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Animal performance and gut microbiota of cattle as affected by the unfermented or fermented total mixed ration

Sihan You[1], Yuqi Zou[1], Yanzi Xiao[2], Lichao He[1], Lili Liu[3], Yuhang Sun[3], Yushan Jia[1], Gentu Ge[1*] and Shuai Du[1*]

Abstract

Diet regulates the gut microbiota, which in turn affects animal performance, but how diet shapes the animal performance and gut microbiota remains largely unknown. To fill this gap, the author conducted a comprehensive study of the influence of total mixed ration (TMR) or fermented TMR (FTMR) on the animal performance and gut microbiome. Sixteen Simmental male cattle were randomly allocated to two treatments (one cattle per pen). The animals were fed with the TMR and FTMR diets respectively. The results showed that the contents of ADF, NDF, cellulose and total cellulose in the FTMR were significantly decreased ($p < 0.05$), the average daily weight gain of the Simmental male cattle shows an increasing trend (TMR: 0.31 vs. FTMR: 0.62), while no significant ($p = 0.2382$) difference was found between the two treatments. The metagenomics analysis showed significant ($p < 0.05$) difference in the α-diversity and β-diversity, and the dominant bacterial genera were *Weissella*, *Lactiplantibacillus*, *Levilactobacillus* and *Companilactobacillus*. The 16S rRNA sequencing indicated that a significant ($p = 0.018$) difference in the bacterial communities between the cattle fed with TMR or FTMR diet, while no significant ($p < 0.05$) differences were detected on the primary genus. It can be found that the FTMR diet increased the average daily gain of cattle by improving the chemical composition and microbial functional profile of the FTMR diet, and affected the growth performance of cattle.

Keywords Cattle, Growth performance, Fermented diet, Fecal microbiota, Metagenomics

Introduction

Corn straw is sources of nutrients and about one billion tons of corn straw are produced every year, but only 30% of the corn straw is utilized for ruminants and the rest is burnt, buried or discarded in the field [15, 39]. Corn stalks processed by ensiling or anaerobic digestion can be used as available raw materials for ruminants [9, 25]. Nevertheless, the low nutritional contents, high polymeric contents and compact properties of lignocellulose results in a low biodigestibility and production performance [39]. Total mixed rations (TMR) could shake off the disadvantage of corn straw and have been globally used for cattle due to they contained sufficient nutrients, minerals and vitamins [39]. Fermented TMR, is not a

*Correspondence:
Gentu Ge
gegentu@163.com
Shuai Du
dushuai_nm@sina.com
[1]Key Laboratory of Forage Cultivation, Processing and High Efficient Utilization, Ministry of Agriculture, Key Laboratory of Grassland Resources, Ministry of Education, College of Grassland Science, Inner Mongolia Agricultural University, Hohhot, Inner Mongolia 010019, China
[2]College of Agriculture, Grass Industry Collaborative Innovation Research Center, Hulunbuir University, Hulunber 021000, China
[3]Naiman Banner Forestry and Grassland Adiministration, Tongliao 028300, China

recent practice, has shown a renewed interest in several countries because they not only obtained the advantages of the TMR but contained some bacteriocin, antimicrobial peptide and organic acids produced by microorganisms during the fermentation process [3, 29].

Previously published studies have shown that the FTMR diet could significantly reduce the fiber content compared to that in the TMR diet due to the hydrolysis [25], resulting in improving the animal performance and health [5, 37]. The sheep and steers fed with the FTMR diet could increase the nutrient utilization, rumen ecology, microbial protein synthesis and better blood profiles [12, 13]. Additionally, the FTMR diet also reduced undesirable microbiome diversity by 16S amplicon sequencing and improved immune status [17, 18]. However, to our knowledge, it is not fully understood how FTMR diet affect the animal performance and gut microbiome.

The author hypothesized that the animal performance and gut microbiome were affected by the microbial compositions and functions of the FTMR diet. Therefore, the present study investigated the animal performance and gut microbiota of cattle fed with the TMR or FTMR diet using 16S rRNA amplicon sequencing, and the metagenomic sequencing analysis were used to characterize the microbial compositions and functions of the TMR or FTMR diet. The goal was to better understand the effects of the microbial functional groups of the FTMR diet on cattle animal performance and gut microbiome. This research could provide deeper insight and a theoretical foundation for FTMR diet utilization.

Materials and methods

Experimental design and diets

A total of sixteen Simmental male cattle (Initial body weight 283.32 ± 9.47 kg; the average age was 15 months) were purchased from the Inner Mongolia Fuchengxiang Animal Husbandry Technology Development Co. Ltd. (Chifeng, China). All cattle were randomly assigned into one of two dietary treatments: total mixed rations (TMR) or fermented TMR (FTMR) diet. The FTMR was packed in polyethylene stretch film at a density of 800 kg/m^3 on the dry matter (DM) basis and stored at an ambient temperature of 25 °C for 21 days. After the fermentation was completed, eight silage wrappings (FTMR) and eight non-silage feeds (TMR) were randomly selected for sampling and analysis. The animal trial utilized 16 cattle, which were divided into two treatment groups with eight animals per group. Each animal was individually housed in separate pens to prevent cross-feeding and behavioral interference. The experiment spanned 60 days, with the initial 15 days designated as an adaptation period. Throughout the trial, cattle were fed their respective diets (FTMR or TMR) twice daily at 7:00 AM and 4:00 PM, and 10% feed refusal were allowed. The cattle had free access to the experimental diet and drinking water.

Chemical analysis

The total mixed ration (TMR) and fermented TMR (FTMR) diet samples were collected for chemical compositions analyses. The dry matter (DM) content of the TMR and FTMR diet samples were measured after drying the samples for at 65 °C 72 h with an oven. The dried samples were ground and through a 1 mm screen for the chemical compositions analysis. The acid detergent fiber (ADF) and neutral detergent fiber (NDF) contents were determined by a ANKOM A200i Fiber Analyzer (ANKOM Technology, Macedon, NY, USA) according to the previous report [34]. The metabolizable energy was calculated according to the previously published method of the following formula: ME = GE – FE – UE – Eg [4]. The acid detergent lignin (ADL) contents were determined by the method of the Association of Official Analytical Chemists [2]. The cellulose, hemicellulose and holocellulose contents were measured followed with Nyang'au [25].

