

Caragana korshinskii tannin improves colonic barrier function through promoting the production of taurocholic acid by *Alistipes onderdonkii* in high concentrate fed lambs

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Abstract

Background

High-concentrate diets can impair colonic barrier function in lambs. Although tannins are considered promising regulators of intestinal health, their protective effects and underlying mechanisms in high-concentrate-induced intestinal injury remain unclear.

Results

We first demonstrated that long-term feeding of a high-concentrate diet impairs the colonic barrier function in lambs, whereas dietary supplementation with *Caragana korshinskii* tannin (CKT) markedly alleviated this impairment. Specifically, CKT improved colonic morphology, increased expression of tight junction proteins in the intestinal mucosa, and enhanced immune and antioxidant capacities. We then showed, through 16S rRNA gene amplicon sequencing and metagenomic analysis, that CKT modulated the colonic microbiota structure and function, altered microbial interactions, and promoted the enrichment of *Alistipes onderdonkii*. Untargeted metabolomics further revealed increased abundances of cholic acid, taurocholic acid, and chenodeoxycholic acid in the colonic digesta of CKT-treated lambs, among which taurocholic acid was positively correlated with *Alistipes onderdonkii*. Then, we verified *in vitro* that *Alistipes onderdonkii* can utilize CKT for growth and taurocholic acid production. Colonic transcriptomic analysis revealed that the PPAR signaling pathway was significantly enriched in CKT group, and taurocholic acid was positively correlated with key genes of this pathway (*FABP*, *PPARα*, *SCD*). In ovine colonic mucosal epithelial cells, taurocholic acid upregulated the expression of PPAR pathway-related genes and tight junction proteins. We also verified that both TCA and *Alistipes onderdonkii* interventions alleviated colonic inflammation, reduced intestinal permeability, and activated PPAR signaling in a DSS-induced mouse colitis model.

Conclusions

This study demonstrates that dietary CKT ameliorates high-concentrate-induced colonic barrier dysfunction in lambs by enriching *Alistipes onderdonkii* and promoting its synthesis of taurocholic acid, which in turn activates the PPAR signaling pathway to enhance intestinal barrier integrity. These findings provide novel mechanistic insights into CKT-mediated protection against intestinal injury and highlight CKT as a promising dietary supplement for maintaining gut health in intensively reared lambs.

Introduction

The intestinal epithelial barrier serves as a critical guardian of intestinal homeostasis, restricting the passage of pathogens, dietary antigens, and microbial toxins into the systemic circulation [1,2]. In sheep production, high-concentrate diets are frequently adopted to meet the elevated energy demands for enhanced milk and meat output. Nevertheless, sustained provision of such diets leads to substantial amounts of incompletely hydrolyzed starch escaping ruminal degradation and entering the hindgut,

where it drives excessive fermentation. This fermentative overload is frequently accompanied by microbial imbalance, acidification of the colonic environment, epithelial damage [3–5]. These alterations are closely associated with reduced colonic pH and elevated lipopolysaccharide (LPS) levels, with LPS being an endotoxin primarily derived from Gram-negative bacteria that can further exacerbate epithelial barrier dysfunction [6,7]. Although antibiotics have been extensively applied to prevent intestinal barrier-related disorders, their use as feed additives has been increasingly restricted due to concerns regarding antimicrobial resistance [8,9]. Therefore, identifying effective nutritional strategies to prevent intestinal epithelial barrier dysfunction is urgently required.

Plant-derived bioactive compounds have received considerable attention as potential nutritional alternatives for improving intestinal health in livestock. Within this category, polyphenolic substances, comprising flavonoids, tannins, and phenolic acids, have been demonstrated to influence gastrointestinal integrity and function, thereby modulating nutrient absorption and barrier properties [10,11]. *Caragana korshinskii* (CK), contains abundant tannins, phenolic acids, syringic acid, gallic acid, proteins, minerals [12,13]. As an important polyphenolic fraction of *C. korshinskii*, *C. korshinskii* tannin (CKT) exhibits antioxidant, anti-inflammatory, and antibacterial properties, indicating its potential application in maintaining intestinal barrier integrity [14,15]. Our previous studies demonstrated that CKT reduced methane emissions by altering the abundance of ruminal methanogens in sheep and improved jejunal physiological status as well as physical and biochemical barrier functions in mice [16,17]. However, whether CKT can alleviate high-concentrate diet-induced intestinal dysfunction in lambs remains unknown.

