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Medium-chain fatty acid triglycerides improve feed intake and oxidative stress of finishing bulls by regulating ghrelin concentration and gastrointestinal tract microorganisms and rumen metabolites

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Abstract

Background As a feed additive, medium-chain fatty acids (MCFAs)/medium-chain fatty acid triglycerides (MCTs) have been used in ruminant production, but mostly added in the form of mixed esters. Studies have shown that MCTs may have a positive effect on feed intake or oxidative stress in animals, but it is unclear which MCT could play a role, and the mechanism has not been elucidated. In this study, the effects of individual MCT on growth performance, serum intake-related hormones, and oxidative stress indices in finishing bulls were investigated and further studied the effects of MCT supplementation on gastrointestinal tract bacteria and rumen fluid metabolomics.

Results Four ruminally fistulated Yanbian cattle (bulls) were selected in 4×4 Latin square designs and allocated to four treatment groups: a control group (CON) fed a basal diet (total mixed ration, TMR), three groups fed a basal diet supplemented with 60 g/bull/day glycerol monocaprylin (GMC, C8), glycerol monodecanoate (GMD, C10), and glycerol monolaurate (GML, C12), respectively. Compared with the CON group, GMD tended to increase the dry matter intake (DMI) of finishing bulls ($P = 0.069$). Compared with the CON group, GMD significantly increased the concentration of ghrelin O-acyl transferase (GOAT), total ghrelin (TG), acylated ghrelin (AG), and orexins ($P < 0.05$) and significantly decreased the concentrations of hydrogen peroxide (H_2O_2), malondialdehyde, reactive oxygen species (ROS), and lipopolysaccharides (LPS) in the serum of finishing bulls ($P < 0.05$). Compared with the CON group, GMD and GML significantly increased the concentrations of total antioxidant capacity (T-AOC), catalase, glutathione peroxidase (GSH-PX), glutathione reductase (GR), and nitric oxide (NO) in the serum of finishing bulls ($P < 0.05$). Compared with the CON group, there were 5, 14, and 6 significantly different bacteria in the rumen digesta in the C8, C10, and C12 groups, respectively; there were 3, 10, and 5 significantly different bacteria in the rumen fluid in the C8, C10, and C12 groups, respectively; and only one differential bacteria (genus level) in the feces among the four treatment groups. Compared with the CON group, there were 3, 14, and 15 significantly differential metabolites identified under positive ionization mode in the C8, C10, and C12 groups, respectively, while under negative ionization mode

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were 3, 11 and 14, respectively. Correlation analysis showed that there was a significant correlation between DMI, GOAT, AG, GSH-PX, LPS, gastrointestinal tract bacteria, and rumen fluid metabolites.

Conclusions Our findings revealed that different types of MCTs have different application effects in ruminants. Among them, GMD may improve the feed intake of finishing bulls by stimulating the secretion of AG. GMD and GML may change gastrointestinal tract microorganisms and produce specific rumen metabolites to improve the oxidative stress of finishing bulls, and ghrelin may also be involved. This study enlightens the potential mechanisms by which MCT improves feed intake and oxidative stress in finishing bulls.

Keywords Feed intake, Gastrointestinal tract bacteria, Medium-chain fatty acid triglycerides, Metabolome, Oxidative stress, Yanbian cattle

Background

Medium-chain fatty acids (MCFAs) are fatty acids with chain lengths between 6 and 12 carbon atoms, mainly including caprylic acid (C8:0), decanoic acid (C10:0), and lauric acid (C12:0), which are easy to be digested and absorbed in animal intestines [1]. At present, as a safe and efficient additive, MCFAs have been used in ruminants to improve performance (such as milk performance, immune function, rumen fermentation, etc.), nutrients digestibility, and inhibit methane (CH_4) production [2–7]. Despite this, MCFAs are mostly added to the diet in the form of mixed esters (such as palm oil and coconut oil), but it is unclear which MCFA could play a role.

MCFAs can be esterified by glycerol to form medium-chain fatty acid triglycerides (MCTs), mainly including glycerol monocaprylin (GMC), glycerol monodecanoate (GMD), and glycerol monolaurate (GML). Some studies have shown that MCTs can improve feed intake and oxidative stress in monogastric animals. The addition of 1% 1: 1: 1 blend of C6:0, C8:0, and C10:0 in nursery pig diets increased average daily feed intake (ADFI) compared with control-fed pigs [8]. Moreover, MCFAs treatment (C8:0, C10:0, and C12:0) significantly increased the total antioxidant capacity (T-AOC), and expression of antioxidant-related genes in AML12 cells under oxidative stress conditions, and reduced reactive oxygen species (ROS) production [9]. Dietary decanoate acid (C10:0) supplementation improved cyclophosphamide-induced intestinal inflammation and oxidative stress in miniature pigs [10], and mustard oil rich in decanoate acid (C10:0) could enhance the antioxidant protection of rat liver and brain [11]. The above results indicated that MCFAs/MCTs may play a positive role in improving feed intake and oxidative stress in animals. However, it is unknown whether or which MCFA/MCT will affect the feed intake and oxidative stress in ruminants, and the mechanism has not been clarified.

It has been reported that MCFAs can be used for the acylation of ghrelin and increase the concentration of acylated ghrelin (AG), and AG can stimulate animal appetite [12, 13], and some studies have shown that ghrelin may also be involved in the regulation of oxidative stress [14–16], which provides new insights into the improvement of feed intake and oxidative stress by MCFAs/MCTs supplementation. Although the effects of mixed forms of MCFA on feed intake and ghrelin secretion in beef/dairy cattle have been studied [13, 17, 18], the effects of individual MCFA/MCT on feed intake, ghrelin secretion, and oxidative stress in beef cattle have not been reported, and the related mechanism has not been revealed. Different omics approaches, especially 16S rRNA gene sequencing and metabolomics, provide powerful tools to identify potential players in a high-throughput manner and to further elucidate mechanisms. Previous studies suggest that gastrointestinal tract microbial, metabolites, or metabolic pattern variations may influence host physiology and behavior via multiple direct or indirect pathways [19–22] and play crucial roles in animal health, hormone secretion, nutrient absorption and metabolism, oxidative stress, gastrointestinal tract development, and immune function [23–26].

Thus, in this study, we aimed to answer two questions. Does the individual MCT (GMC/GMD/GML) supplementation contribute to feed intake or oxidative stress in finishing bulls? If so, is this related to ghrelin and gastrointestinal tract microorganisms and metabolites? This study evaluated the effects of individual MCT (GMC/GMD/GML) supplementation on growth performance, serum intake-related hormones, serum oxidative stress indices, and further studied their effects on gastrointestinal tract bacteria and rumen fluid metabolomics of finishing bulls.

Materials and methods

The experiment was carried out from February 2023 to June 2023 in Yanbian University College of Agriculture Animal Experimental Teaching Base, Jilin Province, China. All procedures involving animals were performed with the approval (approval ID: 20221015) of the Yanbian University Institutional Animal Care and Use Committee.

Animals, feeding, experimental designs, and growth performance

The experiment was conducted by using four ruminally fistulated Yanbian cattle (18-month-old bulls) with initial body weight (BW) 367 ± 32 kg in 4×4 Latin square designs. These bulls were allocated to four treatment groups: a group (CON) fed a basal diet (total mixed ration, TMR), a group (C8) fed a basal diet supplemented with 60 g/bull/day GMC, a group (C10) fed a basal diet supplemented with 60 g/bull/day GMD, and a group (C12) fed a basal diet supplemented with 60 g/bull/day GML. Three monoglycerides (purity $\geq$ 99%) were purchased from Shandong Binzhou Jinsheng New Material Technology Co., Ltd. The selection of the addition amount was based on the appropriate concentration determined in the previous in vitro study [27] and converted according to the volume of rumen fluid in adult cattle. The ingredients and chemical composition of the basal diets are shown in Table 1.

Before the start of the trial, all bulls were ear-tagged, dewormed, and tethered in a tie stall using neck straps. During the study, each bull was kept in an individual pen and fed individually twice a day (0500 and 1500 h) and had free access to fresh water. Each experimental period was composed of 28 days. The first 21 days were considered the adaptation period, and the final 7 days were the sampling period.

Dry matter intake (DMI) was individually measured based on the differences between the amount of diet offered and refused daily. Initial and final BW measurements were repeated on two consecutive days before morning feeding, and then the average of the 2 days was used. Average daily gain (ADG) was calculated as the difference between initial and final live weight divided by 28 days. Feed conversion efficiency (feed:gain) was the ratio of individual DMI to ADG.

Sample collection
Feed and fecal sample collection
Representative feed samples of approximately 200 g were collected during the sampling period. Fecal grab samples were collected once a day for seven consecutive days on days 22–28 of each experimental period by grab sampling after the morning feeding. The feed and feces samples collected from each bull within 7 days were mixed and stored at $-20°C$ for measuring the contents of DM, crude protein (CP), ether extract (EE), ash, neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid insoluble ash (AIA).

