample pretreatment and derivatization methods. After adding 300μL acetonitrile solution (pre-cooled at 4℃) to the sample (0.05–0.15 g), grinding for 3 min (sample tray must pre-cooled at -20℃), and ultrasound extract in an ice water bath for 10 min. After centrifugation for 10 min (4℃, 12000 rpm), transfer 80μL of supernatant to an injection vial, then add 40μL of 200mM 3-NPH (50% acetonitrile solution, v/v) and 40μL of 120mM EDC-6% pyridine (50% acetonitrile solution, v/v), next react at 40℃ for 30 min. After cooling on ice for 1 min, 160μL of the supernatant was filtered by a syringe through an organic phase pinhole filter (0.22 μm), transfer the liquid to a brown injection bottle, and wait for LC-MS/MS system detection. The method for derivatization of standards were the same as above.

An LC-MS/MS system (AB Sciex Qtrap 5500/Waters I-class, ACQUITY UPLC BEH C18 column with 100 mm × 2.1 mm × 1.7 μm) was employed to quantitative measuring the flavor compounds, such as 4-ethyloctanoic acid (EOA), 4-Methylnonanoic acid (MNA), 4-methyloctanoic acid (MOA), as well as short-chain fatty acids (SCFAs, include acetic acid, butyric acid, glutaric acid, hexanoic acid, isobutyric acid, isovaleric acid, lactic acid,

malonic acid, pentanoic acid, propionic acid, succinic acid). The details refer to Table S1 and S2.

Rumen microbial DNA isolation and metagenomics sequencing

Microbial genomic DNA of all samples were isolated by a QIAamp® Fast DNA Stool Mini Kit (Qiagen, Germany) following the manufacturer's instructions. VAHTS Universal PLUS DNA Library Prep Kit for Illumina (Vazyme, China) was used for constructing the libraries. Then, the libraries were sequenced on an DNBSEQ-T7 (MGI, China) and 150 bp paired-end reads were generated (OE Biotech, Shanghai, China).

Data analysis

Based on default parameters, each metabolite was identified using SCIEX OS-MQ software (Sciex, USA). And quantified the flavor compounds and SCFAs through the standard curves obtained from the prepared standard solution.

Fastp (v0.20.1) was used to trim and filter reads, and then bbmap (v38.93-0) was operated to remove the sheep genome to get valid reads, which could be assembled by MEGAHIT (v1.2.9) [17, 18]. Then, prodigal (v2.6.3) was performed to predict and translate ORFs [19]. The non-redundant gene sets were built for all predicted genes using MMSeqs2 (v13.4511). Clean reads aligned against the non-redundant gene set with 95% identity through salmon (v1.8.0), and the abundant information of the gene in the corresponding sample was counted. The taxonomy of the species was obtained from the NR Library, and the abundance of the species was calculated using the corresponding abundance of the genes. In order to construct the abundance profile on the corresponding taxonomy level, abundance statistics were performed at each level. Based on the abundance spectrum, beta diversity was calculated and plotted using R (v4.1.2). The gene set representative sequence (amino acid sequence) was annotated with NR, KEGG database by DIAMOND (v2.1.3) [20], and compared with the CAZy database by hmmscan (v3.1) to obtain the carbohydrate active enzyme abundance of microbes [21].

Differential flavor compounds, SCFAs, alpha estimators, and the relative abundance of phylum, genus, KEGG pathway and CAZymes between ST and TS, were analyzed by *t* test, which statistical significance was declared at $p < 0.05$. LEfSe analysis and Random Forest was employed to screen biomarkers between two breeds of sheep, whereupon according to LDA score (> 2.0) and Mean Decrease Accuracy value, respectively. The correlation among the differential flavor compounds, SCFAs, and the screened genera were analyzed by Pearson Correlation Coefficient ($p < 0.05$, $|r| > 0.6$).

Results

Differential levels of adipose flavor compounds between TS and ST

The concentrations of three BCFAs (EOA, MOA, and MNA) in tail fats were shown in Fig. 1a. The concentration of MNA in tail fats showed no difference between TS and ST. However, the levels of EOA and MOA in