DNA extraction, PCR, sequencing and microbial community analyses

The fecal samples of the cattle were immediately collected after excreting when the experiment is finished and the cattle were selling to the Inner Mongolia Fuchengxiang Animal Husbandry Technology Development. Then the fecal samples were placed in sterile centrifuge tubes and immediately frozen in liquid nitrogen containers, then stored at −80 °C until analysis. The genomic DNA of the microbial community of the fecal samples was extracted from the cattle fed with TMR or FTMR diet by the CTAB method [1, 21]. The concentrations and quality of the extracted genomic DNA was measured with a Nano Drop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The V3–V4 regions of the 16S rDNA gene was targeted with the universal primer pair 341 F (5′-CCTACGGGNGGCWGCAG-3′) and 806R(5′-GGACTACHVGGGTATCTAAT-3′), generating an amplicon length of approximately 465 bp.Sequencing was conducted on the Illumina NovaSeq 6000 platform (IlluminaInc., San Diego, CA, USA) with paired-end 250 bp (PE250) reads. The raw paired-end reads were analyzed by the QIIME2 platform (https://qiime2.org/). Amplicon sequence variants (ASVs) were obtained by removing low-quality data using DADA2 [7]. Subsequently, the ASVs were then taxonomically annotated using the SILVA database (https://www.arb-silva.de/, Release 138) through Mothur [40].

Table 1 Chemical composition of the TMR and FTMR diet

Item	TMR	FTMR	*p*-value
DM (%)	42.96 ± 0.48	41.30 ± 0.68	< 0.0001
ADF (% of DM)	33.63 ± 0.76	29.69 ± 0.64	0.0014
NDF (% of DM)	50.31 ± 0.65	44.37 ± 0.80	< 0.0001
ADL (% of DM)	6.19 ± 0.43	5.79 ± 0.34	0.4735
Cellulose (% of DM)	27.43 ± 0.82	23.90 ± 0.57	0.0032
Hemicellulose (% of DM)	16.68 ± 0.41	14.69 ± 0.96	0.0762
Holocellulose (% of DM)	4411 ± 0.80	38.58 ± 0.85	0.0003

DM dry matter, *CP* crude protein, *ADF* acid detergent fiber, *NDF* neutral detergent fiber, *ADL* acid detergent lignin. Means with unlike letters within a row differ at $p < 0.05$

Metagenome sequencing

The total DNA was extracted from the TMR and FTMR diet samples using the Powersoil® DNA Isolation Kit (MoBio, Carlsbad, CA). The concentrations and quality of the extracted DNA were verified with aNano Drop® Spectrophotometer ND-2000 (Thermo Fisher Scientific, USA). The DNA extraction, sequencing, and bioinformatics analyses were performed by Shanghai Majorbio Biopharm Technology Co., Ltd. (SMBBC, Shanghai, China). The raw reads were trimmed using Fastp (v0.20.0) (https://github.com/OpenGene/fastp) to obtain the clean data. The resulting reads were assembled into contigs individually for each sample through Megahit (v1.1.2) software (https://github.com/voutcn/megahit). The open rea ding frames (ORFs) were predicted using MetaGene [24], and functional and taxonomic analyses annotation of the genes was performed using BLASTP [14].

Statistical analysis

The experiment included two types of samples, namely diet samples and cattle feces samples. Each type consisted of two treatments, TMR feed and FTMR feed, with eight independent biological replicates per treatment. All experimental data were analyzed using the one-way analysis of variance with the general linear models of SAS software (version 9.2, SAS Institute Inc., Cary, NC). Multiple comparisons among the treatments were conducted using the T-test method, with significances were considered at $p < 0.05$ level. The Mantel analysis was used to determine the relationship between the composition of diet and fecal samples using R (version 4.1.3). Microbial data analysis are auspicious creature online platform in the Mei Ji (https://www.majorbio.com/web/www/index), which alpha diversity analysis using the following formu la is analyzed:

$$D_{\text{stimpson}} = \frac{\sum_{i=1}^{S_{\text{obs}}} n_i(n_i - 1)}{N(N-1)}$$

$$H_{\text{shannon}} = -\sum_{i=1}^{S_{\text{obs}}} \frac{n_i}{N} \ln \frac{n_i}{N}$$

Table 2 Ingredient and nutrient compositions of the TMR and FTMR diet (DM basis %)

Item	Treatment	
	TMR diet	FTMR diet
Ingredients		
Corn Straw	70.00	70.00
Corn	16.00	16.00
Soybean meal	08.00	08.00
Distillers dried grains with solubles	02.00	02.00
Mountain flour	01.00	01.00
Salt	01.00	01.00
Premix	02.00	02.00
Nutrient composition		
CP	18.50	18.50
ADF	33.63	29.69
NDF	50.31	44.37
Metabolizable energy (MJ/kg)	9.48	9.54

DM dry matter, *CP* crude protein, *ADF* acid detergent fiber, *NDF* neutral detergent fiber

Table 3 Effects of TMR and FTMR on the growth performance in beef cattle

Item	TMR	FTMR	*p*-value
Initial body weight (kg)	283.57 ± 13.17	283.07 ± 14.66	0.9802
Finish bodyweight (kg)	310.57 ± 13.88	337.29 ± 20.53	0.9466
Average daily gain (kg/d)	0.31 ± 0.19	0.62 ± 0.17	0.2382
Dry matter intake (kg)	12.50 ± 0.07	11.04 ± 0.08	< 0.0001
Feed conversion ratio	40.36 ± 1.41	17.94 ± 0.86	< 0.0001

Means with unlike letters within a row differ at $p < 0.05$

S_{obs} = The actual number of ASVs observed;

n_i = The number of sequences contained in the i-th ASV;

N = The number of all sequences.

Results

Characteristics of diet

The nutrient composition of the TMR and FTMR diet is shown in Table 1. Compared to the TMR diet, the FTMR diet obtained the markedly ($p < 0.05$) lower DM, ADF, NDF, cellulose and holocellulose contents, and no significant ($p > 0.05$) differences were observed on the ADL and hemicellulose contents between the two experimental diets. The ingredient and nutrient composition of these diets used in the current study is shown in Table 2.