Blood sample collection
At the last day of each experimental period, about 10 mL of blood was collected from each bull before morning feeding by venipuncture with vacuum tubes without anticoagulant and were centrifuged at $3186 \times g$ for 20 min at 4°C to harvest serum. The serum samples were immediately stored at $-20°C$ for measurement intake-related hormones, antioxidant indices, oxidative stress indices, and endotoxin lipopolysaccharides (LPS). Intake-related

Table 1 Ingredient and nutritional composition of basal diets (%, dry matter basis)

Ingredient composition	Content (% of DM)	Nutritional composition[b]	Content (% of DM)
Corn meal	45.00	NEg[c] (Mcal/kg DM)	1.16
Cornstalk	40.00	Crude protein	11.47
Soybean meal	6.75	Crude fat	3.17
Corn germ meal	2.25	Crude ash	5.62
DDGS	2.25	Neutral detergent fibers	34.89
Calcium carbonate	0.68	Acid detergent fibers	20.62
Molasses cane	0.60	Calcium	0.51
Sodium bicarbonate	0.45	Phosphorus	0.34
Calcium hydrogen phosphate	0.37	Sodium chloride	0.45
Urea	0.45		
Salt	0.45		
Premix[a]	0.75		
Total	100.00		

[a] Premix provided the following per kg of the diet: Fe 400 mg, Cu 600 mg, Zn 400 mg, Mn 20 mg, Se 10 mg, Co 10 mg, vitamin A 350 000 IU, vitamin D 300 000 IU, vitamin E 5000 IU

[b] Conventional nutrients in feed were calculated based on the actual content of feed ingredients

[c] NEg (net energy for gain) was estimated from the analyzed value of the dietary ingredients (based on Ministry of Agriculture of P.R. China (2018))

hormones included GOAT, total ghrelin (TG), deacylated ghrelin (DAG), growth hormone (GH), insulin (INS), orexins, and corticotropin-releasing hormone (CRH). Antioxidant indices included T-AOC, superoxide dismutase (SOD), catalase, glutathione peroxidase (GSH-PX), and glutathione reductase (GR). Oxidative stress indices included hydrogen peroxide (H_2O_2), malondialdehyde, nitric oxide (NO), and ROS.

Gastrointestinal tract sample collection

At the end of each experimental period, rumen samples were collected from four different (the ventral sac, the atrium or reticulum, and two samples from the feed mat) locations in the rumen, filtered through four layers of gauze to separate solid and fluid fractions; the collecting methods were referred to Pitta et al. [28]. Rumen fluid was snap frozen in liquid nitrogen and stored at $-80°C$ for measurement fermentation parameters (such as pH, ammoniacal nitrogen [NH_3-N], lactic acid, LPS, and volatile fatty acids [VFAs]), bacterial diversity, and liquid chromatography-mass spectrometry (LC–MS) metabolomics. Moreover, rumen digesta (solid) and fresh rectum fecal samples were also snap frozen in liquid nitrogen, and stored at $-80°C$ for measurement of bacterial diversity.

Analysis of nutrients' apparent digestibility

The feed and fecal samples collected above were dried at 65°C for 72 h and ground to pass through a 1-mm screen for nutrients' apparent digestibility analysis. DM, CP, EE, ash, NDF, ADF, and AIA contents of feed and feces were determined according to standard procedures [29]. The apparent digestibility of DM, CP, EE, organic matter (OM), NDF, and ADF was calculated by the endogenous indicator (AIA) method according to Luan et al. [30].

Analysis of serum intake-related hormones

The serum GOAT, TG, DAG, GH, INS, orexins, and CRH were determined using commercial ELISA Kits—YJ623074, YJ446287, YJ233015, YJ921050, YJ016134, YJ180841, and YJ405202, respectively—according to the manufacturer's instructions (Shanghai Enzyme-linked Biotechnology Co., Ltd., Shanghai, China), and a microplate reader (CMax Plus, Molecular Devices, SV, USA). Using the same measurement method and principle, details about the method description of the measurement refer to Luan et al. [30]. The intra-assay and inter-assay coefficients of variation (CV) were < 10 and 15%, respectively.

Analysis of antioxidant and oxidative stress indices

Antioxidant indices in the serum samples of bulls were determined in a service corporation (Beijing Sino-UK

of Biological Technology, Beijing, China) using commercial kits (Beijing Sino-UK of Biological Technology, Beijing, China) by the method of colorimetry and an A6 semi-automatic biochemistry analyzer (Beijing Shiningsun Technology Inc., China). Details about the method description of the measurement of T-AOC, SOD, catalase, and GSH-PX refer to Luan et al. [31]. GR was determined using an HY-60010 commercial kit. Briefly, under the condition that reduced coenzyme II (nicotinamide adenine dinucleotide phosphate, NADPH) provides hydrogen, GR can reduce oxidized glutathione (GSSG) to reduced glutathione (GSH). Meanwhile, NADPH forms $NADP^+$. NADPH has an absorption peak at 340 nm, and the amount of NADPH production is proportional to the activity of GR. Therefore, the activity of GR can be calculated by measuring the absorbance of samples at 340 nm. For T-AOC, catalase, GSH-PX, and GR, the intra-assay coefficients of variation were less than 7.3%; the inter-assay coefficients of variation were less than 10%. For SOD, the intra-assay coefficients of variation were less than 8.3%; the inter-assay coefficients of variation were less than 10%.

Oxidative stress indices in the serum samples of bulls were determined in a service corporation (Beijing Sino-UK of Biological Technology, Beijing, China) using commercial kits (Beijing Sino-UK of Biological Technology, Beijing, China) by method of colorimetry and an A6 semi-automatic biochemistry analyzer (Beijing Shiningsun Technology Inc., China) (H_2O_2, malondialdehyde, and NO) or a microplate reader (DR-200BS, Wuxi Hiwell-Diatek Instruments Co., Ltd., Wuxi, China) (ROS). Details about the method description of the measurement of H_2O_2, malondialdehyde, NO, and ROS refer to Luan et al. [31] and Geng et al. [32]. Moreover, the measurement method and principle of LPS (YJ600359) are the same as those of serum intake-related hormones. For H_2O_2, malondialdehyde, and NO, the intra-assay coefficients of variation were less than 7.3%; the inter-assay coefficients of variation were less than 10%. For ROS, the intra-assay coefficients of variation were less than 8.3%, the inter-assay coefficients of variation were less than 10%. For LPS, the intra-assay coefficients of variation were less than 10%; the inter-assay coefficients of variation were less than 15%.

Analysis of rumen fluid fermentation parameters

The pH value of the rumen fluid was measured instantly by a rapid pH analyzer (ST3100, Ohaus, NJ, USA) after collection. NH_3-N and lactic acid concentrations in rumen fluid were determined by a microplate reader (CMax Plus, Molecular Devices, SV, USA) according to the method of Barker and Summerson [33] and Broderick and Kang [34]. VFA was determined by gas

chromatograph (GC-1120, Sunny Hengping Instrument, Shanghai, China). Rumen fluid in a volume of 1 mL was mixed with 0.2 mL 25% (w/v) metaphosphoric acid solution containing 2-ethylbutyrate and centrifuged at $10,621 \times g/min$ for 15 min for VFA analysis.

Analysis of gastrointestinal tract bacterial diversity
High-throughput 16S rRNA gene sequencing
Total genomic DNA was extracted from rumen digesta, rumen fluid, and fecal samples using the TGuide S96 Magnetic Soil/Stool DNA Kit (Tiangen Biotech (Beijing) Co., Ltd.) according to the manufacturer's instructions. The quality and quantity of the extracted DNA were examined using electrophoresis on a 1.8% agarose gel, and DNA concentration and purity were determined with a NanoDrop 2000 UV–vis spectrophotometer (Thermo Scientific, Wilmington, USA). After quantitative measurement of DNA samples, a total of 48 samples were qualified. There were 16 samples of rumen digesta, rumen fluid, and feces, respectively (4 samples in each group [CON, C8, C10, C12]). The hypervariable region V3–V4 of the bacterial 16S rRNA gene was amplified with primer pairs 338F: 5'- ACTCCTACG GGAGGCAGCA-3' and 806R: 5'- GGACTACHVGGG TWTCTAAT-3'. PCR products were checked on agarose gel and purified through the Omega DNA purification kit (Omega Inc., Norcross, GA, USA). The purified PCR products were collected and the paired ends (2×250 bp) were performed on the Illumina Novaseq 6000 platform (Beijing Biomarker Technologies Co., Ltd., Beijing, China).

Sequence data bioinformatic analysis
Analysis of sequence data followed by Gao and Geng [35]. The qualified sequences with more than 97% similarity thresholds were allocated to one operational taxonomic unit (OTU) using USEARCH (v.10.0). Taxonomy annotation of the OTUs was performed based on the Naive Bayes classifier in QIIME2 (v.2020.6) using the SILVA database (release 138.1) with a confidence threshold of 70%. The Venn diagram, rarefaction curve, Shannon curves, and rank abundance curves (OTU level) were created using R (v.3.1.1), Mothur (v.1.22.2), and Python (v.2.7.8). QIIME2 (v.2020.6) was used to determine beta diversity to evaluate the degree of similarity of microbial communities from different samples, and three-dimensional principal component analysis (3D-PCA) and principal coordinates analysis (PCoA) for analysis of beta diversity. Species distribution histograms at phylum, family, and genus levels were created using Python (v.2.7.8).

Liquid chromatography-mass spectrometry (LC–MS) metabolomics and data analysis
The pretreatment of rumen fluid samples was referred to Gao and Geng [35]. The LC–MS system for metabolomics analysis is composed of Waters Acquity I-Class PLUS ultra-high performance liquid tandem Waters Xevo G2-XS QTof high-resolution mass spectrometer with a Waters Acquity UPLC HSS T3 column ($1.8~\mu m \times 2.1$ mm $\times 100$ mm). Samples were eluted in 0.1% formic acid aqueous solution (mobile phase A) and 0.1% formic acid acetonitrile (mobile phase B), injection volume 1 μL. A flow rate of 400 μL/min was applied to the elution gradient (A%: B%) 98:2 for 0–0.25 min, 2:98 at 10–13 min, and 98:2 at 13.1–15 min. In each data acquisition cycle, dual-channel data acquisition was performed on both low collision energy and high collision energy at the same time. The low collision energy is 2 V, the high collision energy range is 10–40 V, and the scanning frequency is 0.2 s for a mass spectrum. The parameters of the ESI ion source are as follows: capillary voltage: 2000 V (positive ion mode) or -1500 V (negative ion mode); cone voltage: 30 V; ion source temperature: 150℃; desolvent gas temperature 500℃; backflush gas flow rate: 50 L/h; desolventizing gas flow rate: 800 L/h. The raw data collected using MassLynx (v.4.2) is processed by Progenesis QI software for peak extraction, peak alignment, and other data processing operations. After normalizing the original peak area information with the total peak area, the follow-up analysis was performed. Metabolites were preliminarily identified in an in-house secondary mass spectrometry database using R (v.3.3.2), then compared with the Kyoto Encyclopedia of Genes and Genomes Database (KEGG), Human Metabolome Database (HMDB), and lipid maps to identify known metabolites.