Gut microbiota serve as important intermediates connecting plant-derived polyphenols with host intestinal health. Polyphenols can selectively shape gut microbial communities, while specific bacterial members can further transform dietary compounds or regulate host-associated metabolites, thereby affecting epithelial barrier function [18,19]. Microbial metabolites represent key functional links in host–microbiota interactions, and previous studies have demonstrated that dietary polyphenols can regulate intestinal barrier integrity [20]. Among gut bacteria, members of the genus *Alistipes* have been increasingly associated with intestinal homeostasis and inflammation regulation. For example, polyphenol-rich extracts from *Sanguangporus* were shown to alleviate DSS-induced colitis, at least in part, by enriching *Alistipes onderdonkii* to elevate the microbial metabolite 5-hydroxyindole-3-acetic acid, thereby activating aromatic hydrocarbon receptor signaling [21]. In addition, *Alistipes fingoldii* has been identified as a protective bacterial species against colitis. However, whether CKT modulates intestinal barrier function through regulation of gut microbiota and microbiota-derived metabolites remains unclear.

In the current study, we used high-concentrate-fed lambs as an animal model to investigate the protective effects of CKT on colonic barrier function and to elucidate whether its actions are mediated through gut microbiota and microbiota-derived metabolites. Specifically, we (I) assessed the effects of CKT supplementation on intestinal barrier injury induced by a high-concentrate diet; (II) profiled the alterations in colonic microbial composition using 16S rRNA gene sequencing and metagenomic

analysis; (III) identified differential microbial metabolites associated with CKT intervention using untargeted metabolomics; (IV) examined host colonic transcriptional responses and candidate regulatory pathways by transcriptomic analysis; and (V) verified the barrier-protective effects of the key bacterium and metabolite in primary ovine colonic mucosal epithelial cells and a DSS-induced mouse model.

Materials and methods

Preparation and identification of CKT

The collected material was air-dried in a well-ventilated indoor environment, cut into 3–5 cm segments, ground into powder, and freeze-dried. CKT was then extracted using the ultrasound-assisted solvent extraction method as previously described [22]. Briefly, 20 g of CK powder was mixed with a 62% acetone at a solid-to-solvent ratio of 1:20 (w/v), followed by ultrasonic extraction at 52°C for 95 minutes with an ultrasonic power of 450 W. After extraction, the mixture was vacuum-filtered to obtain the CK tannin solution. The filtrate was concentrated to 50 mL by rotary evaporation at 55°C; after cooling, the concentrated solution was freeze-dried at -80°C to obtain condensed tannins from CK. The total condensed tannin content in CKT was determined, and the measured content was 265.62 mg/g. The monomer composition of CKT was analyzed, which detected a total of 2556 metabolites, including 552 tannins, 762 flavonoids, 302 phenolic acids, 129 terpenoids, 66 lignans, and 745 metabolites of other classes. As shown in Table S1, among the top 30 components ranked by peak area, 11 were tannins, indicating that tannins were the predominant active constituents of CKT, catechin showing the highest relative abundance. To further clarify the monomer composition of condensed tannin in CKT, targeted metabolite detection of condensed tannins was conducted. The high-performance liquid chromatography (HPLC) chromatogram of the condensed tannin monomers in CKT is shown in Fig. S1.

Lamb experiment, design, and diets

Eighteen male Dumont crossbred lambs (3 months old; initial body weight 29 ± 2 kg) were assigned to three dietary treatments (n = 6): control diet (CON; forage-to-concentrate ratio 70:30), high-concentrate diet (HC; 30:70), and high-concentrate diet supplemented with CKT (CKT; 30:70 plus 2 g/kg CKT). Animals were individually housed and received the corresponding diets for 75 days, including a 15-day adaptation period and a 60-day feeding period. Diet formulations are presented in Table S2. At the end of the trial, all lambs were slaughtered and samples were collected for subsequent analyses.