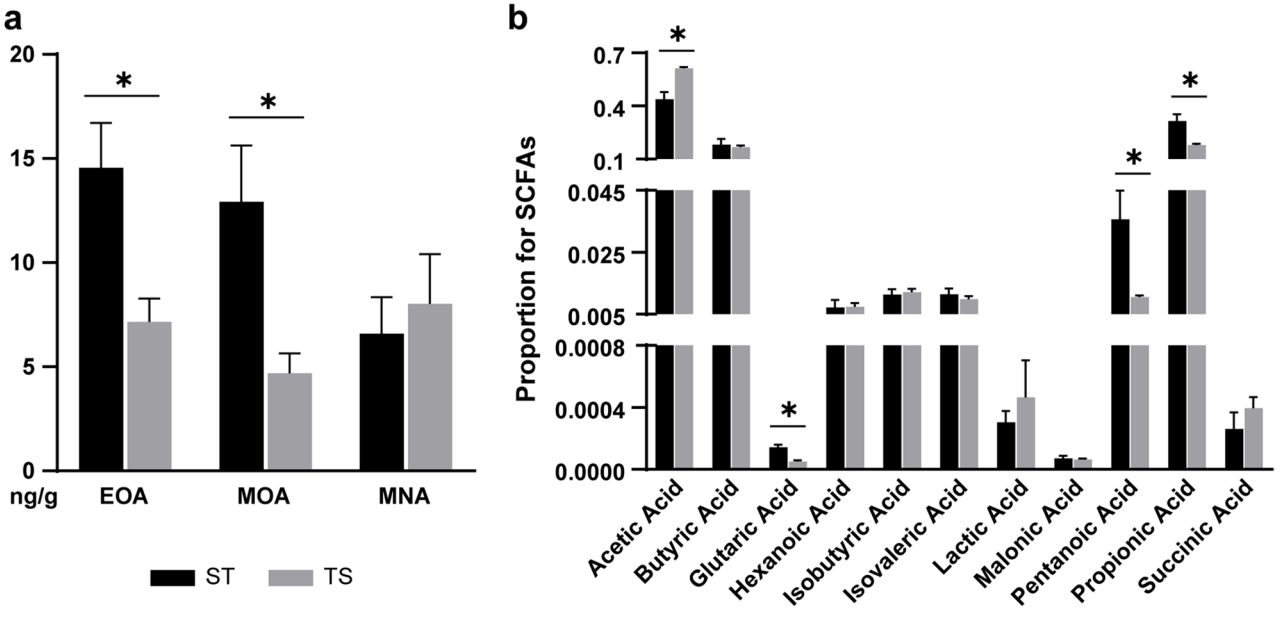

Fig. 1 Three BCFAs of fats **a** and SCFAs of rumen contents **b** in TS and ST. MOA, 4-methyloctanoic acid; EOA, 4-ethyloctanoic acid; MNA, 4-methylnonanoic acid (MNA); TS, Tibetan sheep; ST, Small-tail Han Sheep. Six sheep per group. * means significant difference between 2 group based on *t* test. Values are expressed as the mean ± SEM

ST were significantly higher than those in TS ($p < 0.05$, log2FoldChange > 1.0).

Rumen SCFAs in TS and ST

Fig. 1b depicted the relative content of SCFAs (acetic acid, butyric acid, glutaric acid, hexanoic acid, isobutyric acid, isovaleric acid, lactic acid, malonic acid, pentanoic acid, propionic acid, succinic acid) in rumen in TS and ST. The total SCFAs, the levels of butyrate, hexanoate, isobutyrate, isovalerate, lactate, malonate, and succinate in rumen of the two sheep breeds were similar and showed no significant difference. The level of pentanoate in rumen of ST was significantly higher than that of TS ($p < 0.05$). The relative amount of glutarate and propionate were also significantly higher in ST compared to that in TS ($p < 0.01$). However, the acetate content was higher in TS ($p < 0.01$).

Differences of rumen microbes between TS and ST

Rumen contents samples from 12 sheep were performed metagenomic sequencing and generated 46.9 million clean reads per sheep on average. The Good's Coverage index in each individual was over 0.9999. Besides, there was no significant difference on Coverage index and Shannon indices between ST and TS group. Yet Chao1 richness was much lower in ST than that in TS ($p < 0.01$, Figure S1). The distinct distribution of 2 group in the PCoA plot based on Bray-Curtis distance represented dissimilar patterns for rumen microbial communities in ST and TS ($p < 0.01$, Figure S2).

Also, metagenome analysis revealed that the rumen microbiome in sheep was dominated by the Bacteria, followed by Archaea and the Fungi of Eukarya. As shown in Fig. 2a, the bacterial community at the phylum level was dominated by Bacteroidota (formerly Bacteroidetes, 46.51–51.25%) and Bacillota (formerly Firmicutes, 21.31–22.76%), followed by Fibrobacterota (5.06–6.07%),