The overall metabolic differences between groups of samples (including quality control samples) and the degree of variation between samples in the group were preliminarily understood by PCA. Additionally, partial least squares-discriminant analysis (PLS-DA) was applied to screen the variables related to the differences among groups.

Statistics and analysis
The experimental data (i.e., growth performance, serum intake-related hormones, antioxidant indices, oxidative stress indices, rumen fluid fermentation parameters, and nutrients apparent digestibility) were analyzed by the general linear model of SPSS 21.0 (SPSS Inc., Chicago, IL, USA) according to the model: $Y_{ijk} = \mu + D_i + A_j + S_k + e_{ijk}$, where Y_{ijk} is the observation of dependent variables, μ is the overall mean, D_i represents the fixed effect of treatment, A_j represents the fixed effect of period, S_k represents the random effect of bulls (animal ID), and e_{ijk} is the residual error of the model. $P < 0.05$ means a significant difference, while differences with $P > 0.05$ to $P < 0.10$ are considered as a trend.

Moreover, for the analysis of alpha diversity indices (ACE, Chao1, Simpson, Shannon, and coverage)

of gastrointestinal tract bacteria, the raw data were extracted and analyzed by the general linear model of SPSS 21.0 (SPSS Inc., Chicago, IL, USA). The raw data of relative abundance (more than 0.1%) of bacteria were extracted, and species abundance data between groups were analyzed by the general linear model of SPSS 21.0 (SPSS Inc., Chicago, IL, USA), and species were screened according to *P*-value. The raw data of metabolite abundance were also extracted to determine the significant differences of metabolites by the general linear model of SPSS 21.0 (SPSS Inc., Chicago, IL, USA) and plotted KEGG classification maps of significant differential metabolites. The parameter threshold for differential metabolite screening was set at VIP (variable importance in the projection) > 1, and $P < 0.05$. Before the data analysis, the alpha diversity indices, species abundance, and metabolite abundance were not performed data transformation, and a Shapiro–Wilk normality test found that these data conform to the normality distribution.

Partial correlation matrix (groups are used as control variables) and *P*-value matrix were calculated using SPSS 21.0 (SPSS Inc., Chicago, IL, USA) for the differential conventional indices (i.e., DMI, GOAT, TG, AG, Orexins, GHS-PX, MDA, NO, LPS), differential gastrointestinal tract bacteria (all levels), and differential metabolites, plotting the correlation heat maps. Correlation coefficients ranging from −1 to +1 represented strong negative correlations to strong positive correlations. Correlation $P < 0.05$ (*) and 0.01 (**) represented significant and extremely significant correlations, respectively.

Results
Growth performance
The effects of MCT supplementation on the growth performance of finishing bulls are shown in Table 2. The average initial BW of four bulls was 367 ± 32 kg, and the average final BW of four bulls was 519 ± 39 kg. Compared with the CON group, there were no significant effects on ADG and feed conversion efficiency of finishing bulls

among the C8, C10, and C12 groups ($P > 0.05$), but the C10 group tended to increase the DMI of finishing bulls ($P = 0.069$).

eSerum intake-related hormones
The effects of MCT supplementation on serum intake-related hormones of finishing bulls are shown in Table 3. Compared with the CON group, the C8 and C10 groups significantly increased the concentration of GOAT in the serum of finishing bulls ($P < 0.05$). The C10 group significantly increased the concentrations of TG and AG in the serum of finishing bulls compared with the other three groups ($P < 0.05$). The C10 group significantly increased the AG/TG and the concentration of orexins in the serum of finishing bulls compared with the CON and C8 groups ($P < 0.05$). There were no significant effects on serum DAG, GH, INS, and CRH of finishing bulls among the four treatment groups ($P > 0.05$).

Serum antioxidant and oxidative stress indices, and endotoxin LPS
Effects of MCT supplementation on serum antioxidant and oxidative stress indices, and endotoxin LPS of finishing bulls are shown in Table 4. Compared with the CON group, the C10 and C12 groups significantly increased the concentrations of TAOC, GHS-PX, and NO in the serum of finishing bulls ($P < 0.05$). The C12 group significantly increased the concentration of SOD in the serum of finishing bulls compared with the CON and C8 groups ($P < 0.05$). Compared with the CON group, the concentrations of catalase and GR significantly increased in the serum of finishing bulls among the other three groups ($P < 0.05$). The C10 and C12 groups significantly decreased the concentrations of H_2O_2, malondialdehyde, and ROS in the serum of finishing bulls compared with the CON group ($P < 0.05$). The C10 group significantly decreased the concentrations of LPS in the serum of finishing bulls compared with the CON and C8 groups ($P < 0.05$).

Table 2 Effects of medium-chain fatty acid triglycerides supplementation on growth performance of finishing bulls

Items[a]	Dietary treatment[b]				SEM[c]	*P*-value
	CON	C8	C10	C12		
ADG (kg/day)	1.39	1.26	1.52	1.25	0.167	0.393
DMI (kg/day)	9.73	9.98	10.51	9.50	0.304	0.069
Feed conversion efficiency (feed: gain)	7.15	8.05	7.15	7.62	0.723	0.574

[a] *ADG* average daily gain, *DMI* dry matter intake

[b] CON, the control group cattle fed control diets; C8, the treatment group cattle fed control diets containing glycerol monocaprylin; C10, the treatment group cattle fed control diets containing glycerol monodecanoate; C12, the treatment group cattle fed control diets containing glycerol monolaurate

[c] *SEM* standard error of the means

Table 3 Effects of medium-chain fatty acid triglycerides supplementation on serum intake-related hormones of finishing bulls

Items[c]	Dietary treatment[d]				SEM[e]	*P*-value
	CON	C8	C10	C12		
GOAT (ng/mL)	2.471[b]	2.916[a]	3.000[a]	2.818[ab]	0.183	0.043
TG (pg/mL)	139.231[b]	156.649[b]	188.471[a]	158.082[b]	14.244	0.018
AG (pg/mL)	37.635[b]	30.785[b]	66.714[a]	43.093[b]	6.875	0.001
DAG (pg/mL)	101.596	125.864	121.757	114.989	13.624	0.327
AG/TG	0.250[b]	0.190[b]	0.353[a]	0.272[ab]	0.040	0.005
GH (ng/mL)	2.435	2.435	2.538	2.358	0.102	0.392
INS (mIU/L)	4.472	5.017	5.460	4.917	0.387	0.118
Orexins (ng/mL)	1.312[b]	1.254[b]	1.538[a]	1.385[ab]	0.077	0.008
CRH (pg/mL)	11.648	10.800	10.170	11.796	1.210	0.511

[a,b] Means bearing different superscripts in the same row differ significantly (*P*-value < 0.05)

[c] *GOAT* ghrelin O-acyl transferase, *TG* total ghrelin, *AG* acyl ghrelin, *DAG* des-acyl ghrelin, *GH* growth hormone, *INS* insulin, *CRH* corticotropin-releasing hormone

[d] *CON*, the control group cattle fed control diets, *C8* the treatment group cattle fed control diets containing glycerol monocaprylin, *C10* the treatment group cattle fed control diets containing glycerol monodecanoate, *C12* the treatment group cattle fed control diets containing glycerol monolaurate

[e] *SEM* standard error of the means

Table 4 Effects of medium-chain fatty acid triglycerides supplementation on serum antioxidant and oxidative stress indices, and endotoxin LPS of finishing bulls

Items[e]	Dietary treatment[f]				SEM[g]	*P*-value
	CON	C8	C10	C12		
TAOC (U/mL)	7.50[c]	8.21[bc]	9.13[ab]	9.87[a]	0.607	0.032
SOD (U/mL)	49.49[b]	57.12[b]	68.22[ab]	82.72[a]	7.855	0.024
Catalase (U/mL)	33.42[d]	41.99[c]	46.23[b]	53.69[a]	1.124	<0.001
GHS-PX (U/mL)	115.78[b]	142.97[b]	205.95[a]	220.70[a]	15.508	0.001
GR (U/L)	3.65[c]	5.21[b]	6.04[a]	6.72[a]	0.326	<0.001
H_2O_2 (U/mL)	56.08[a]	50.35[ab]	44.60[bc]	39.30[c]	3.025	0.007
Malondialdehyde (nmol/mL)	4.14[a]	3.66[b]	3.17[c]	2.32[d]	0.189	<0.001
NO (µmol/L)	49.75[c]	49.76[c]	55.42[b]	59.65[a]	0.817	<0.001
ROS (Fluorescence intensity/mL)	1150.09[a]	986.48[b]	686.51[c]	511.23[d]	44.987	<0.001
LPS (EU/mL)	1.131[a]	1.040[a]	0.698[b]	0.868[ab]	0.145	0.033

[a−d] Means bearing different superscripts in the same row differ significantly (*P*-value < 0.05)

[e] *T-AOC* total antioxidant capacity, *SOD* superoxide dismutase, *GSH-PX* glutathione peroxidase, *GR* glutathione reductase, *H_2O_2* hydrogen peroxide, *NO* nitric oxide, *ROS* reactive oxygen species, *LPS* lipopolysaccharides

[f] *CON* the control group cattle fed control diets, *C8*, the treatment group cattle fed control diets containing glycerol monocaprylin; *C10*, the treatment group cattle fed control diets containing glycerol monodecanoate; *C12*, the treatment group cattle fed control diets containing glycerol monolaurate

[g] *SEM*, standard error of the means

Rumen fermentation parameters

The effects of MCT supplementation on rumen fermentation parameters of finishing bulls are shown in Table S1. Compared with the CON group, the C10 group significantly increased the pH in the rumen fluid of finishing bulls (*P* < 0.05), the C10 and C12 groups significantly decreased the concentrations of NH_3-N in the rumen fluid of finishing bulls (*P* < 0.05), and the C8 and C10 groups significantly decreased the concentrations of

lactic acid in the rumen fluid of finishing bulls (*P* < 0.05). There were no significant effects on the concentrations of LPS, TVFA, individual VFA, and A/P in the rumen fluid of finishing bulls among the four treatment groups (*P* > 0.05).