Animal growth performance

The growth performance of the cattle is depicted in Table 3. The cattle had similar initial body weight ($p > 0.05$) at 283.07 to 283.57 kg and finish bodyweight ($p > 0.05$) of 310.57 to 337.29 between the TMR and FTMR diet fed cattle. No significant ($p > 0.05$) difference was observed on the average daily gain between the TMR (0.31 kg) and FTMR (0.62 kg) diet fed cattle. The DMI was significantly ($p < 0.05$) affected and the markedly

($p < 0.05$) lower DMI was observed in the FTMR diet fed cattle. There was also a significant impact of the diet on the feed conversion ratio (FCR), and the FCR in FTMR were statistically ($p < 0.05$) lower than that in the TMR ($p < 0.05$) diet fed cattle.

Profiling of the diet metagenome

The application of metagenome has greatly expanded to characterize the real functions of the microbiome rather than its potential. The sequencing of all 16 diet samples generated 693,799,744 raw reads. Then, a total of 688,186,838 clean reads were obtained by removing low-quality and the clean reads were integrated to form 8,957,670 contigs and 7,077,255 open reading frames (ORFs) via metagenomic assembly and ORF prediction (Table 4). The analysis of the functional diversity of the diet microbiota at the metagenomics level showed markedly significant difference in the Shannon ($p < 0.0001$) and Simpson ($p = 0.009$) indexes between the TMR and FTMR groups (Fig. 1A). The principal co-ordinates analysis (PCoA) scatter plot showed the notably distinct separation between the TMR and FTMR groups (Fig. 1B), and the Bray–Curtis-based β-diversity analysis (corrected by permutational multivariate analysis of variance, permutations = 999, R = 1, $p = 0.003$) revealed the significant differences in the microbial composition and functions between the two treatments. Then, the distribution of dominant bacterial was also investigated and assigned into MAGs (Metagenome-Assembled Genomes) (Fig. 1C). Of these MAGs, the TMR and FTMR groups had approximately 30.08% and 3.76% MAGs at phylum level, respectively. The most abundant phyla were Pseudomonadota (66.00%), Bacillota (10.95%), Actinomycetota (6.96%) and Bacteroidota (6.88%) in the TMR diet, while a considerable portion of Bacillota (94.14%) dominant in the FTMR group (Fig. 1D). The FTMR diet was significantly enriched with species having higher relative abundance including *Weissella hellenica* (14.09%), *Lactiplantibacillus plantarum* (8.84%), *Lactiplantibacillus sp.* (8.10%), *Levilactobacillus brevis* (5.87%), *Weissella cibaria* (3.12%), and *Companilactobacillus alimentarius* (3.09%) belonged to the genus *Weissella*, *Lactiplantibacillus*, *Levilactobacillus* and *Companilactobacillus* in Fig. 1E, Fig. 1F and Supplementary Fig. 1A and B, while the TMR diet was significantly enriched with species having higher relative abundance including Escherichia coli (11.54%), *Klebsiella pneumoniae* (5.89%), *Acinetobacter baumannii* (5.01%), *Enterobacter hormaechei* (3.66%), and *Pantoea sp.* (3.62%) belonged to *Escherichia*, *Salmonella*, *Pantoea*, *Klebsiella*, *Enterobacter* and *Acinetobacter* (5.27%) in Fig. 1E, Fig. 1F and Supplementary Fig. 1A and B.

The author also annotated the functional and metabolic pathways of the TMR and FTMR diet microbiome based on the KEGG databases and Carbohydrate-Active EnZymes database (Fig. 2). Moreover, the PCoA of the functional diversity revealed the dramatic separations between the TMR and FTMR diets, explaining the 99.09% variation ($p = 0.005$, Fig. 2A). The categories of 'Metabolism' were the most abundant in the TMR and FTMR diets at level 1, especially in the FTMR diet (Fig. 2B), significant difference was also found between the two diets (Fig. 2C). The metabolic pathways were enriched in the global and overview maps, carbohydrate metabolism, metabolism transport and energy metabolism in FTMR diet than that in the TMR diets (Fig. 2D). Then, the carbohydrate active enzymes were also analyzed. The significant changes in the number of total CAZy enzymes families were observed (Fig. 2E), and the predicted function in the TMR and FTMR diets composed of six classes of CAZy-annotated enzymes. The relative abundance of glycoside hydrolase (GH) was markedly increased in the FTMR diet compared to that in the TMR diet (Fig. 2E). The LEfSe analysis was used to characterized the differential abundance of the CAZy families that changed in the two diets (Fig. 2F). GH17, GH28, GH9, GH152, GH 14 GH13, GH23 and GH100 showed markedly increased on the FTMR diet.

Profiles of the fecal microbiome and taxonomic differences

To further explore whether feeding the TMR or FTMR diet changes the fecal microbiome, the author next instigated the changes on the fecal microbiome of cattle fed with of the TMR or FTMR diet. The diversity indices of fecal microbiota of cattle fed with the TMR and FTMR diet was analyzed by 16S rRNA sequencing (Fig. 3A). In total, 1,063,277 raw reads were obtained and 656,680 valid reads were obtained from the 16 fecal samples. These valid reads were clustered into 25,457 ASVs with a confidence threshold value of 90%. The Goods' coverage

Table 4 Genome statistics of the TMR and FTMR diet

Item	TMR	FTMR	*P*-value	Total number
Raw reads	57,937,989 ± 3,208,668	57,695,301 ± 3,011,015	0.8950	693,799,744
Clean reads	57,409,491 ± 3,164,346	57,288,315 ± 2,940,044	0.9470	688,186,838
Contigs	1,289,636 ± 41,552	203,309 ± 82,334	< 0.0001	
ORFs number	921,520 ± 41,056	258,022 ± 25,125	< 0.0001	
Total Length (bp)	261,806,057 ± 12,311,034	137,258,206 ± 7,157,050	< 0.0001	