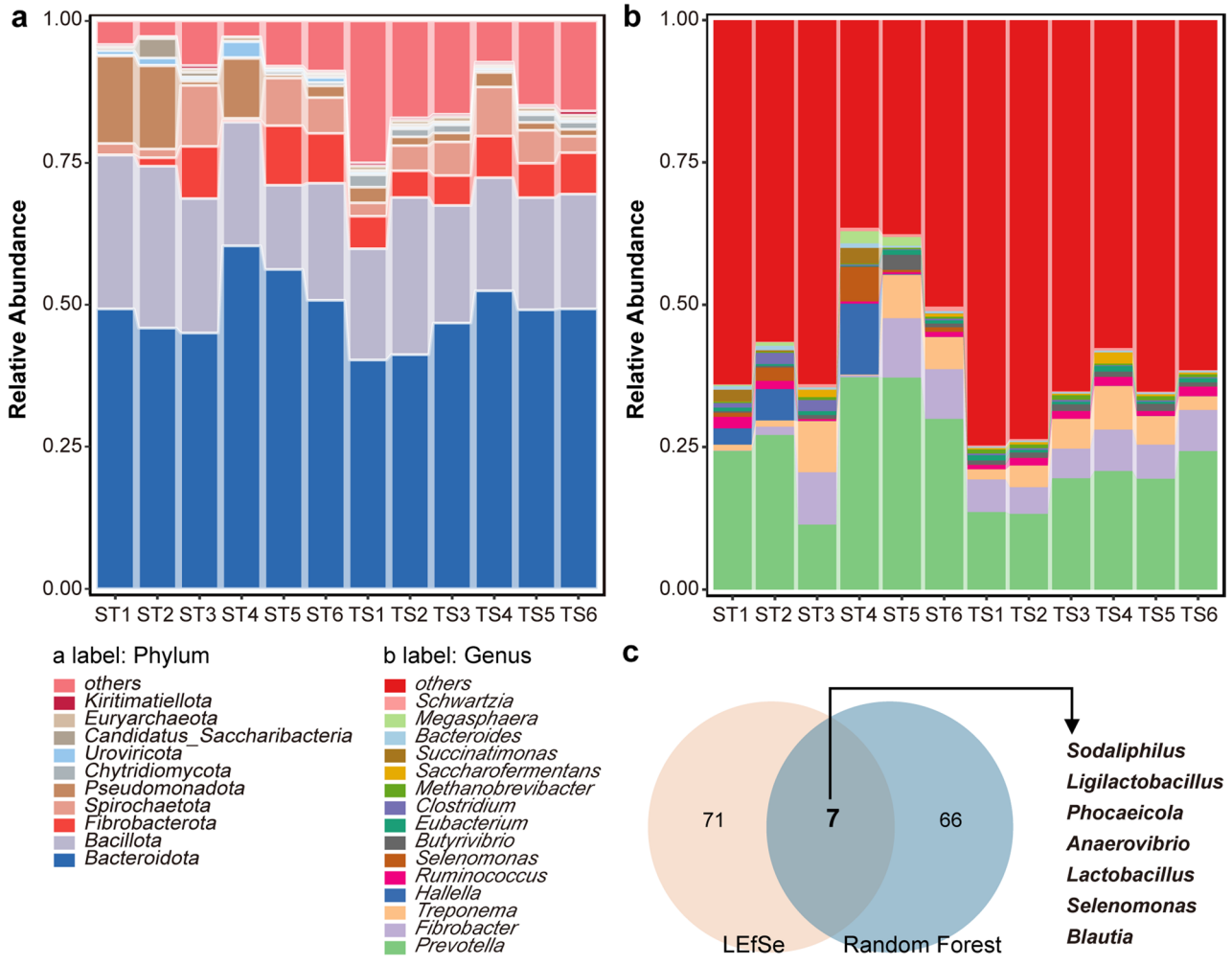

Fig. 2 The rumen microbial taxa in sheep. The microbial taxonomic composition at phylum **a** and genus **b** level of all rumen content samples. Venn diagram indicates the numbers of biomarkers (genera) screened by Random Forest and LEfSe **c**. TS, Tibetan sheep; ST, Small-tail Han Sheep

Spirochaetota (4.87-5.00%), Pseudomonadota (formerly Proteobacteria, 1.84–7.34%), Candidatus_Saccharibacteria (0.30–0.89%), and Kiritimatiellota (0.23–0.51%). The relative abundance of Bacteroidota, Bacillota, Pseudomonadota and Candidatus_Saccharibacteria were higher, while Fibrobacterota, Spirochaetota and Kiritimatiellota were lower in ST compared with TS. Conspicuously, the differential relative abundance of Kiritimatiellota was presented between two group ($p < 0.05$). Besides, Chytridiomycota (0.33–1.36%, belongs to Fungi) and Euryarchaeota (0.46–0.69%, belongs to Archaea) with high relative abundance were more abundant in ST. At the genus level (Fig. 2b), the top 15 most prominent genera were *Prevotella* (18.49–27.88%), followed by *Fibrobacter* (5.03-6.00%), *Treponema* (4.09–4.32%), *Hallella* (0.008-3.50%), *Ruminococcus* (0.90–1.26%), *Selenomonas* (0.02–1.74%), *Butyrivibrio* (0.77–0.96%), *Eubacterium* (0.52–0.65%), *Clostridium* (0.24–0.90%), *Methanobrevibacter* (0.28–0.63%), *Saccharofermentans* (0.32–0.58%), *Succinatimonas* (0.004–0.85%), *Bacteroides* (0.28–0.56%), *Megasphaera* (0.005–0.75%) and *Schwartzia* (0.24–0.46%). Among the prominent genera, *Bacteroides* was more abundant in ST ($p < 0.05$). Specially, *Methanobrevibacter* belongs to Archaea, and with higher relative abundance in TS remarkably ($p < 0.05$).

The Random Forest analysis was conducted on 73 important genera, alongside the LEfSe analysis identifying 78 differentiated genera between TS and ST. Of the above biomarkers, 7 common bacterial genera, *Lactobacillus*, *Ligilactobacillus*, *Blautia*, *Anaerovibrio*, *Selenomonas*, *Phocaeicola*, *Sodaliphilus*, were shared by Random Forest and LEfSe (Fig. 2c). Besides, all of the 7 genera were more abundant in ST rather than in TS.