Sample collection

Blood samples were collected from the jugular vein of each lamb before slaughter on day 75, serum samples were obtained and stored at -20°C until analysis. Following slaughter, colonic digesta were collected, homogenized, and immediately subjected to pH determination. Colonic digesta were stored at

– 80°C for metabolomic analysis, and SCFA measurement. Approximately 6 cm of colonic tissue was collected from each lamb. The tissues were separated into two portions: one portion was fixed with 4% paraformaldehyde or 2.5% glutaraldehyde for histological assessment of epithelial morphology, while the mucosal layer from the other portion was scraped using sterile slides.

Growth performance

All lambs were weighed on days 0, 30, and 60 of the treatment periods, and average daily gain (ADG) was calculated based on body weight changes over time. Feed intake was recorded daily for each lamb throughout the trial to calculate the average feed daily intake (ADFI), and the feed conversion rate was calculated for further analysis.

Serum samples analysis

Serum biochemical parameters (TP, ALB, AST, ALT, CREA, CHOL, TG, and Glu) were analyzed using a biochemical analyzer (Hitachi 3100). Cytokines, immunoglobulins, antioxidant-related indices, and intestinal permeability markers were quantified using commercial ELISA kits according to the corresponding instructions.

Colonic histological analysis

Colon tissue morphology was evaluated using hematoxylin and eosin (H&E) staining. Briefly, tissue samples were fixed with 4% paraformaldehyde, paraffin-embedded, sectioned, and examined under a light microscope.

Transmission electron microscopy (TEM) of colonic tissue

Colonic ultrastructure was evaluated by TEM. Colon specimens were fixed in 2.5% glutaraldehyde, followed by resin embedding, sectioning, and double staining. The prepared sections were examined using a TEM system (HT7700, Hitachi, Tokyo, Japan).

Colonic Alcian blue-PAS staining

Goblet cell abundance was evaluated using Alcian blue-PAS staining. Images were captured using a microscope, and goblet cells were quantified based on epithelial length. The number of goblet cells per unit epithelial length was calculated as goblet cell number divided by epithelial length.

Quantification of inflammatory and antioxidant indexes in colon mucosa

Colonic mucosa tissues were lysed in RIPA buffer to extract total proteins. The levels of inflammatory cytokines (IL-1 β and IL-6) were evaluated using ELISA methods, whereas antioxidant status was assessed by measuring T-AOC, T-SOD, CAT, GSH-Px, and MDA with commercial detection kits following the manufacturers' instructions.

Total RNA isolation and quantitative real-time PCR

Total RNA from colonic mucosa was isolated with TRIzol reagent and subsequently converted into cDNA through reverse transcription. Gene expression analysis was conducted by quantitative PCR using a SYBR Green-based fluorescence detection system. Relative transcript abundance was determined using the $2^{-\Delta\Delta Ct}$ calculation method, with β -actin serving as the internal reference. The primer information is provided in Table S3.

Western blotting analysis

Colon mucosa samples were lysed, and total proteins were quantified. Equal amounts of protein were separated by SDS-PAGE, transferred onto PVDF membranes, and blocked before incubation with primary antibodies against Claudin-1 (1:500), Occludin (1:500), ZO-1 (1:500), FABP (1:1000), PPAR- α (1:1000), RXR (1:500), SCD (1:500), and ACO (1:500). After incubation with secondary antibodies (1:5000), protein signals were detected using an Odyssey Infrared Imaging System (LI-COR Biosciences, Lincoln, NE).

Short chain fatty acids (SCFAs) analysis

SCFAs in colonic digesta were quantified using gas chromatography (Agilent 5975C, USA). Samples were extracted with double-distilled water and acidified with 25% metaphosphoric acid (1:5, w/v), followed by centrifugation and GC analysis of the supernatants.