Diversity of gastrointestinal tract bacteria

Each sample produced at least 67,815 clean reads, with an average of 85,989 ± 23,684 clean reads (Supplementary

Table 3). The results of the OTUs were used to create histograms (Supplementary Fig. 1 A, B, C) and Venn diagrams (Fig. 1A, B, C), showing the numbers of gastrointestinal tract microbes and variances of different groups of finishing bulls.

Alpha diversity analysis showed that the rarefaction curves of each group first raised sharply and then tended to be gentle (Supplementary Fig. 2 A, D, G), the Shannon curve of each group had a plateau period (Supplementary Fig. 2 B, E, H), and the rank abundance curves were relatively wide (Supplementary Fig. 2 C, F, I), indicating that the sampling was sufficient. To measure the diversity within the microbial community of each sample, we compared the alpha diversity of the gastrointestinal tract bacteria in all samples by calculating the ACE, Chao1, Simpson, and Shannon indices (Table 5). The coverage of all samples in this experiment was higher than 99.9%, indicating that the sequencing coverage was sufficient for further analysis. For rumen digesta, the Shannon index of the C12 group was significantly decreased compared with the other three groups ($P < 0.05$), but there were no significant effects on ACE, Chao 1, and Simpson indices among the four treatment groups ($P > 0.05$). For rumen fluid, the ACE and Chao 1 indices of the C8 group were significantly increased compared with the CON and C12 groups ($P < 0.05$), Shannon index of the C12 group was significantly decreased compared with the other three groups ($P < 0.05$), but there were no significant effects on Simpson index among four treatment groups ($P > 0.05$). For feces, The alpha diversity was not significantly different among the four treatment groups ($P > 0.05$).

Beta diversity was used to compare the microbial community in different samples by calculating the 3D-PCA and PCoA. As shown in Fig. 2A, B, C, the 3D-PCA showed that the points representing gastrointestinal tract microorganisms in the four treatment groups were not independent, which indicated that the community structure of gastrointestinal tract bacteria had no difference among treatments. In the PCoA plots (Fig. 2D, E, F), the points representing gastrointestinal tract bacteria in four treatment groups were clustered in separate quadrants.

Composition of gastrointestinal tract bacteria

At the phylum level, *Firmicutes* and *Bacteroidota* were the dominant phylum among the four treatment groups and occupied more than 80% of the microbial community (Supplementary Fig. 3 A, D, G). At the family level, for rumen digesta and rumen fluid, the bacteria with relative abundance greater than 10% were *Prevotellaceae* and *Lachnospiraceae*, while for feces were *Lachnospiraceae*, *Oscillospiraceae* and, *Rikenellaceae* (Supplementary Fig. 3 B, E, H). At the genus level, for rumen digesta, the bacteria with relative abundance in the top 5 were *Prevotella*, *uncultured_rumen_bacterium*, *Rikenellaceae_RC9_gut_group*, *Succiniclasticum*, and *Saccharofermentans* (Supplementary Fig. 3C); for rumen fluid, the bacteria with relative abundance in the top 5 were *Prevotella*, *uncultured_rumen_bacterium*, *Rikenellaceae_RC9_gut_group*, *NK4A214_group*, and *Succiniclasticum* (Supplementary Fig. 3 F); for feces, the bacteria with relative abundance in the top 5 were *unclassified_Lachnospiraceae*, *UCG_005*, *unclassified_UCG_010*, *Rikenellaceae_RC9_gut_group*, and *Succinivibrio* (Supplementary Fig. 3I).

Furthermore, the significantly different gastrointestinal tract bacteria among the four treatment groups were analyzed by the general linear model. As shown in Table 6, compared with the CON group, there were 5 (including 2 order level, 2 family level, and 1 genus level), 14 (including 1 phylum level, 2 class level, 2 order level, 4 family level, and 5 genus level) and 6 (including 1 phylum level, 1 class level, 1 order level, 1 family level, and 2 genus level) significantly different bacteria in the rumen digesta
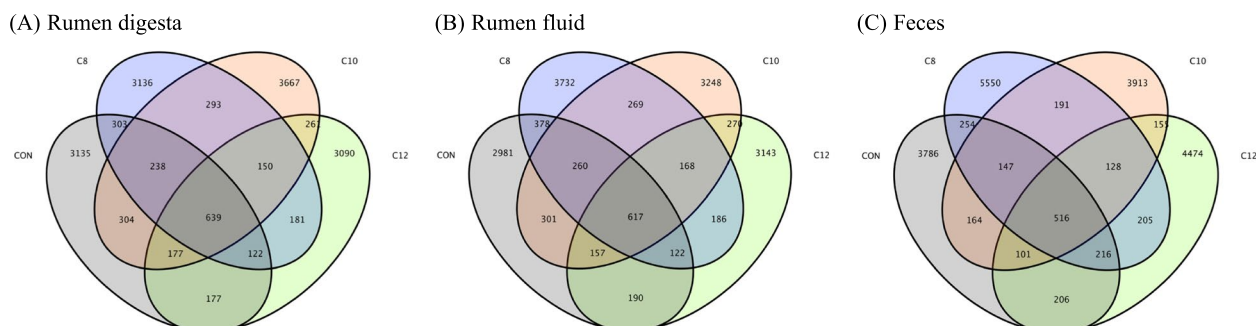

Fig. 1 The number of operational taxonomic units (OTUs) of gastrointestinal tract bacteria in finishing bulls. Venn diagram (**A**) rumen digesta, (**B**) rumen fluid, and (**C**) feces. CON, the control group cattle fed control diets (*n* = 4); C8, the treatment group cattle fed control diets containing glycerol monocaprylin (*n*= 4); C10, the treatment group cattle fed control diets containing glycerol monodecanoate (*n* = 4); C12, the treatment group cattle fed control diets containing glycerol monolaurate (*n* = 4)

Table 5 Effects of medium-chain fatty acid triglycerides supplementation on alpha diversity of gastrointestinal tract bacteria of finishing bulls

Alpha diversity indices	Dietary treatment[d]				SEM[e]	*P*-value
	CON	C8	C10	C12		
Rumen digesta						
ACE	1598.27	1575.59	1807.17	1480.44	107.443	0.100
Chao1	1583.60	1561.65	1791.29	1467.60	106.676	0.101
Simpson	0.9963	0.9961	0.9966	0.9952	0.00061	0.256
Shannon	9.162[a]	9.149[a]	9.211[a]	8.953[b]	0.066	0.030
Coverage	0.9992	0.9993	0.9993	0.9993	0.00012	0.689
Rumen fluid						
ACE	1570.03[bc]	1745.06[a]	1647.51[ab]	1485.83[c]	40.029	0.003
Chao1	1559.93[bc]	1730.22[a]	1635.17[ab]	1472.76[c]	40.696	0.004
Simpson	0.9972	0.9968	0.9970	0.9944	0.00132	0.213
Shannon	9.344[a]	9.335[a]	9.312[a]	8.889[b]	0.140	0.045
Coverage	0.9994	0.9991	0.9993	0.9993	0.00011	0.284
Feces						
ACE	1588.49	2098.54	1581.11	1728.13	317.253	0.396
Chao1	1581.93	2089.61	1573.65	1721.08	317.524	0.400
Simpson	0.9950	0.9925	0.9948	0.9934	0.001	0.291
Shannon	8.869	8.853	8.822	8.759	0.172	0.918
Coverage	0.9997	0.9996	0.9995	0.9997	0.00009	0.497

[a] −[c]Means bearing different superscripts in the same row differ significantly (*P*-value < 0.05)

[d] *CON* the control group cattle fed control diets (*n* = 4); C8, the treatment group cattle fed control diets containing glycerol monocaprylin (*n* = 4); C10, the treatment group cattle fed control diets containing glycerol monodecanoate (*n* = 4); C12, the treatment group cattle fed control diets containing glycerol monolaurate (*n* = 4)

[e] *SEM* standard error of the means

in the C8, C10, and C12 groups, respectively; there were 3 (including 1 class level, 1 order level, and 1 genus level), 10 (including 1 phylum level, 1 class level, 3 order level, 2 family level, and 3 genus level) and 5 (including 1 order level, 2 family level, and 2 genus level) significantly different bacteria in the rumen fluid in the C8, C10, and C12 groups, respectively; but only one differential bacteria at the genus level (g_*Ruminococcus*) was observed in the feces of the four treatment groups (*P* < 0.05).

Analysis of rumen metabolites

In this study, based on the LC-QTOF platform, 16 rumen fluid samples were analyzed qualitatively and quantitatively. A total of 13,655 peaks were detected, of which 3484 metabolites were annotated. Unsupervised multivariate statistical analysis (PCA) of all samples provided an overview of rumen metabolites in the four groups (Fig. 3A, B). Differential rumen metabolites across the groups were clearly distinguished using a supervised discriminant analysis method (PLS-DA) (Fig. 3C, D).