Means with unlike letters within a row differ at $p < 0.05$

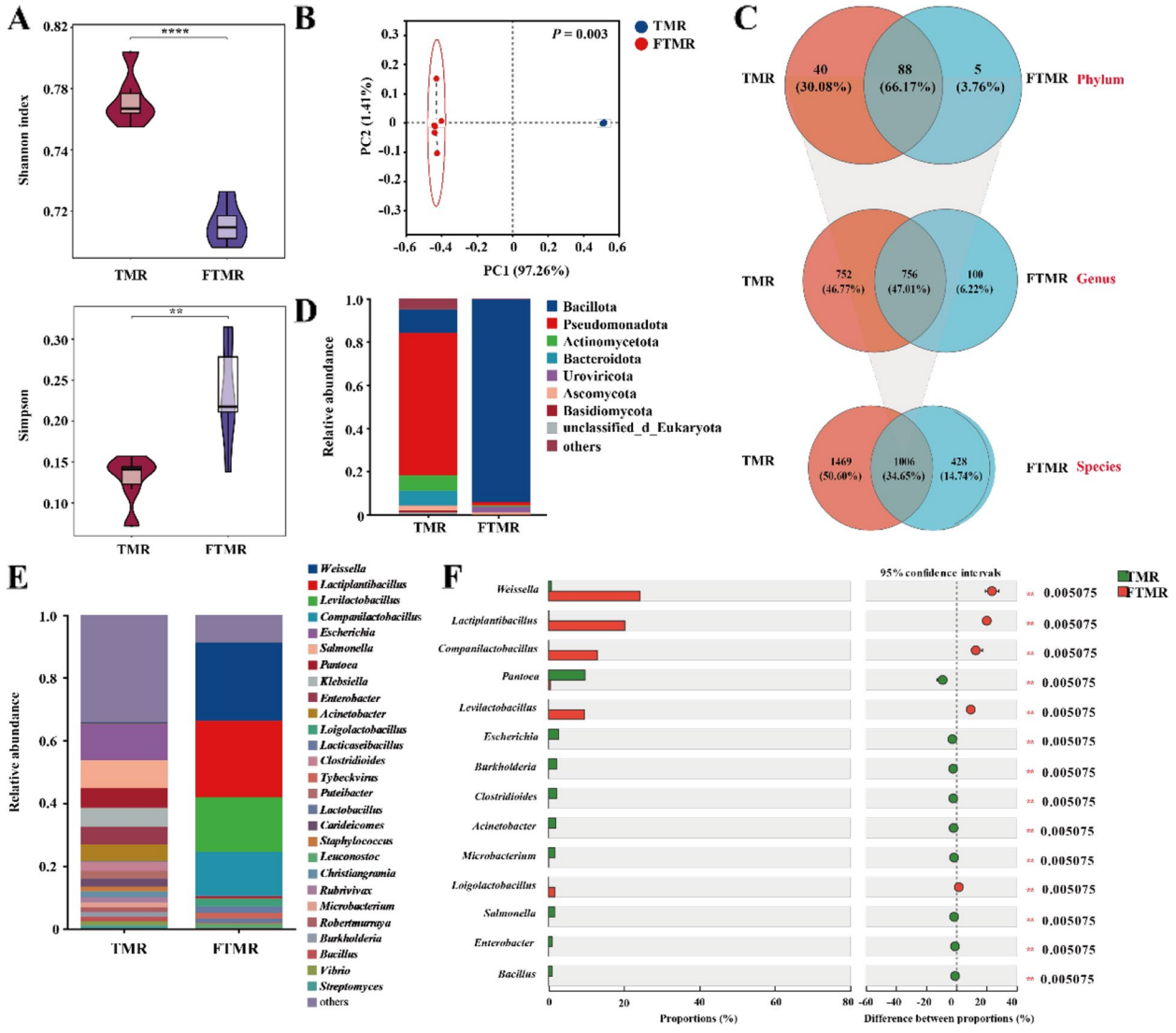

Fig. 1 Comparison of the structure and function of the metagenome in the TMR and FTMR diet. **A** The Alpha diversity differences between the TMR and FTMR diet groups. **B** The Principal Coordinate Analysis (PCoA) using Bray–Curtis distances. **C** The specific numbers and proportions of genome bins at the phylum, genus, and species levels. **D** The bacterial abundances at the phylum level. **E** The bacterial abundances at the genus level. **F** Comparison of bacterial variations of the TMR and FTMR diet

of the fecal samples was higher than 0.99, indicating that the sequencing depth was sufficient for further analysis (Table 5). Briefly, analysis of the 16S rRNA sequencing revealed that the microbial community was affected by the diet. After processing and quality filtering, an average of 41,042 effective tag sequences for per fecal sample (Data are not shown). The bacterial community stability of the fecal samples was evaluated by average variation degree (AVD), the lower AVD value indicates higher microbiome stability [38], the lower AVD value was obtained in the FTMR-fed group compared to that in the TMR-fed group (Fig. 3B). The FTMR-fed cattle also displayed markedly decreasing trend in Shannon index when compared to the TMR-fed group (Fig. 3C). The reads with 0.8 similarity were clustered into the amplicon sequence variants (ASVs). A total of 14,899ASVs for the TMR and FTMR groups, with an average of 1637 (ranging from 1,413 to 1788) and 1215 (ranging from 1,209 to 1,801) ASVs per sample for the TMR- and FTMR- fed cattle, the Venn diagram also showed the TMR and FTMR groups had 6011 and 7051 exclusive ASVs, respectively (Fig. 3D). The PCoA plot showed a significant ($p = 0.018$) difference in the bacterial communities between the two groups of cattle (Fig. 3E). After taxonomic annotation, a total of 656,680 effective tag sequences taxa at five levels (including 17 phyla, 39 classes, 94 orders, 186 families and 446 genera, Fig. 3F and 3G). At the genus level, there were ten significantly different genera were identified between the treatments, *Paenicolostridium*, *Clostridium_sensu_stricto*_1, *Turicibacter* and *Blautia* were