Functional metagenomic profiles of TS and ST

The differences at KEGG pathway were conducive to understand the functional differences between TS and ST rumen microbes. A total of 259 differential categories at KEGG pathway level 3 were detected. Therein, 50 categories belonged to the metabolism pathway, and were involved in amino acid, nucleotide, carbohydrate, Glycan, cofactors and vitamins, lipid, terpenoids and polyketides and xenobiotics metabolism. Apart from Inositol phosphate metabolism, Ether lipid metabolism, Arachidonic acid metabolism, Steroid biosynthesis, and Steroid degradation, the relative abundance of the other 45 pathways in ST microbes were higher than in TS microbes (Fig. 3a).

To further explore and compare the microbial potential for dietary degradation between TS and ST, the professional CAZy database for studying carbohydrate enzymes was employed. As shown in Fig. 3b, the relative abundance of Auxiliary activities (AA), and Glycosyl transferases (GT) in the ST were higher than in the TS ($p < 0.05$). Conversely, the relative abundance of Carbohydrate-binding modules (CBM) and Polysaccharide lyases (PL) were higher in the TS ($p < 0.05$). At the genus level, *Prevotella* and *Fibrobacter* predominantly contributed to the six classes of CAZy database. The relative contribution of *Prevotella* to the CBM, CE, GH, GT, and PL classes of CAZy was higher in ST than TS ($p < 0.05$). Also, the opposite was observed for *Fibrobacter* ($p > 0.05$). *Ruminococcus* was distributed in all classes except for AA, and was more abundant in TS, but instead in PL class. *Bacteroides*, *Butyrivibrio*, *Clostridium*, *Eubacterium*, *Hallella*, *Selenomonas*, and *Treponema* were contributed to the six classes. *Bacteroides*, *Clostridium*, *Hallella*, and *Selenomonas* were higher, while *Butyrivibrio* and *Eubacterium* were lower in the ST compared to TS. Ultimately, the relative contribution of *Treponema* in the AA, CBM, GT classes was higher in ST, but converse results in the CE, GH and PL classes (Fig. 3c).

Relationship of flavor compounds, rumen microbes and SCFAs

Pearson correlation analysis was employed to explore the relevance among the differential flavor compounds (EOA and MOA), SCFAs (acetic acid, glutaric acid, pentanoic acid and propionic acid), and the 7 screened biomarkers. All significant correlations were represented in Fig. 4 ($p < 0.05$), MOA was positively correlated to rumen propionic acid ($r = 0.83$) and *Blautia* ($r = 0.68$), whereas negatively related to acetic acid (r = -0.66). EOA had positive correlation with rumen glutaric acid ($r = 0.84$) and *Anaerovibrio* ($r = 0.73$). Additionally, pentanoic acid correlated positively to *Blautia*, *Phocaeicola*, *Lactobacillus*, *Ligilactobacillus*, *Sodaliphilus*, *Selenomonas* ($r = 0.64$–0.91). Propionic acid was also positive associated with 4 biomarkers (*Phocaeicola*, *Sodaliphilus*, *Selenomonas*, and *Blautia*) with correlation coefficient (r) from 0.67 to 0.88. Still, glutaric acid was positively correlated with *Phocaeicola* ($r = 0.71$) and *Anaerovibrio* ($r = 0.81$). There was a strong inverse correlation (r = -0.84 to -0.67) between acetic acid and 6 specific genera (*Blautia*, *Selenomonas*, *Anaerovibrio*, *Sodaliphilus*, *Ligilactobacillus*, *Lactobacillus*) yet. The raw data were provided in the supplementary material.

Discussion

Variations in flavor profiles can significantly influence consumer preferences and marketability of livestock products. Various chemical compounds have been implicated in contributing to 'mutton flavor'. Of those compounds, BCFAs, principally EOA, MOA and MNA, have been confirmed to be the most important [1, 2, 22]. Additionally, several studies indicated that EOA, MOA and MNA of adipose were easier to detect rather than in muscle tissue [23, 24]. Previous studies have indicated that BCFAs accumulation is affected by various