Furthermore, the significantly different metabolites in rumen fluid among the four treatment groups were analyzed by the general linear model. Compared with the CON group, the C8 group significantly up-regulated

S-(2-hydroxyethyl)glutathione, bis(glutathionyl)spermine, significantly down-regulated 25-hydroxyvitamin D3 under positive ionization mode; the C10 group significantly up-regulated 6 metabolites (e.g., L-threonine, 22-hydroxydocosanoic acid, cortisol etc.), significantly down-regulated 8 metabolites (e.g., S-(2-hydroxyethyl) glutathione, (-)-jasmonic acid, (S)-2-aceto-2-hydroxybutanoate, etc.) under positive ionization mode; the C12 group significantly up-regulated 7 metabolites (e.g., imidazole-4-acetate, linoleic acid, 3-(3'-methylthio) propylmalic acid, etc.), significantly down-regulated 8 metabolites (e.g., S-(2-hydroxyethyl)glutathione, iso-maltotriose, 3-methylbutanoic acid, etc.) under positive ionization mode (Supplementary Table 4). Compared with the CON group, the C8 group significantly up-regulated gamma-hydroxy-3-pyridinebutanoate, significantly down-regulated dopamine quinone, 12-Oxo-9(Z)-dodecenoic acid under negative ionization mode; the C10 group significantly up-regulated 7 metabolites (e.g., creatine, succinic acid, 11(R)-HPETE, etc.), significantly down-regulated 4 metabolites (e.g., L-dopachrome, dopamine quinone, docosanedioate, etc.) under negative ionization mode; the C12 group significantly up-regulated 10 metabolites (e.g., creatine, 3-hexenal, 11(R)-HPETE, etc.),
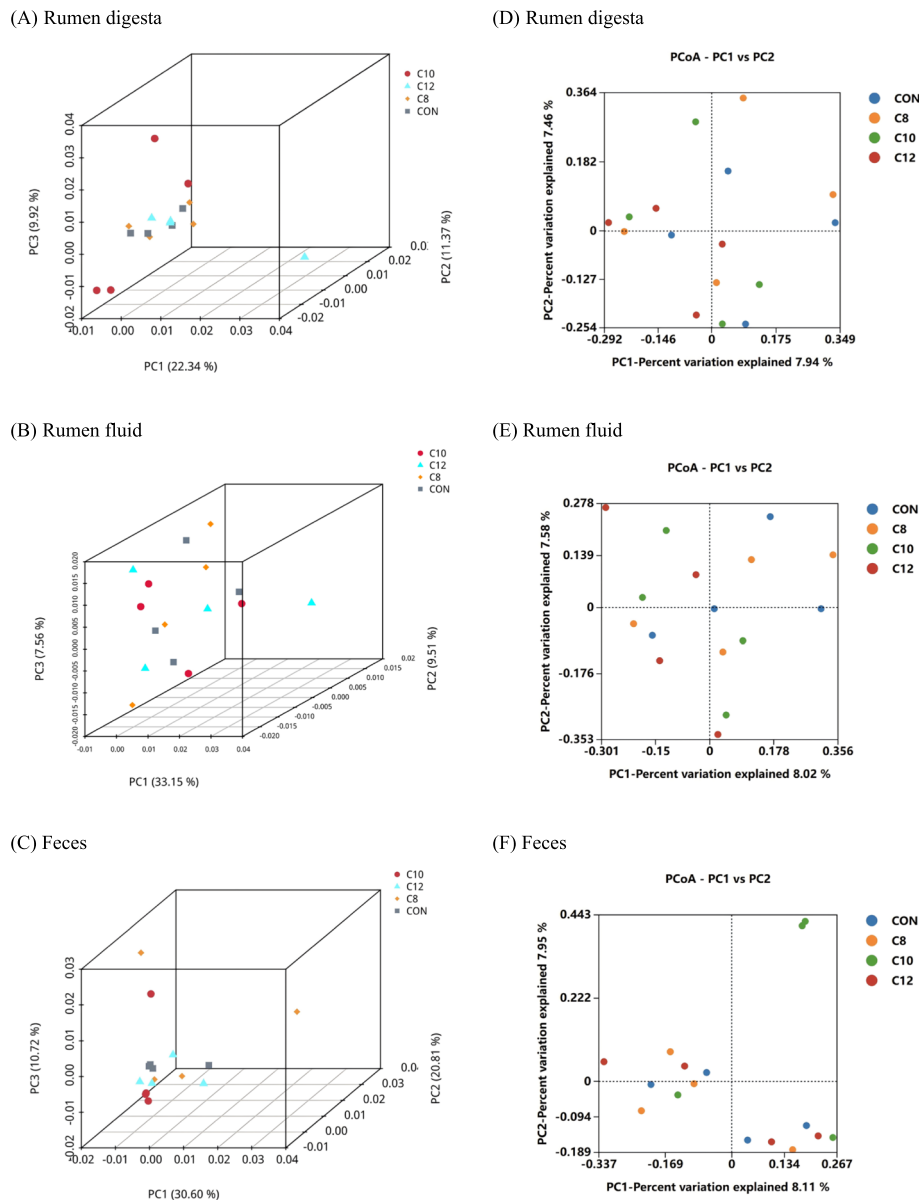
Fig. 2 Beta diversity analysis of gastrointestinal tract bacteria. Three-dimensional principal component analysis (3D-PCA) (**A**) rumen digesta, (**B**) rumen fluid, and (**C**) feces, and principal coordinates analysis (PCoA) (**D**) rumen digesta, (**E**) rumen fluid, and (**F**) feces. *CON* the control group cattle fed control diets ($n=4$); C8, the treatment group cattle fed control diets containing glycerol monocaprylin ($n=4$); C10, the treatment group cattle fed control diets containing glycerol monodecanoate ($n=4$); C12, the treatment group cattle fed control diets containing glycerol monolaurate ($n=4$)

significantly down-regulated 5 metabolites (e.g., dopamine quinone, docosanedioate, zidovudine, etc.) under negative ionization mode (Supplementary Table 5).

KEGG classification maps showed that under positive ionization mode, the differential metabolite pathway was rich in amino acid metabolism, biosynthesis of other secondary metabolites, cancer: overview, cancer: specific types, carbohydrate metabolism, digestive system, endocrine and metabolic disease, endocrine system, energy metabolism, excretory system, glycan biosynthesis and metabolism, infectious disease: bacterial, lipid metabolism, membrane transport, metabolism of cofactors and vitamins, metabolism of other amino acids, nervous system, nucleotide metabolism, sensory system, signaling molecules and interaction, translation, and xenobiotics biodegradation and metabolism (Supplementary Fig. 4A). Besides, under negative ionization mode, the differential metabolite pathway was rich in amino acid

Table 6 Effects of medium-chain fatty acid triglycerides supplementation on significantly differential gastrointestinal tract bacteria composition of finishing bulls

Items[d]	Dietary treatment[e]				SEM[f]	*P*-value
	CON	C8	C10	C12		
Rumen digesta (%, relative abundance)						
p_Proteobacteria	0.0116[b]	0.0148[b]	0.0110[b]	0.0460[a]	0.0092	0.023
p_Spirochaetota	0.0112[ab]	0.0126[a]	0.0035[c]	0.0100[b]	0.0009	<0.001
c_Gammaproteobacteria	0.0107[b]	0.0136[b]	0.0103[b]	0.0454[a]	0.0089	0.020
c_Negativicutes	0.0595[b]	0.0594[b]	0.0697[a]	0.0564[b]	0.0031	0.021
c_Spirochaetota	0.0112[ab]	0.0126[a]	0.0035[c]	0.0100[b]	0.0009	<0.001
o_Enterobacterales	0.0095[b]	0.0128[b]	0.0092[b]	0.0445[a]	0.0089	0.019
o_Oscillospirales	0.2041[bc]	0.2190[a]	0.2158[ab]	0.1916[c]	0.0059	0.012
o_RF39	0.0023[b]	0.0036[a]	0.0009[c]	0.0020[b]	0.0003	<0.001
o_Spirochaetales	0.0112[ab]	0.0126[a]	0.0035[c]	0.0100[b]	0.0009	<0.001
f_Bacteroidales_RF16_group	0.0012[b]	0.0016[b]	0.0027[a]	0.0017[b]	0.0004	0.047
f_Oscillospiraceae	0.0679[b]	0.0790[ab]	0.0903[a]	0.0679[b]	0.0066	0.039
f_Prevotellaceae	0.2061[a]	0.1687[b]	0.2079[a]	0.1827[ab]	0.0118	0.042
f_Rikenellaceae	0.0659[b]	0.0787[a]	0.0536[c]	0.0748[ab]	0.0048	0.007
f_Spirochaetaceae	0.0112[ab]	0.0126[a]	0.0035[c]	0.0100[b]	0.0009	<0.001
f_Succinivibrionaceae	0.0092[b]	0.0126[b]	0.0088[b]	0.0442[a]	0.0089	0.019
g_Christensenellaceae_R_7_group	0.0239[b]	0.0272[a]	0.0288[a]	0.0267[ab]	0.0012	0.038
g_Desulfovibrio	0.0019[b]	0.0028[ab]	0.0032[a]	0.0025[ab]	0.00035	0.047
g_Lachnospiraceae_UCG_006	0.0020[b]	0.0020[b]	0.0024[a]	0.0025[a]	0.00016	0.040
g_Lachnospiraceae_XPB1014_group	0.0172[a]	0.0153[a]	0.0163[a]	0.0071[b]	0.00128	<0.001
g_NK4A214_group	0.0386[b]	0.0455[ab]	0.0532[a]	0.0382[b]	0.0038	0.023
g_Rikenellaceae_RC9_gut_group	0.0610[ab]	0.0730[a]	0.0495[b]	0.0701[a]	0.0051	0.014
g_Treponema	0.0110[ab]	0.0125[a]	0.0034[c]	0.0098[b]	0.00089	<0.001
Rumen fluid (%, relative abundance)						
p_Fibrobacterota	0.0026[bc]	0.0039[ab]	0.0054[a]	0.0018[c]	0.00079	0.016
p_Synergistota	0.0012[ab]	0.0016[a]	0.0013[ab]	0.0008[b]	0.00021	0.033
c_Fibrobacterota	0.0026[bc]	0.0039[ab]	0.0054[a]	0.0018[c]	0.00079	0.016
c_Gracilibacteria	0.0095[b]	0.0175[a]	0.0091[b]	0.0063[b]	0.0024	0.016
c_Synergistota	0.0012[ab]	0.0016[a]	0.0013[ab]	0.0008[b]	0.00021	0.033
o_Absconditabacteriales__SR1	0.0095[b]	0.0175[a]	0.0091[b]	0.0063[b]	0.0024	0.016
o_Erysipelotrichales	0.0101[ab]	0.0087[bc]	0.0078[c]	0.0116[a]	0.0008	0.012
o_Fibrobacterales	0.0026[bc]	0.0039[ab]	0.0054[a]	0.0018[c]	0.00079	0.016
o_Peptococcales	0.0013[a]	0.0010[ab]	0.0009[b]	0.0005[c]	0.0002	0.009
o_Synergistales	0.0012[ab]	0.0016[a]	0.0013[ab]	0.0008[b]	0.00021	0.033
f_Fibrobacteraceae	0.0026[bc]	0.0039[ab]	0.0054[a]	0.0018[c]	0.0008	0.016
f_Peptococcaceae	0.0013[a]	0.0010[ab]	0.0009[b]	0.0005[c]	0.0002	0.009
f_Prevotellaceae	0.2115[a]	0.1949[a]	0.2007[a]	0.1633[b]	0.0128	0.040
f_Synergistaceae	0.0012[ab]	0.0016[a]	0.0013[ab]	0.0008[b]	0.0002	0.033
g_Anaerovorax	0.0062[a]	0.0060[ab]	0.0045[bc]	0.0042[c]	0.00065	0.047
g_Fibrobacter	0.0026[bc]	0.0039[ab]	0.0054[a]	0.0018[c]	0.00079	0.016
g_Prevotellaceae_NK3B31_group	0.0122[a]	0.0061[b]	0.0069[b]	0.0036[b]	0.00207	0.029
Feces (%, relative abundance)						
g_Ruminococcus	0.0043[a]	0.0042[a]	0.0028[b]	0.0035[ab]	0.00043	0.043