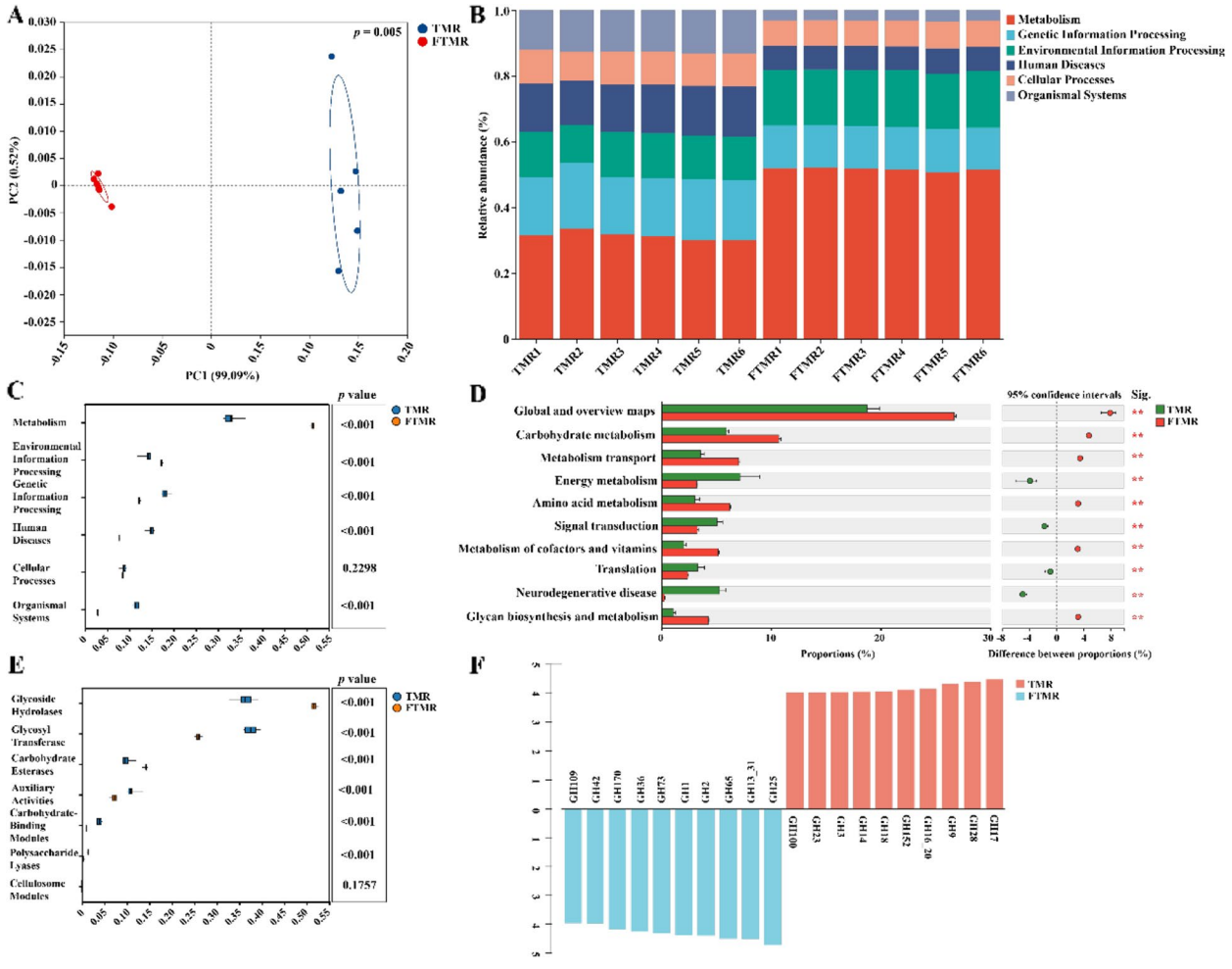

Fig. 2 The functional annotation of predicted non-redundant gene catalog based on the KEGG annotation and Carbohydrate-Active EnZymes database are from metagenomic sequence. **A**β diversity. **B** Compositions of the main metabolic pathways in the TMR and FTMR diets. **C** The KEGG level-1 pathways with significances in the TMR and FTMR diets. **D** The KEGG level-2 pathways with significances in the TMR and FTMR diets. **E** The main Carbohydrate-Active EnZymes database classes in the TMR and FTMR diets. **F** The main Carbohydrate-Active EnZymes database classes with significant differences by LEfSe analysis in the TMR and FTMR diets

enriched in the fecal microbiome of the FTMR diet calves ($p < 0.05$; Fig. 3H). The relative abundance of the top 15 in the fecal samples of the cattle fed with the TMR and FTMR diet were displayed in Fig. 3H, the fecal microbiota of TMR diet calves was enriched with *Monoglobus*, *Prevotellaceae*_UCG-004, Family_XIII_AD3011_group, *Bacteroides*, norank_o_*Gastranaerophilales* and *Prevotellaceae*_UCG-003 ($p < 0.05$).

Relations between MAG species and fecal microbiome

The Mantel test revealed a positive and significant association ($p < 0.05$, $r \geq 0.5$) between the MAG species and fecal microbiome (Fig. 4). The s_*Lactiplantibacillus_paraplantarum*, s_*Lactiplantibacillus_pentosus*, s_*Lactiplantibacillus_plantarum*, s_*Levilactobacillus_brevis*, s_*Weissella_cibaria* and s_*Weissella_hellenica* correlated positively with g_*Prevotellaceae*_UCG-003. Interestingly, the s_*Lactiplantibacillus_paraplantarum*, s_*Lactiplantibacillus_pentosus*, s_*Lactiplantibacillus_plantarum*, s_*Levilactobacillus_brevis*, s_*Weissella_cibaria* and s_*Weissella_hellenica* also showed positive relationships with g_*Prevotellaceae*_UCG-004.

Discussion

Fermentation technology is gaining attention as a potential processing method to improve the nutritional properties, and health benefits of diet for ruminants [17]. The fermented diet modulates the animal performance and gut microbiome through the taxonomic composition and functional potential of the microbiome in the fermented diet by the metagenomic sequencing technology [6, 18]. In the current study, the high-throughput metagenomic sequence technology was employed to evaluate the effects of TMR or FTMR diet on the growth performance and gut microbiome of cattle.