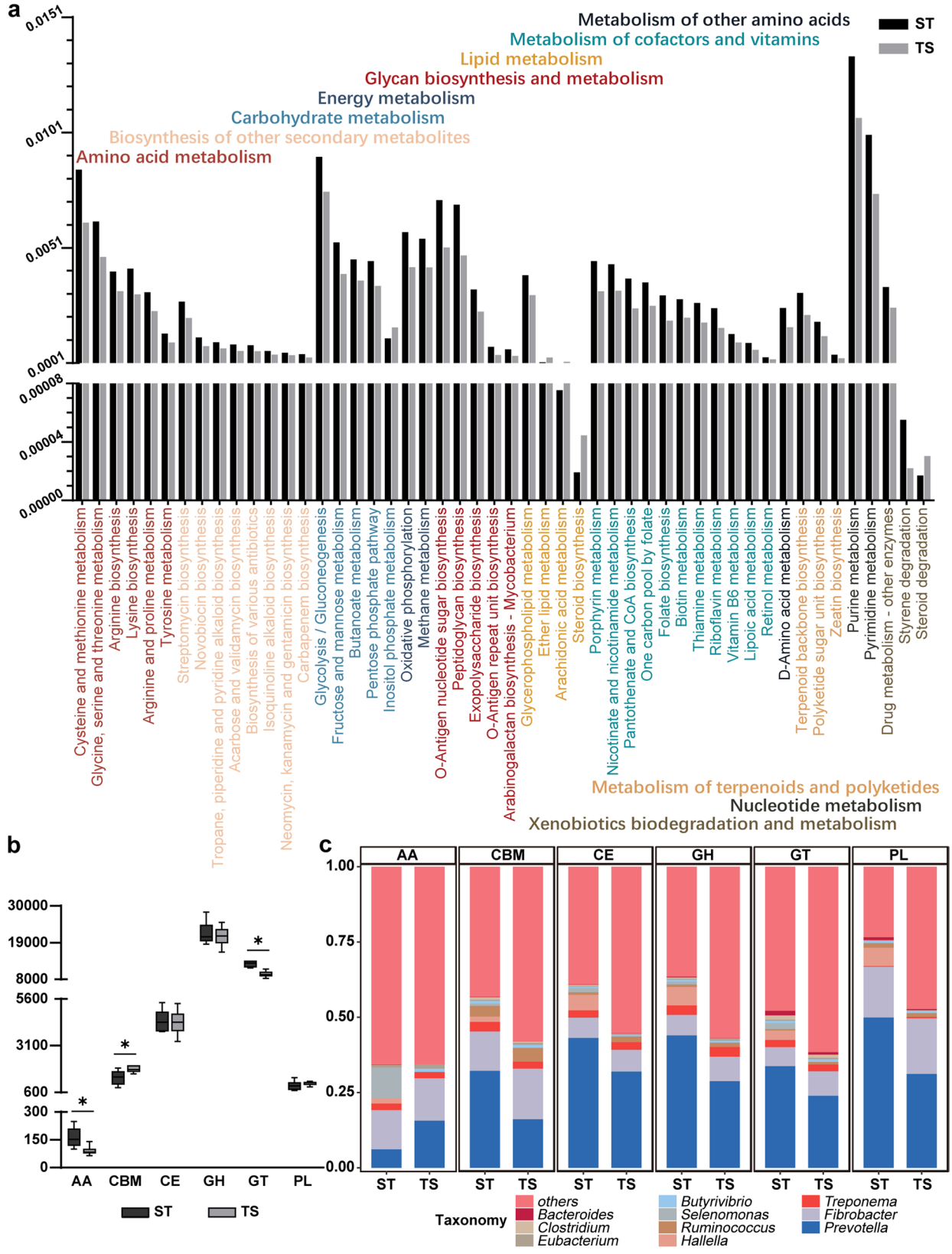

Fig. 3 Comparative functional analysis of rumen microbiomes from TS and ST. The Functional enrichment analysis showing the differential KEGG metabolism pathways (KEGG_3, < 0.05) between TS and ST **a**. Differences in the abundant genes of CAZy functional terms between rumen microbes of TS and ST **b**. The bar plot shows the top 10 abundant genera contributed to six CAZy classes in sheep **c**. TS, Tibetan sheep; ST, Small-tail Han Sheep. Six sheep per group. * means significant difference between 2 group according to *t* test

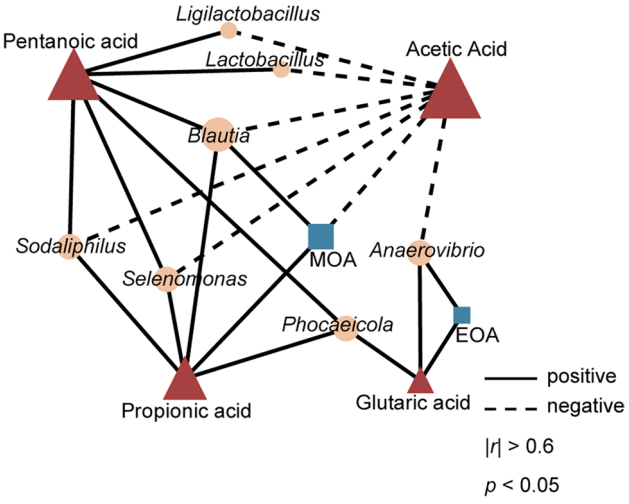

Fig. 4 The correlation of EOA and MOA, the differential SCFAs, and biomarkers in sheep. Pearson correlation coefficient with $|r|>0.6$ and $p<0.05$ are shown. The solid and dotted edge are stand for positive and negative correlations. MOA, 4-methyloctanoic acid; EOA, 4-ethyloctanoic acid; TS, Tibetan sheep; ST, Small-tail Han Sheep

non-genetic factors, including dietary composition and grazing conditions [8, 25], which could lead to significant differences in the fatty acid profiles present in the meat. Further, consumer panels were conducted to compare the sensory characteristics of meat from sheep of different age, sex or breeds [5, 26]. The difference of BCFAs levels among sheep breeds was barely reported. In this study, the higher concentrations of EOA and MOA in ST compared to TS, which suggested a more intense odor of meat in ST.