[a –c]Means bearing different superscripts in the same row differ significantly (*P*-value < 0.05)

[d] *p* phylum, *c* class, *o* order, *f* family, *g* genus

[e] *CON* the control group cattle fed control diets (*n*=4); C8, the treatment group cattle fed control diets containing glycerol monocaprylin (*n*=4); C10, the treatment group cattle fed control diets containing glycerol monodecanoate (*n*=4); C12, the treatment group cattle fed control diets containing glycerol monolaurate (*n*=4)
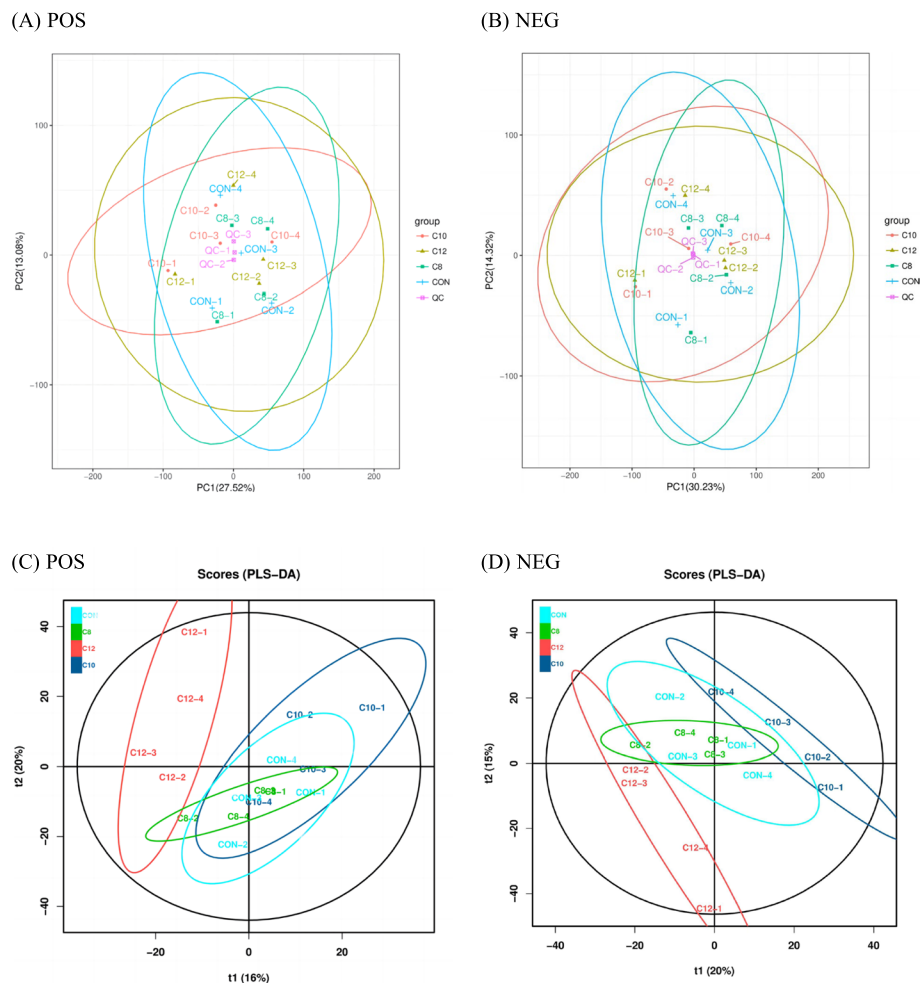
[f] *SEM* standard error of the means

Fig. 3 Principal component analysis (PCA) POS (positive ionization mode) (**A**), NEG (negative ionization mode) (**B**), and partial least squares discriminant analysis (PLS-DA) POS (positive ionization mode) (**C**), NEG (negative ionization mode) (**D**) of rumen fluid metabolites. QC quality control samples. *CON* the control group cattle fed control diets (*n* = 4); C8, the treatment group cattle fed control diets containing glycerol monocaprylin (*n* = 4); C10, the treatment group cattle fed control diets containing glycerol monodecanoate (*n* = 4); C12, the treatment group cattle fed control diets containing glycerol monolaurate (*n* = 4)

metabolism, biosynthesis of other secondary metabolites, cancer: overview, carbohydrate metabolism, digestive system, endocrine system, energy metabolism, infectious disease: bacterial, lipid metabolism, membrane transport, metabolism of cofactors and vitamins, metabolism of terpenoids and polyketides, nervous system, neurodegenerative disease, nucleotide metabolism, signal transduction, and xenobiotics biodegradation and metabolism (Supplementary Fig. 4 B).

Correlation analysis of differential conventional indicators, differential gastrointestinal bacteria, and differential metabolites

Correlation of differential conventional indices and differential gastrointestinal tract bacteria (all levels) showed that DMI and GOAT were positively associated with rumen digesta g_*Desulfovibrio*, rumen fluid p_*Fibrobacterota*, c_*Fibrobacteria*, o_*Fibrobacterales*, f_*Fibrobacteraceae*, and g_*Fibrobacter*. AG was positively associated with rumen digesta o_*Oscillospirales*, f_*Prevotellaceae*, g_*Christensenellaceae_R_7_group*, rumen fluid p_*Fibrobacterota*, c_*Fibrobacteria*, o_*Fibrobacterales*, f_*Fibrobacteraceae*, f_*Prevotellaceae*, and g_*Fibrobacter*, and negatively associated with rumen digesta p_*Proteobacteria*, p_*Spirochaetota*, c_*Gammaproteobacteria*, c_*Spirochaetia*, o_*Enterobacterales*, o_*Spirochaetales*, f_*Spirochaetaceae*, f_*Succinivibrionaceae*, and g_*Treponema*. LPS was positively associated with ruemen fluid p_*Synergistota*, c_*Gracilibacteria*, c_*Synergistia*, o_*Absconditabacteriales__SR1*, o_*Synergistales*, f_*Synergistaceae*,

and negatively associated with rumen fluid o_*Erysipelotrichales* (Fig. 4A).

Correlation of differential conventional indices and differential metabolites showed that, under positive ionization mode, DMI and GOAT were positively associated with L-threonine, 3'-phosphoadenylyl sulfate, and 3-methylbutanoic acid. AG was positively associated with L-threonine, isomaltotriose, and negatively associated with fluvastatin. LPS was positively associated with cytidine, 3-beta-D-galactosyl-sn-glycerol, and negatively associated with (2R,3R)-3-methylglutamyl-5-semialdehyde-N6-lysine (Fig. 4B).Under negative ionization mode, DMI and GOAT were positively associated with dTDP-4-oxo-alpha-D-xylose, P1,P4-Bis(5'-uridyl) tetraphosphat, gamma-hydroxy-3-pyridinebutanoate, and docosanedioate. AG was negatively associated with L-homophenylalanine. Orexins was positively associated with Maleylpyruvate. LPS was positively associated with succinic acid and negatively associated with methylimidazole acetaldehyde (Fig. 4C).

Besides, the results of the correlation of differential gastrointestinal tract bacteria (all levels) and differential metabolites are shown in Fig. 4D and E.