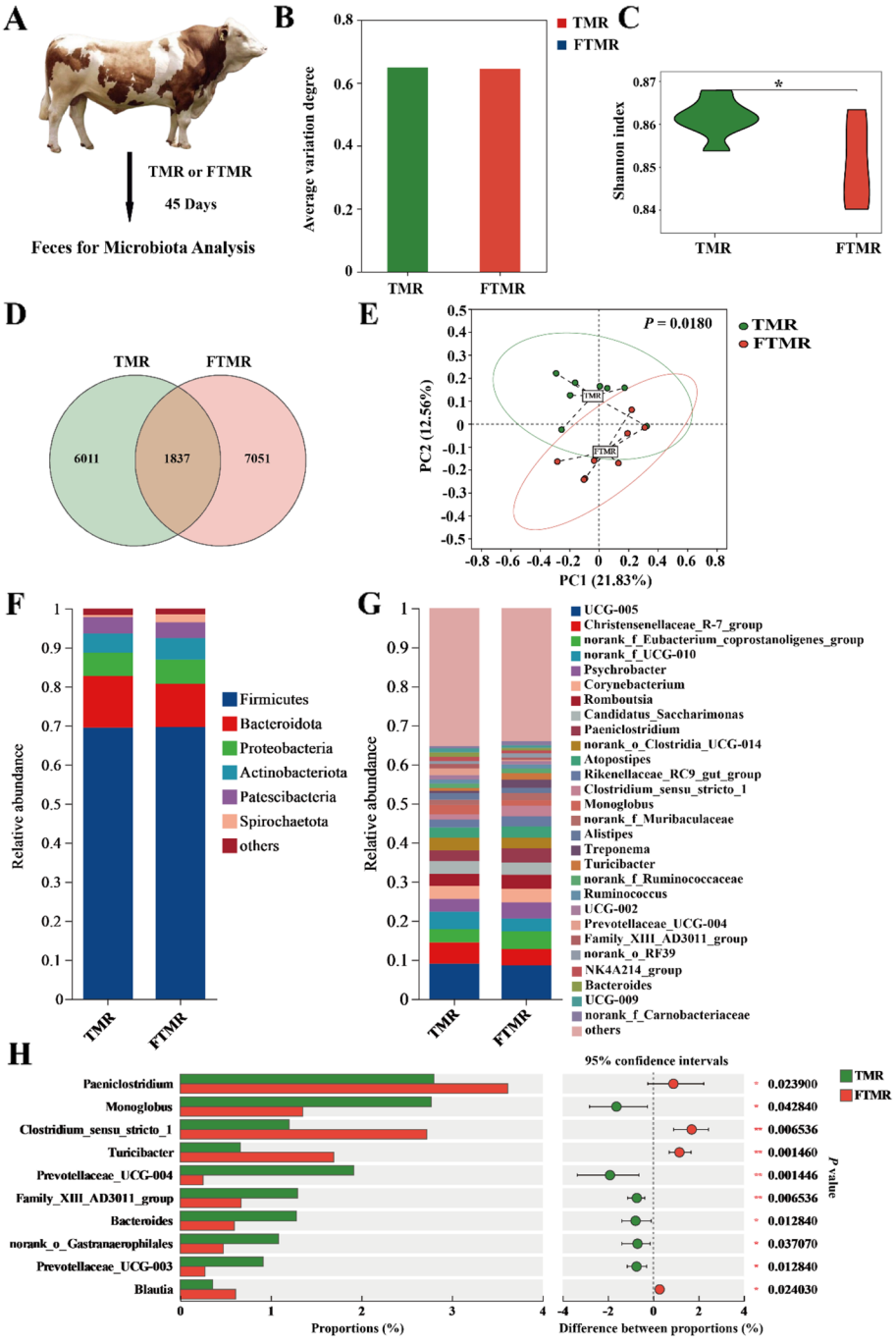

Fig. 3 Effects of TMR or FTMR on the gut microbiota of cattle. **A** Experimental layout to study the changes in gut microbiota profile in TMR or FTMR fed cattle. Same age cattle ($n=7$ per treatment) maintained either on TMR or FTMR diet for 45 days. Feces were aseptically collected and used for 16S rRNA sequencing. **B** The average variation degree in the fecal samples. **C** The Shannon index differences in the fecal samples. **D** Venn diagram representing the common and unique ASVs found at fecal samples. **E** The bacterial community dissimilarities in different ensiling time, calculated by Bray–Curtis distances, with coordinates calculated by principal coordinates analysis. **F** Relative abundances of bacterial phylum at fecal samples. **G** Relative abundances of bacterial genus at fecal samples. **H** Extended error bar plot showing the bacteria at the genus level that had significant differences in the fecal samples

Fermented foods are beneficial for human and animal health because of the probiotics and their metabolites produced by the microbial fermentation [8, 16]. It has been reported that the fermented diet could improve the animal growth performance [33]. Some studies also indicated that fermented diet had no significant effect on the DMI [23, 27]. In this study, cattle fed FTMR exhibited higher ADG compared to the TMR group, while demonstrating lower DMI and FCR than the TMR group, which indicating a revolutionary improvement the FCR

Table 5 Diversity indices of fecal microbiota of cattle fed with TMR and FTMR diet

Item	TMR	FTMR	*P*-value	Total number
Number of sequences	61,202 ± 1,178	71,707 ± 1,643	< 0.0001	1,063,277
Number of valid sequences	38,714 ± 9,554	43,370 ± 990	< 0.0045	656,680
Observed ASV number	1,525 ± 68	1,656 ± 46	< 0.1366	
Goods' coverage	> 0.99	> 0.99		

Means with unlike letters within a row differ at $p < 0.05$

in the FTMR diet. The fermentation process is inherently a form of "pre-digestion" and nutritional enhancement. At the microbial level, FTMR accumulated substantial amounts of *Lactiplantibacillus* and *Weissella* during fermentation. These bacteria rapidly produce lactic acid under lower pH conditions, initiating the partial hydrolysis of structural carbohydrates. This process partially degrades the ADF, NDF, cellulose, and total cellulose content in plant cell walls components that are difficult for cattle to fully digest via their own secreted enzymes when consuming unfermented TMR. Furthermore, feed with lower fiber content and lower pH favors the growth of the fiber-hydrolyzing bacterium *Paenicolostridium* within the cattle's digestive system. This leads to the release of more fermentable sugars and improves energy utilization efficiency ([33], Du et al., 2025). During the fermentation progress, the fibers in the diet were decomposed by the bacterial CAZymes and metabolic pathways into water soluble carbohydrate and further converted into organic acids and increasing the palatability and digestibility of silages [26]. Furthermore, the main carbohydrate-Active EnZymes also investigated and the glycoside hydrolase was enriched in the FTMR diet. These results indicated that the fermentation process utilized the water-soluble carbohydrate in the materials and decomposed the fibers into water-soluble carbohydrate, while inhibiting the hydrolase of nonstructural carbohydrate, these results also explained the decreased of the ADF and NDF contents, and improved animal performance [19].