Our study investigated that the level of propionate, as well as EOA and MOA were markedly higher in ST, which was in concordance with previous research that indicated SCFAs, such as propionate and butyrate, were the precursors of BCFAs synthesis [27]. Researchers have shown that propionate can synthesize BCFAs in the preen gland of geese [28]. Evidence indicated that the incomplete metabolism of excessive propionate and its derivative resulted to the accumulation of BCFAs in adipose tissue of sheep and goat. Excess propionate is converted into methylmalonyl-CoA via hepatic gluconeogenesis. Then methylmalonyl-CoA serves as a primer for fatty acid synthase, which produces MOA and EOA in adipose tissue [29]. This might explain the high levels of propionic acid and EOA, MOA observed in ST. Likewise, the correlation analysis in this study also indicated the positive relationship of MOA and rumen propionic acid in sheep. Barely report demonstrated BCFAs associated with acetic acid, glutaric acid and pentanoic acid. Yet it was clear that acetyl-CoA carboxylase (ACC) and fatty acid synthase (FASN) utilized acetyl-CoA, which captured acetate as one of the carbon sources, to synthesize fatty acids in mammalian cells [30]. Our research revealed that acetate directly or indirectly negative regulation with MOA and EOA levels. Thereby, the higher level of acetate increased the concentration of acetyl-CoA, which led TS preferred to traditional fatty acid synthesis, perchance. Likewise, pentanoic acid and glutaric acid might be also as the carbon skeleton for MOA and EOA, respectively.

Additionally, the distinct microbiome observed in ST and TS suggested that specific microbial taxa might play pivotal roles in the fermentation processes that contributed to BCFAs and SCFAs production. Anaerobic gut fungi from herbivores can generate cellulosomes for fiber degradation to produce SCFAs. However, there are few reports on Chytridiomycota contributing to fiber degradation. *Methanobrevibacter* was commonly found in the rumen of sheep, and in this study, it with prominently higher relative abundance in TS. Presently, *Methanobrevibacter* can reduce the hydrogen partial pressure in the environment by consuming hydrogen. Low hydrogen partial pressure favors the continuous decomposition of organic matter by hydrogen-consuming microorganisms, such as cellulose-degrading bacteria, resulting in the production of acetic acid [31, 32]. By competing with propionic acid-producing bacteria for hydrogen, *Methanobrevibacter* may indirectly inhibit propionic acid production [32]. This is consistent with our results. Furthermore, *Methanobrevibacter* has been reported having a positive relationship with ethyl caproate and hexanoic acid in pit mud [33], as well as 2-phospho-D-glyceric acid [34]. There is a report that speculate 2-phospho-D-glyceric acid may contribute to the glyoxylate and dicarboxylate, and pentose phosphate pathway to modulate muscle fatty acids in TS and Oula sheep [34]. But a direct regulatory relationship has still not been established. It is possible that the glyoxylate and dicarboxylate metabolism and the pentose phosphate pathway contribute to muscle fatty acid synthesis by generating substantial acetic acid. *Bacteroides* were not only significantly more abundant, but also contributed more to GT class in ST compared to TS. The genera *Bacteroides* and *Prevotella* were well known in ability to metabolize carbohydrates just substrate specificity, and contributed to carbohydrates degraded classes (GH, CE and PL) [35]. It was concordant with the present results for *Prevotella*. Carbohydrates were fermented by the abundant *Prevotella* to provide energy and SCFAs, then prospective generate more BCFAs in ST. Of the 7 screened biomarkers, the genera *Blautia* and *Anaerovibrio* had direct positive relationship with BCFAs. *Blautia* was known as a butyrate producer, but still was observed for associating with propionic acid of several reports [36], what correlation also was detected in this study. Thereby the observed positive correlation between *Blautia* and MOA may stem from the role of propionic acid in the synthesis of BCFAs.

Although *Anaerovibrio* was involved in lipid metabolism and could increase lipase activity [37, 38], the relationship of *Anaerovibrio* and BCFAs was barely reported. It was a very definite possibility that previously paying few attentions to glutaric acid when exploring rumen SCFAs. Here the results offered a new perspective on the regulation of EOA by rumen *Anaerovibrio* and glutaric acid. Furthermore, the special biomarker in this research was *Selenomonas*, which also was one of the most abundant genera in rumen, as well as one of the primarily contributed to CAZy classes. Among the above analysis, the relative abundance of *Selenomonas* was always higher in ST compared to TS. *Selenomonas* can decarboxylate succinate to propionate [39], and a positive association between *Selenomonas* and propionate also persisted in the rumen of lambs [27]. Thus, propionate was the potentially pivotal SCFAs fitting several rumen genera and fat MOA together.

In conclusion, this study highlights significant differences in meat flavor related BCFAs, rumen SCFA profiles, and microbial communities between Small-tail Han Sheep (ST) and Tibetan Sheep (TS). By elucidating the complex interactions between rumen microbial communities and flavor compounds, this research paves the potential way for microbes-mediated nutritional manipulation strategies that aimed at altering meat flavor in sheep farming. However, this study primarily reveals correlations rather than establishes causal relationships, highlighting the need for more detailed mechanistic investigations, such as isotope-tracing experiments, in the future.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12866-026-04755-9.

Supplementary Material 1.

Supplementary Material 2.

Acknowledgements
Not applicable.