Discussion

As a safe and efficient additive, MCTs have been shown to have a positive effect on growth performance and health status in animals [6–8, 36, 37], but the reports of direct addition of individual MCT (GMC/GMD/GML) are limited. Murayama et al. [38] found that MCTs (C8:0 and C10:0) supplementation in the milk replacer may improve the growth performance and gut health of dairy calves. However, some studies have shown that supplementation of glycerides of MCFAs (C8:0, C10:0, and C12:0) had no effects on the health and growth of calves [39], and even decreased DMI when supplementing coconut oil (~ 75% MCFAs) [40] or lauric acid [41]. In the present study, compared with the CON group, there were no significant effects on ADG or feed conversion efficiency of finishing bulls among the treatment groups, similar to the results of Masmeijer et al. [39]. However, compared with the CON group, the C10 group tended to increase the DMI of finishing bulls (*P* = 0.069), indicating that the addition of GMD may have a positive effect on

feed intake of finishing bulls. The differences in growth performance of livestock responses to MCTs supplementation in different studies may be due to differences in the types and doses of MCT, age and breed, animal stress, or supplementation period in different trials.

At present, although the mechanism of MCTs on the feed intake of beef cattle has not been fully elucidated, it may be related to MCFAs' involvement in the acylation modification of ghrelin [42]. Ghrelin is a 28-amino acid acylated peptide mainly synthesized and secreted by X/A-like cells of the gastric fundus mucosa, and it acts as a brain–gut peptide hormone that stimulates feed intake [43, 44]. It mainly exists in the form of acylated and deacylated in ruminants, and AG is its active form, which has been proved that stimulate animal appetite through orexins [12, 45]. Studies have shown that MCFAs mainly act on the stomach and can participate in the acylation of ghrelin in the stomach, which is achieved by GOAT [46]. Not only the amount of fatty acids but also the length of fatty acids can impact the efficiency of ghrelin acylation, and fatty acids derived from C6:0 to C14:0 may affect the acylation process of ghrelin [47]. In the present study, compared with the CON group, the C8 and C10 groups significantly increased the concentration of GOAT in the serum of finishing bulls, the C10 group significantly increased the AG/TG, and the concentrations of TG, AG, and orexins in the serum of finishing bulls. Studies have shown that supplementation of MCFAs-Ca (containing 25% C8:0, 20% C10:0, and 55% C12:0) in the diet could significantly increase the level of AG in dairy cows [13], which is consistent with the result of AG in blood after feeding mice with MCFAs (mainly C6:0, C8:0, and C10:0) [42]. Our results are consistent with the above results. It was reported that exogenous AG can increase the DMI in beef cattle [48], and AG concentration and AG/TG were positively correlated with DMI in beef cattle [49]. This may be the reason for the increasing trend compared to the CON group of DMI in the C10 group.

It has been reported that the high-concentrate diet mode can lead to the imbalance of oxidation and antioxidation in the ruminants, which makes the animals in an oxidative stress state and causes oxidative damage [50]. At present, some studies have shown that MCFAs/ MCTs can improve oxidative stress in organs or tissues of

(See figure on next page.)

Fig. 4 Correlation analysis between significantly differential conventional indices and differential gastrointestinal tract bacteria (**A**); significantly differential conventional indices and differential metabolites under positive ionization mode (**B**), and negative ionization mode (**C**); significantly differential gastrointestinal tract bacteria and differential metabolites under positive ionization mode (**D**), and negative ionization mode (**E**). RD rumen digesta, RF rumen fluid, F feces. POS positive ionization mode, NEG negative ionization mode. Correlation coefficients ranging from − 1 to + 1 represented strong negative correlations to strong positive correlations. Correlation *P* < 0.05 (*) and 0.01 (**) represented significant and extremely significant correlations, respectively. In this analysis, the bacteria with a relative abundance of 0.1% or higher were considered; rumen metabolites VIP (variable importance in the projection) > 1, and *P* < 0.05 were considered significant
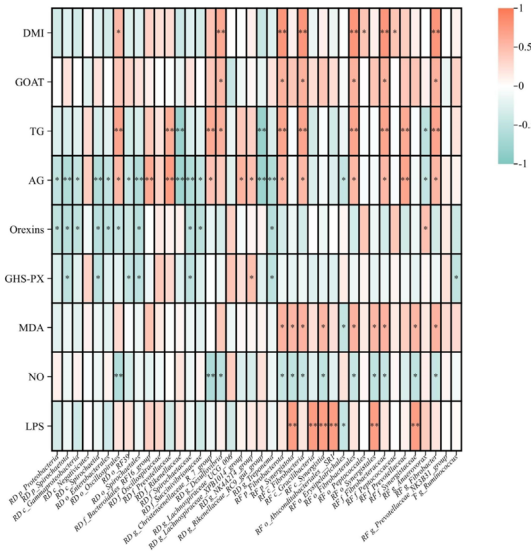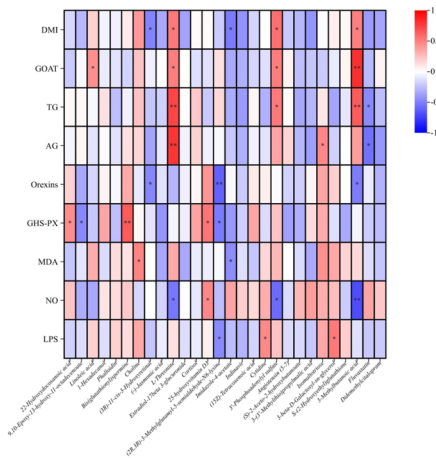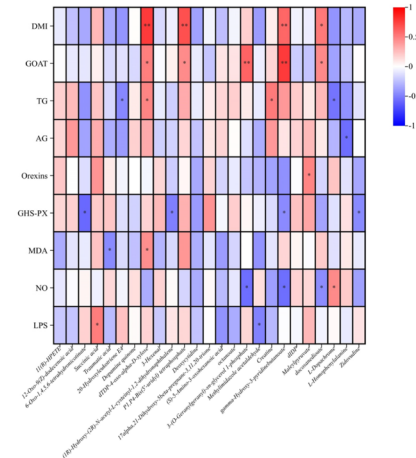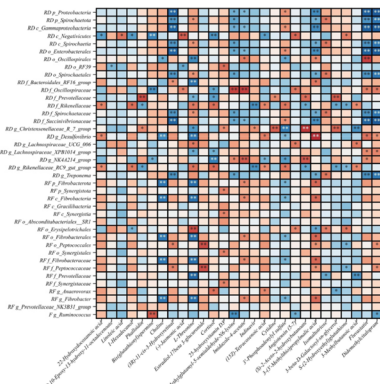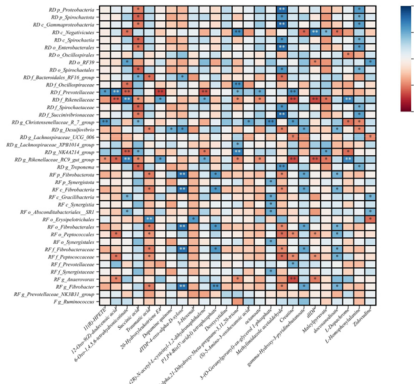
Fig. 4 (See legend on previous page.)

mice/pigs [9, 51, 52], but there are relatively few reports in the ruminants. In the present study, compared with the CON group, the C10 and C12 groups significantly increased the concentrations of TAOC, catalase, GHS-PX, GR, and NO in the serum of finishing bulls, while the C10 group significantly decreased the concentrations of H_2O_2, malondialdehyde, ROS, and LPS. This indicated that supplementation of GMD/GML may improve oxidative stress in finishing bulls under high-concentrate feeding conditions, but the mechanism is still unclear.

Słupecka et al. [14] showed that the intestinal administration of AG could enhance the autophagy of piglet intestinal epithelial cells, promote the renewal of intestinal mucosa in newborn piglets, and protect the intestine. Ghrelin can improve $Na_2S_2O_5$-induced apoptosis and oxidative stress-induced gastric mucosal injury in rats [53]. Previous and our studies have found that MCFAs have a stimulating effect on the secretion of ghrelin in the ruminants [13, 30], which provides a new idea to further reveal the mechanism of MCTs improving oxidative stress in beef cattle. In this study, we found that AG concentration was significantly negatively correlated with the oxidative stress marker ROS (Supplementary Fig. 5). This indicated that MCFAs may mediate ghrelin acylation through GOAT, and AG is involved in the regulation of oxidative stress in high-concentrate-fed finishing bulls. This may be one of the reasons why GMD/GML may improve oxidative stress in finishing bulls in this trial.

There are complex and diverse microbial communities in the gastrointestinal tract of ruminants. The C10 group significantly increased the relative abundance of g_*Christensenellaceae_R_7_group* in the rumen digesta, p_*Fibrobacterota* and its subordinate classification in the rumen fluid. *Fibrobacterota* is considered the main bacteria that degrade dietary starch polysaccharides and wood fiber [54]. Moreover, *Christensenellaceae_R_7_group* belongs to the Firmicutes [55], which are mainly involved in the degradation of cellulose and hemicellulose in the rumen [56, 57]. This may be the reason for the increasing trend compared to the CON group of NDF digestibility in the C10 group of finishing bulls in this trial (Supplementary Table 2, $P = 0.057$). Increased abundance and activity of cellulose-degrading bacteria are reported to decrease NH_3-N concentration in the rumen [58]; this also explained the reason why the C10 and C12 groups significantly reduced NH_3-N concentration in the rumen fluid of finishing bulls (Supplementary Table 1). Furthermore, GMD supplementation reduced the relative abundance of g_*Rikenellaceae_RC9_gut_group* in the rumen digesta, which may affect intestinal permeability, oxidative stress and energy metabolism, and might contribute to the pathogenesis of inflammation [59]. Additionally, although *Treponema* includes harmless

commensal bacterial species, they are primarily known as potential pathogens [60]; one species of g_*Treponema* (*T. brennaborense*) was found to be associated with digital dermatitis in dairy cows [61]. Our study found that the addition of GMD significantly reduced the relative abundance of g_*Treponema* in the rumen digesta*.* These results indicated that the addition of MCTs did not adversely affect the function and structure of the gastrointestinal tract in finishing bulls.