The 16S rRNA sequencing technology has been widely applied in silage fermentation research due to its high efficiency and accuracy, and can deeply analyze the structure and dynamic changes of microbial communities. Despite extensive research on the succession and composition of bacteria and fungi throughout the fermentation process of feed and grass-fermented diets. Nevertheless, systematic analyses the potential function

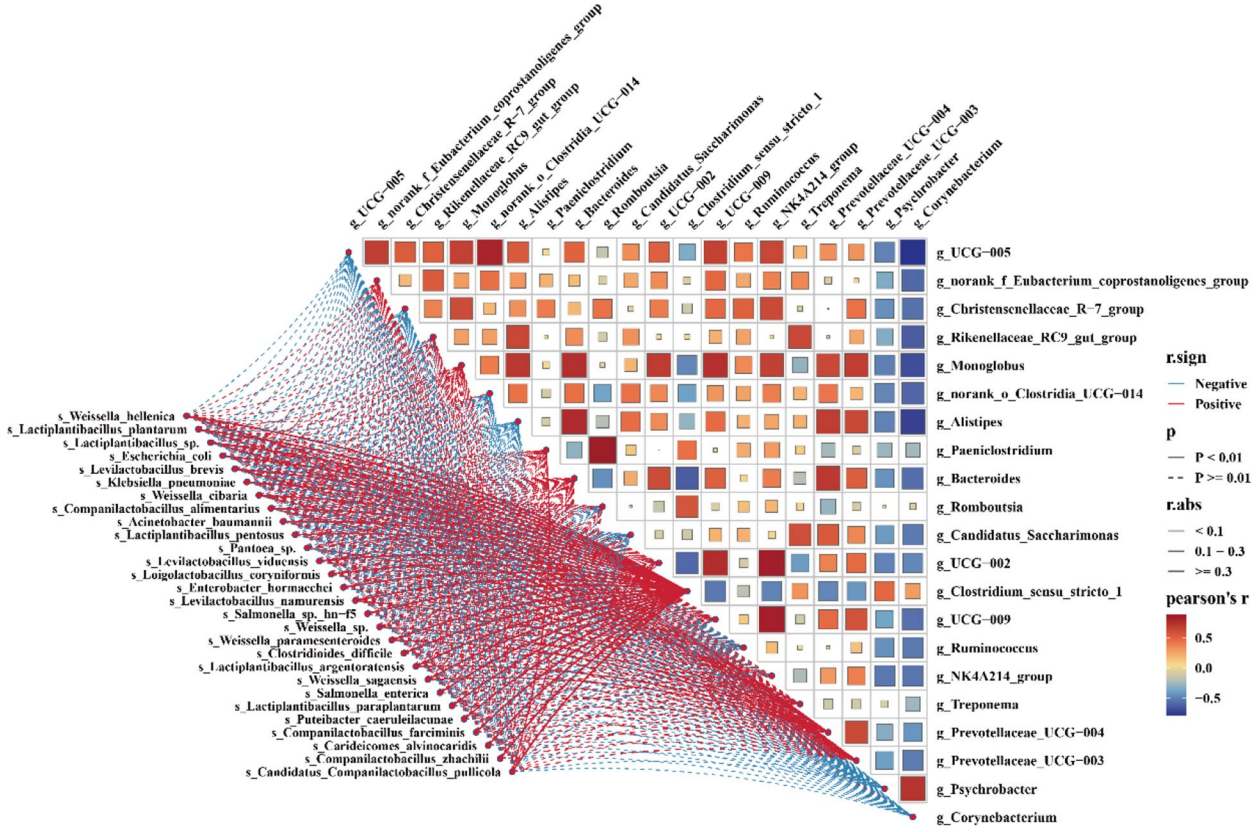

Fig. 4 Mantel analysis between the composition of diet and fecal samples. Red, positive correlation; blue, negative correlation. The square chart indicates the magnitude of the correlation coefficients, and the shade of color indicates the significance of the correlation coefficients

of the microbiome in the fermented diet remain insufficient. In the current study, the author explored the microbiome of the TMR and FTMR diet by the metagenomics sequencing. This is to enable us to gain insights into the function of the microbial genomes that could be driving the variation in animal performance between the TMR and FTMR diet fed cattle. The Shannon ($p < 0.001$) and Simpson ($p < 0.01$) indexes, and PERMANOVA ($p = 0.003$) revealed significant differences and separation between the two diets fed cattle.

The Two-tailed test showed significant differences in several microbiomes, including s_*Escherichia_coli*, s_*Lactiplantibacillus_plantarum*, s_*Clostridioides_difficile*, s_*Weissella_cibaria*, s_*Levilactobacillus_brevis*, s_*Lactiplantibacillus_pentosus* and s_*Lactiplantibacillus_paraplantarum*, between the two treatments. At the metagenomics read level, the FTMR diet obtained a higher composition of 4 genera and 19 species g_*Weissella* (s_*Weissella_cibaria*), g_*Lactiplantibacillus* (s_*Lactiplantibacillus_plantarum*, s_*Lactiplantibacillus_pentosus*), g_*Levilactobacillus*, s_*Levilactobacillus_brevis*) and g_*Companilactobacillus* (s_*Companilactobacillus_alimentarius*) compared to these in the TMR diet. The specific function of the majority of the enriched genera in the fermented diet has been documented [11], such as the *Lactiplantibacillus plantarum* and *Weissella cibaria* could to produce an acid and anaerobic environment that limited the microorganisms to continuously consume the nutrients [36]. Others like *Escherichia* and *Clostridioides* have been reported to cause gastrointestinal infection or inflammation [35]. In the current study, the undesirable microorganisms s_*Escherichia_coli*, s_*Clostridioides_difficile* and s_*Klebsiella_pneumoniae* showed higher relative abundances in the FTMR diet, the beneficial bacteria such as s_*Lactiplantibacillus* and s_*Levilactobacillus* proliferate in large quantities in the FTMR, inhibiting the growth of other bacteria might be the main reason. The bacterial colonies such as s_*Escherichia_coli* antagonize beneficial bacteria such as s_*Lactiplantibacillus* and s_*Levilactobacillus* through multiple mechanisms, and thus survive and thrive. [17, 28, 30, 31].