Authors' contributions
Wang.XQ: conceptualization, methodology, formal analysis, original draft. Hao. WJ: validation. Tian.DH, Han.BY, Zhao.K, Du, K: resources. Li.X and Duan.ZY: resources, supervision.

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Data availability
The raw sequence data reported in the current study have been deposited in the Genome Sequence Archive in China National Genomics Data Center (GSA: CRA025234), https://ngdc.cncb.ac.cn/gsa.

Declarations

Ethics approval and consent to participate
We have obtained informed consent from the sheep owners for this research. Animal experiments were performed in accordance with animal welfare and ethics, and approved by the Animal Care and Use Committee of Northwest Institute of Plateau Biology, Chinese Academy of Sciences (approval no. NWIPB2023015).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. Wong E, Johnson CB, Nixon LN. The contribution of 4-methyloctanoic (hircinoic) acid to mutton and goat meat flavour. New Z J Agricultural Res. 1975;18(3):261–6.
2. Wong E, Nixon LN, Johnson CB. Volatile medium chain fatty acids and mutton flavor. J Agric Food Chem. 1975;23(3):495–8.
3. Man L, Ren W, Qin H, Sun M, Yuan S, Zhu M, Liu G, Wang C, Li M. Characterization of the relationship between lipids and volatile compounds in donkey, bovine, and sheep meat by UHPLC–ESI–MS and SPME–GC–MS. LWT. 2023;175:114426.
4. Sha Y, He Y, Liu X, Shao P, Wang F, Xie Z, Li W, Wang J, Li S, Zhao S et al. Interactions of rumen microbiota and metabolites with meat quality-related genes to regulate meat quality and flavor of Tibetan sheep under nutrient stress in the cold season. J Appl Microbiol. 2023;134(8):lxad182.
5. Jaborek JR, Zerby HN, Wick MP, Fluharty FL, Moeller SJ. Effect of energy source and level, animal age, and sex on the flavor profile of sheep meat. Translational Anim Sci. 2020;4(2):1140–7.
6. Zhang X, Han L, Hou S, Raza SHA, Gui L, Sun S, Wang Z, Yang B, Yuan Z, Simal-Gandara J, et al. Metabolomics approach reveals high energy diet improves the quality and enhances the flavor of black Tibetan sheep meat by altering the composition of rumen microbiota. Front Nutr. 2022;9:915558.
7. Xiao C, Liu Y, Zhao W, Liang Y, Cui C, Yang S, Fang W, Miao L, Yuan Z, Lin Z, et al. The comparison of meat yield, quality, and flavor between small-tailed Han sheep and two crossbred sheep and the verification of related candidate genes. Front Nutr. 2024;11:1399390.
8. Zhang Z, Wang X, Jin Y, Zhao K, Duan Z. Comparison and analysis on sheep meat quality and flavor under pasture-based fattening contrast to intensive pasture-based feeding system. Anim Bioscience. 2022;35(7):1069–79.
9. Wang J, Lin H, Wang Q, Wu Y, Zhang C. 4D DIA proteomic analysis of gender and age influences on meat quality and flavor in Hetian white sheep. Food Chem. 2025;464(Pt 3):141851.
10. Zhang Z, Wang X, Bao Q, Li H, Wei L, Zhao K, Duan Z. Analysis of the longissimus dorsi meat quality and the content OfMutton flavor substances of F1 hybrid sheep between AustralianWhite sheep and native Small-Tail Han sheep. Sci Technol Food Ind. 2021;42(14):272–7.
11. Li H, Yu Q, Li T, Shao L, Su M, Zhou H, Qu J. Rumen Microbiome and metabolome of Tibetan sheep (Ovis aries) reflect animal age and nutritional requirement. Front Veterinary Sci. 2020;7:609.
12. Wu X, Zhang G, Zhang W, Zhou J, Cong H, Yang G, Liu G. Rumen microbiota helps Tibetan sheep obtain energy more efficiently to survive in the extreme environment of the Qinghai-Tibet plateau. Front Microbiol. 2024;15:1431063.
13. Wang X, Zhang Z, Wang X, Bao Q, Wang R, Duan Z. The impact of host Genotype, intestinal sites and probiotics supplementation on the gut microbiota composition and diversity in sheep. Biology. 2021;10(8):769.
14. Pang D, Laferriere C. Review of intraperitoneal injection of sodium pentobarbital as a method of euthanasia in laboratory rodents. J Am Association Lab Anim Science: JAALAS. 2020;59(3):346.
15. Evans RG, Iguchi N, Cochrane AD, Marino B, Hood SG, Bellomo R, McCall PR, May CN, Lankadeva YR. Renal hemodynamics and oxygenation during experimental cardiopulmonary bypass in sheep under total intravenous anesthesia. Am J Physiol Regul Integr Comp Physiol. 2020;318(2):R206–13.