Studies have found that some metabolites produced by gastrointestinal tract microorganisms in mammals can affect mitochondrial balance, ROS levels, and gastrointestinal tract health [62]. In the rumen, amino acids are degradation products of dietary or microbial proteins, precursors of peptide and protein synthesis, and regulate certain metabolic pathways in the body [63]. Lysine is the first or second limiting amino acid in beef cattle [64]. Lysine in the rumen fluid is derived mainly from dietary degradation and synthesis by rumen microorganisms [65]. In this study, the metabolites (2R,3R)-3-methylglutamyl-5-semialdehyde-N6-lysine under the lysine biosynthesis pathway (ko00300) in the rumen fluid of the C10 group was significantly down-regulated; this may suggest the up-regulation of the lysine synthesis pathway, which may have a positive effect on the growth of beef cattle. L-Threonine participates in the glycine, serine and threonine metabolism (ko00260), biosynthesis of amino acids (ko01230), protein digestion and absorption (ko04974), and other metabolic pathways. As the reaction substrate of L-threonate 3-dehydrogenase, it can improve the animal's antioxidant status [35]. Therefore, the up-regulation of rumen fluid metabolite L-threonine in the C10 group may be the reason for the improvement of oxidative stress compared to the CON group.

Dopamine quinone is the oxidation product of dopamine oxidation and is an important biologically active intermediate. It plays an important role in physiological processes such as nerve conduction and oxidative stress in organisms, which may lead to oxidative damage and cytotoxicity [66]. Therefore, the down-regulation of dopamine quinone in the rumen fluid of finishing bulls can also explain the improvement of oxidative stress by the C10 and C12 groups. Besides, tyrosinase catalyzes the conversion of L-tyrosine to L-DOPA and then to dopachrome, L-DOPA can increase the secretion of growth hormone in steers [67]. In this study, L-dopachrome in the rumen fluid of the C10 group was significantly down-regulated, but it did not adversely affect the growth of beef cattle. Moreover, bis(glutathionyl)spermine in antioxidant-related pathway glutathione metabolism (ko00480) in the rumen fluid of the C10 group was significantly up-regulated. The changes in above rumen metabolites indicated that the

improvement of MCTs (GMD/GML) on oxidative stress in beef cattle may also be related to its regulation on metabolites. However, how MCTs regulate metabolites remains to be further studied.

The interaction between gastrointestinal tract flora and oxidative stress depends on a variety of metabolites. Accumulating evidence indicates that the interaction of gastrointestinal tract microorganisms and rumen fluid metabolites can shape phenotypic traits in the host [21, 68, 69]. In the present study, bis(glutathionyl) spermine, a metabolite annotated to the glutathione metabolic pathway (an antioxidant-related pathway) was significantly positively correlated with rumen digesta c_*Negativicutes* ($r = 0.657$, $P = 0.004$), o_*Oscillospirales* ($r = 0.621$, $P = 0.007$), g_*NK4A214_group* ($r = 0.485$, $P = 0.034$) and significantly negatively correlated with feces g_*Ruminococcus* ($r = -0.618$, $P = 0.007$). Therefore, we speculated that GMD/GML may also improve the oxidative stress of finishing bulls under high-concentrate feeding conditions by reshaping the gastrointestinal tract microorganisms and producing specific metabolites related to oxidative stress.

In addition, positive ionization metabolites L-threonine, 3'-phosphoadenylyl sulfate, negative ionization metabolites dTDP-4-oxo-alpha-D-xylose, P1,P4-bis(5'-uridyl) tetraphosphate, gamma-hydroxy-3-pyridinebutanoate, and docosanedioate were significantly correlated with rumen digesta g_*Desulfovibrio* and rumen fluid *Fibrobacteres*, and they were all significantly positively correlated with GOAT. This indicated that gastrointestinal tract microorganisms may also produce specific metabolites closely related to GOAT, indirectly affecting GOAT-mediated ghrelin acylation, and thus improving the feed intake or oxidative stress of finishing bulls. It also indicated that ghrelin, as a brain–gut peptide, may be an important link of information transmission between gastrointestinal tract microorganisms, metabolites and the body function on the brain–gut axis, and participated in the regulation of the body by MCT. However, these assumptions need to be further verified.

Moreover, studies have shown that ghrelin and its receptors are widely expressed in the gastrointestinal tract and various tissues and organs, and can regulate related signaling pathways (such as p38-MAPK; TLR4/NFκB; PI3K/Akt, etc.), reduce oxidative stress and inflammatory response of animal brain or other tissues and organs [16, 70–73]. These important signaling pathways can activate nuclear factor erythroid 2-related factor 2 (Nrf2), regulate the related factors in the Nrf2 pathway, and improve the damage caused by oxidative stress to animal bodies [16, 73, 74]. However, in this experiment, it is unknown whether GMD/GML activated the Nrf2 signaling pathway through GOAT-mediated ghrelin acylation. All in all, our results provided a foundation for the rational use of individual MCT in ruminant and provided new ideas for the improvement of feed intake and oxidative stress in finishing bulls through the microbiota–gut–brain axis. In the future, some in-depth research on the effect of MCTs on feed intake and oxidative stress should be carried out to explain more about its mechanism in finishing bulls.

Conclusion

Under the dietary conditions of our study, the addition of MCTs (GMC/GMD/GML) did not have an adverse effect on the growth of finishing bulls. Compared to the CON group, GMD tended to increase the DMI of finishing bulls, GMD increased the concentration of GOAT, TG, AG, and orexins in the serum of finishing bulls, GMD and GML increased the concentrations of TAOC, catalase, GHS-PX, GR, and NO in the serum of finishing bulls, while GMD decreased the concentrations of H_2O_2, malondialdehyde, ROS, and LPS. This indicated that different types of MCTs have different application effects in ruminants. Among them, GMD may improve the feed intake of finishing bulls by stimulating the secretion of AG. GMD and GML may change gastrointestinal tract microorganisms and produce specific rumen metabolites to improve the oxidative stress of finishing bulls, and ghrelin may also be involved. This study enlightens the potential mechanisms by which MCT improves feed intake and oxidative stress in finishing bulls.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40168-024-01946-2.

Additional file 1: Table S1. Effects of medium-chain fatty acid triglycerides supplementation on ruminal fermentation parameters of finishing bulls. Table S2. Effects of medium-chain fatty acid triglycerides supplementation on nutrient digestibility of finishing bulls. Table S3. Summary of sequence statistics for each sample. Table S4. Significant differential metabolites in rumen fluid of four treatment groups ($n = 4$) under positive ionization mode. Table S5. Significant differential metabolites in rumen fluid of four treatment groups ($n = 4$) under negative ionization mode.

Additional file 2: Fig. S1. The number of operational taxonomic units (OTUs) of gastrointestinal tract bacteria in finishing bulls. (A) rumen digesta, (B) rumen fluid, and (C) feces. CON, the control group cattle fed control diets ($n = 4$); C8, the treatment group cattle fed control diets containing glycerol monocaprylin ($n = 4$); C10, the treatment group cattle fed control diets containing glycerol monodecanoate ($n = 4$); C12, the treatment group cattle fed control diets containing glycerol monolaurate ($n = 4$). Fig. S2. Alpha diversity curve. Rumen digesta (A) rarefaction curves, (B) shannon curves, (C) rank abundance curves, rumen fluid (D) rarefaction curves, (E) shannon curves, (F) rank abundance curves, and feces (G) rarefaction curves, (H) shannon curves, (I) rank abundance curves (OUT level). CON, the control group cattle fed control diets ($n = 4$); C8, the treatment group cattle fed control diets containing glycerol monocaprylin ($n = 4$); C10, the treatment group cattle fed control diets containing glycerol monodecanoate ($n = 4$); C12, the treatment group cattle fed control diets containing glycerol monolaurate ($n = 4$). Fig. S3. The relative abundances

of gastrointestinal tract bacterial community at rumen digesta (A) phylum, (B) family, (C) genus, rumen fluid (D) phylum, (E) family, (F) genus, and feces (G) phylum, (H) family, (I) genus levels (top 20). The different colors of the bars represent different species, and the length of the bars represents the proportion of the species. CON, the control group cattle fed control diets (n = 4); C8, the treatment group cattle fed control diets containing glycerol monocaprylin (n = 4); C10, the treatment group cattle fed control diets containing glycerol monodecanoate (n = 4); C12, the treatment group cattle fed control diets containing glycerol monolaurate (n = 4). Fig. S4. KEGG (Kyoto Encyclopedia of Genes and Genomes) classification maps of significant differential metabolites in the rumen fluid of four treatment groups (n = 4) under positive ionization mode (A) and negative ionization mode (B). Fig. S5. Correlation between serum acylated ghrelin (AG) level and serum reactive oxygen species (ROS) level in finishing bulls. r, correlation coefficient.

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Authors' contributions
CYG, JML and XF conceived and designed the study. JML, XF, YLD and DXY conducted the experiments and collected the samples. JML and XF analyzed the data and wrote the manuscript. CYG revised the manuscript. All authors read and approved the final manuscript.

Data availability
The gastrointestinal tract microbiome sequencing reads (including rumen digesta, rumen fluid, and feces) are available in the Sequence Read Archive (SRA) of NCBI under accession project number PRJNA1111285 (https://dataview.ncbi.nlm.nih.gov/object/PRJNA1111285?reviewer = uo63pvg3kuigl56msror69nrb0).

Declarations

Ethics approval and consent to participate
All procedures involving animals were performed with the approval (approval ID: 20221015) of the Yanbian University Institutional Animal Care and Use Committee.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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