DNA extraction, 16S rRNA gene sequencing, and microbiome analysis

Microbial DNA was extracted from colonic digesta using the E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, Norcross, GA, USA). The V3–V4 region of bacterial 16S rRNA genes was amplified with primers 338F and 806R and sequenced using a high-throughput sequencing platform. Sequence data were analyzed using QIIME2 with the DADA2 workflow for ASV generation, followed by taxonomic annotation against the SILVA database. Microbial community analyses were performed using the Majorbio cloud platform [23,24].

Metagenomic sequencing and data processing

Microbial DNA was extracted from lamb colonic digesta using the E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, USA). Metagenomic sequencing was performed on an Illumina NovaSeq platform at Majorbio Bioinformatics Technology Co., Ltd. The sequencing data were assembled using MEGAHIT, followed by ORF prediction with MetaGene and construction of a nonredundant gene catalog using CD-HIT. Taxonomic annotation was performed using DIAMOND against the NCBI-NR database, and functional profiles were assigned based on KEGG pathways [25–28].

Metabolomic analysis and data processing

Untargeted metabolomics was performed to analyze metabolites in lamb colonic digesta. Derivatized samples were analyzed using an Agilent 8890B-5977B GC-MS system, and the resulting data were processed using MassHunter and MetWare Cloud. Multivariate analysis (PCA and OPLS-DA) was conducted with the ropls package in R. Differential metabolites were screened according to VIP values, fold changes, and Student's t-test, with $VIP > 1.5$ and $P < 0.05$ used as significance criteria. Metabolic pathways were annotated using the KEGG database, and enrichment analysis was performed as previously described [29].

Transcriptomics analysis and data processing

Total RNA was isolated from lamb colonic epithelial tissues using TRIzol reagent. RNA samples were used for library preparation and sequencing on an Illumina HiSeq2500 platform at Majorbio Co., Ltd. (Shanghai, China). RNA-seq reads were mapped to the sheep reference genome using HiSat2 and TopHat2. Transcript abundance was quantified as TPM, and differentially expressed genes (DEGs) were identified using DESeq2 with adjusted $P < 0.05$ and $|\text{fold change}| \geq 2$. Functional enrichment analysis was performed using GSEA with clusterProfiler and enrichplot packages.

Effect of CKT on the growth of *Alistipes onderdonkii*

To determine if *Alistipes onderdonkii* can utilize CKT to grow and produce taurocholic acid (TCA), a medium based on BHI/GAM broth was used as the base medium (BM, $\text{pH} = 7.0 \pm 0.2$, Table S4). In sterile centrifuge tubes, 10 mL of sterilized fastidious anaerobic broth medium was added respectively. For the experimental groups, CKT was added to final concentrations of 0.1%, 0.2%, and 0.4% respectively; for the control group, an equal volume of sterile ultrapure water was added to eliminate solvent interference. All groups were inoculated with 500 μL of activated *Alistipes onderdonkii* bacterial suspension using a sterile pipette. After thorough mixing, the tubes were placed in an anaerobic incubator for constant-temperature cultivation at 37°C . Samples were taken every 2 hours within 24 hours for subsequent growth curve determination (OD_{600} value). Based on the growth curve results, the culture medium with the optimal CKT concentration was selected for bile acid metabolomics analysis.

Culture of *Alistipes onderdonkii* and targeted bile acid analysis

Bacterial culture samples (50 μL) were mixed with internal standards and extracted using 20% methanol-acetonitrile. After centrifugation, the supernatants were collected and analyzed using an UPLC-MS/MS system equipped with an ExionLC™ AD UPLC and QTRAP® 6500 + mass spectrometer (Sciex). Qualitative identification of bile acid metabolites was performed based on an in-house MWDB database, and quantitative analysis was conducted using multiple reaction monitoring (MRM) with standard curves. Multivariate analysis was performed using PCA and OPLS-DA to characterize metabolite differences between groups. The reliability of the OPLS-DA model was evaluated by permutation testing.

Differential bile acid metabolites were identified based on VIP values, Student's t-test, and fold-change analysis. Metabolites with $VIP > 1$, $P \leq 0.05$, and $|\log_2 FC| \geq 1$ were considered significantly different.