16. Maham M, Sarrafzadeh-Rezaei F. Cardiovascular effects of adonis aestivalis in anesthetized sheep. Veterinary Res Forum: Int Q J. 2014;5(3):193–9.
17. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for illumina sequence data. Bioinformatics. 2014;30(15):2114–20.
18. Li D, Luo R, Liu CM, Leung CM, Ting HF, Sadakane K, Yamashita H, Lam TW. MEGAHIT v1.0: A fast and scalable metagenome assembler driven by advanced methodologies and community practices. Methods. 2016;102:3–11.
19. Hyatt D, Chen GL, Locascio PF, Land ML, Larimer FW, Hauser LJ. Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinformatics. 2010;11:119.
20. Buchfink B, Xie C, Huson DH. Fast and sensitive protein alignment using DIAMOND. Nat Methods. 2015;12(1):59–60.
21. Cantarel BL, Coutinho PM, Rancurel C, Bernard T, Lombard V, Henrissat B. The Carbohydrate-Active enzymes database (CAZy): an expert resource for glycogenomics. Nucleic Acids Res. 2009;37(Database issue):D233–8.
22. Young OA, Berdagué JL, Viallon C, Rousset-Akrim S, Theriez M. Fat-borne volatiles and sheepmeat odour. Meat Sci. 1997;45(2):183–200.
23. Jamora JJ, Rhee KS. Flavor of Lamb and Mutton. In: *Quality Attributes of Muscle Foods*. Edited by Xiong YL, Chi-Tang H, Shahidi F. Boston, MA: Springer US; 1999: 135–145.
24. Johnson CB, Wong E, Birch EJ. Analysis of 4-methyloctanoic acid and other medium chain-length fatty acid constituents of ovine tissue lipids. Lipids. 1977;12(4):340–7.
25. Vasta V, Priolo A. Ruminant fat volatiles as affected by diet. A review. Meat Sci. 2006;73(2):218–28.
26. Villatoro KM, Yang F, Duarte T, Phillips CR, Woerner DR, Chao MD, Yang X. Quality, proximate composition, and sensory characteristics of Dorper, domestic commercial crossbred, and Australian sheep meat: a comparative study. Translational Anim Sci. 2021;5(1):txab024.
27. Zhao Y, Zhang Y, Khas E, Ao C, Bai C. Effects of allium Mongolicum Regel ethanol extract on three flavor-related rumen branched-chain fatty acids, rumen fermentation and rumen bacteria in lambs. Front Microbiol. 2022;13:978057.
28. Noble RE, Stjernholm RL, Mercier D, Lederer E. Incorporation of propionic acid into a Branched-chain fatty acid of the preen gland of the Goose. Nature. 1963;199(4893):600–1.
29. Ørskov ER. Starch digestion and utilization in ruminants. J Anim Sci. 1986;63(5):1624–33.
30. Heil CS, Wehrheim SS, Paithankar KS, Grininger M. Fatty acid biosynthesis: Chain-Length regulation and control. Chembiochem: Eur J Chem Biology. 2019;20(18):2298–321.
31. Misiukiewicz A, Gao M, Filipiak W, Cieslak A, Patra AK, Szumacher-Strabel M. Review: methanogens and methane production in the digestive systems of nonruminant farm animals. Animal. 2021;15(1):100060.
32. Smith NW, Shorten PR, Altermann EH, Roy NC, McNabb WC. Hydrogen Cross-feeders of the human Gastrointestinal tract. Gut Microbes. 2019;10(3):270–88.
33. Han B, Gong H, Ren X, Tian S, Wang Y, Zhang S, Zhang J, Luo J. Analysis of the differences in physicochemical properties, volatile compounds, and microbial community structure of pit mud in different time spaces. PeerJ. 2024;12:e17000.
34. Xu X, Liu H, Guo T, Zhang Q, Wang X, Wang Y, Wei L, Jia Y, Hu L, Xu S. Insight into the differences in meat quality among three breeds of sheep on the Qinghai-Tibetan plateau from the perspective of metabolomics and rumen microbiota. Food Chemistry: X. 2024;23:101731.
35. Aakko J, Pietilä S, Toivonen R, Rokka A, Mokkala K, Laitinen K, Elo L, Hänninen A. A carbohydrate-active enzyme (CAZy) profile links successful metabolic specialization of prevotella to its abundance in gut microbiota. Sci Rep. 2020;10(1):12411.
36. Rowe JC, Winston JA, Parker VJ, McCool KE, Suchodolski JS, Lopes R, Steiner JM, Gilor C, Rudinsky AJ. Gut microbiota promoting propionic acid production accompanies diet-induced intentional weight loss in cats. Res Square 2023;rs.3.rs-3273531.
37. Ren Z, Yao R, Liu Q, Deng Y, Shen L, Deng H, Zuo Z, Wang Y, Deng J, Cui H, et al. Effects of antibacterial peptides on rumen fermentation function and rumen microorganisms in goats. PLoS ONE. 2019;14(8):e0221815.
38. Zhu Y, Cidan Y, Sun G, Luo C, Duan J, Shi B, Ma T, Tang S, Zhong R, Chen L, et al. Different feeding patterns affect meat quality of Tibetan pigs associated with intestinal microbiota alterations. Front Microbiol. 2022;13:1076123.
39. Guangsheng C, Plugge CM, Roelofsen W, Houwen FP, Stams AJM. Selenomonas Acidaminovorans sp. nov., a versatile thermophilic proton-reducing anaerobe able to grow by decarboxylation of succinate to propionate. Arch Microbiol. 1992;157(2):169–75.

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