The gut microbiota plays an important role in promoting the food digestion and intestinal health, and could be affected by many factors including diet, genes and age [10]. Among these factors, diet is one of the decisive factors affecting the dynamic balance of the gut microbiota [20]. To further explore the contribution of the TMR or FTMR diet on the gut microbiota. The author employed the 16S rRNA sequencing to characterized the influence of the TMR or FTMR diet on the gut microbiota. Compared to the TMR diet fed cattle, the Shannon index in the FTMR diet fed cattle was statistically decreased. The beta-diversity of fecal microbiota showed a significant separation. These results indicated that the FTMR diet modulated the composition of gut microbiota. At the phylum level, Firmicutes and Bacteroidetes exhibited the highest relative abundance among all phyla; however, no significant differences were observed in their abundance between cattle fed the two diets. At the genera level, there were only several statistical difference between the two diet fed cattle. The genus *Prevotellaceae*_UCG-003 and *Prevotellaceae*_UCG-004 were enriched in the TMR diet fed cattle fecal samples compared to that the FTMR diet fed cattle fecal samples, which was in accordance with the previous report that the lower relative abundance of *Prevotellaceae* was existed in the animal received the fermented diet [32, 41]. Interestingly, these results were contrary with the previous report that the higher relative abundance of *Prevotellaceae*_UCG-003 and *Prevotellaceae*_UCG-004 were found in the animal fed with silage diet [42]. The genera *Prevotellaceae*_UCG-003 and *Prevotellaceae*_UCG-004 are members of the Prevotellaceae family. The Prevotellaceae family can break down proteins and carbohydrates in feed [22, 41]. However, the absorption and utilization of proteins by animals actually depend on the composition and proportion of amino acids. The variation in the relative abundance of Prevotellaceae might be the main reason.

Correlations between fecal microbiome measures and fecal metabolite concentrations were evaluated using Mantel test. The g_*Prevotellaceae*_UCG-003, g_*Prevotellaceae*_UCG-004 and g_*Paenicolostridium* were positively correlated with s_*Lactiplantibacillus_paraplantarum*, s_*Lactiplantibacillus_pentosus*, s_*Lactiplantibacillus_plantarum*, s_*Levilactobacillus_brevis*, s_*Weissella_cibaria* and s_*Weissella_hellenica*, which could be contributed to the FTMR group in the feed is enriched with s_*Weissella*, s_*Lactiplantibacillus*, and s_*Levilactobacillus*, which may secrete lactic acid and thereby inhibit the growth of pathogenic bacteria s_*Escherichia* and s_*Klebsiella*. It may have promoted the colonization of g_*Paenicolostridium* and g_*Turicibacter,* which are related to fiber degradation, in the FTMR group of the intestinal tract. The degradation of fiber generated short-chain fatty acids (SCFAs), which promoted the nutritional transformation of feed by cattle. As a result, under the same DMI, the daily weight gain was increased compared with the TMR group. Interestingly, the genus g_*Prevotellaceae*_UCG-003 and g_*Prevotellaceae*_UCG-004 were concentrated in the TMR diet fed cattle fecal samples.

The bacteriocin, antimicrobial peptide and organic acids produced by lactic acid bacteria (*Lactiplantibacillus*, *Levilactobacillus* and *Weissella*) could depress the growth of *Prevotellaceae* [17].

Conclusions

In conclusion, this study identified the differences in animal performance, microbial composition and functions of cattle fed with the TMR and FTMR diet. The FTMR diet significantly reduced the DMI and simultaneously increased the average daily gain of cattle. The fecal microbiome of cattle fed TMR or FTMR was mostly unchanged. One limitation of this study was the numbers of the animals and the shorter feeding period for analyzing and reflecting the animal performance and microbial community of cattle. Additionally, the fecal microbiota analysis primarily represents the hindgut; nonetheless, it remains the most practical and informative method for studying fiber fermentation. Rumen fermentation parameters and rumen microbial community structure will conduct in-depth research later. Regardless, the results of the current study could provide a direct perspective and fundamental knowledge of the animal performance and microbial community in response to the influence of the FTMR diet.

Abbreviations

DM	Dry matter
CP	Crude protein
ADF	Acid detergent fiber
NDF	Neutral detergent fiber
ADL	Acid detergent lignin
MAGs	Metagenome-Assembled Genomes

Supplementary Information

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Supplementary Material 1.

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Authors' contributions

Sihan You: Data curation, Writing–original draft, Validation. Yuqi Zou: Investigation. Yanzi Xiao: Data curation. Lichao He: Investigation. Lili Liu: Writing–review and editing. Yuhan Sun: Data curation. Wenge Li: Funding acquisition. Yushan Jia: Funding acquisition. Gentu Ge: Funding acquisition. Shuai Du: Conceptualization, Data curation, Investigation, Writing–original draft, Writing–review and editing, Validation.

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Data availability

The sequence raw data associated with this project have been deposited in the National Center for Biotechnology Information Short Read Archive database (With the accession number PRJNA1243703 (https://submit.ncbi.nlm.nih.gov/subs/sra/SUB15215540/overview) for the feces amples and PRJNA1246253 (https://submit.ncbi.nlm.nih.gov/subs/sra/SUB15215559/overview) for the TMR or FTMR Metagenome samples).

Declarations

Ethics approval and consent to participate

The research protocol used in the current research was according to the Institutional Guidelines for Animal Experiments of the Inner Mongolia Agricultural University protocol number: NND2024056, Huhhot, China.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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