Primary ovine colonic mucosal epithelial cell preparation and taurocholic acid treatments

Primary ovine colonic mucosal epithelial cells (OCMECs) obtained from BeNa Culture Collection (BNCC, China) were cultured in DMEM/F12 medium (Gibco, USA) containing 1% penicillin–streptomycin, 2.5% fetal bovine serum (FBS), 5 $\mu\text{g/mL}$ insulin, and 20 ng/mL epidermal growth factor. Cells were maintained at 37°C under 5% CO_2 , with medium replacement every 2 days. After reaching approximately 80% confluence, OCMECs were treated with TCA (Macklin, China) at final concentrations of 0, 5, 10, 20, 40, and 80 $\mu\text{g/mL}$ for 24 h. Subsequently, the total RNA was extracted for further analysis.

Molecular docking

The crystal structure of human FABP was obtained from the Protein Data Bank (PDB ID: 7e25). Docking analysis between TCA and the FABP ligand-binding domain was conducted using AutoDock Vina 1.2.0 [30]. The binding mode with the highest affinity was selected for further analysis. Three-dimensional visualization of the ligand–protein complex was performed using PyMOL Molecular Graphics System (version 2.5.4) [31].

Molecular dynamics (MD) simulation

MD analysis was performed with GROMACS 2023. The protein and ligand parameters were generated using the AMBER14SB force field and GAFF2, respectively. The complex was placed in a periodic cubic box, solvated with TIP3P water, and adjusted to physiological ionic strength (0.15 M Na^+/Cl^-). After minimization and equilibration under NVT/NPT conditions, a 100 ns production run was carried out at 310 K and 1 bar. Binding free energy was estimated by MMPBSA, and structural stability was evaluated using RMSD, RMSF, Rg, SASA, hydrogen-bond interactions, and free energy landscape analyses.

DSS-induced murine colitis model

Male C57BL/6J mice (6 weeks old) were acclimated under SPF conditions for one week and randomly assigned to four groups ($n = 8/\text{group}$). To reduce intestinal microbiota, mice received drinking water containing an antibiotic cocktail for 10 days. Colitis was subsequently induced by providing 2.5% DSS (Coolaber, Beijing, China) in drinking water for 11 days, except for the CON group. During DSS treatment, mice in the DSS + *A. onderdonkii* group received daily intragastric administration of 2×10^8 CFU *A. onderdonkii* suspended in 0.2 mL PBS, whereas the DSS + TCA group received TCA (50 mg/kg) in 0.2 mL PBS. CON and DSS groups were administered with an equal volume of PBS.

Body weight was recorded daily, and disease activity index (DAI) was calculated based on body weight loss, stool consistency, and fecal bleeding scores [32,33]. On day 11, mice were euthanized after anesthesia, and colon length was measured. Colon tissues were collected for histological examination and subsequent molecular analyses. Intestinal barrier-related markers (DAO, D-lactic acid, and LPS) and

tight junction gene expression were determined as described previously. Primer sequences are listed in Table S5.

Statistical analysis

Statistical analyses were performed to evaluate the effects of dietary treatments on physiological, biochemical, and molecular parameters. Each sheep was considered as the experimental unit. Data distribution was assessed using normality tests, and nonparametric methods were applied when normality assumptions were not met. Differences among the three dietary groups were analyzed using one-way ANOVA in R software (version 4.3.1, R Core Team, Vienna, Austria), followed by Tukey's multiple comparison test when appropriate. Data from *Alistipes onderdonkii* growth and TCA assays were analyzed using the GLM procedure of SAS in a completely randomized design. Results are presented as means $\pm$ SEM, and $P < 0.05$ was considered statistically significant. Microbial community differences were evaluated by principal coordinate analysis based on Bray–Curtis distances, and differential abundance was assessed using the Wilcoxon rank-sum test. Microbe–metabolite associations were determined using Spearman correlation analysis with $|r| > 0.35$ and $P < 0.05$ as significance criteria.

Results

CKT regulates growth performance and colonic barrier function in lambs fed a high-concentrate diet

Compared with the CON group, the FW, ADG and feed conversion rate of lambs in the HC and CKT groups were significantly increased ($P < 0.05$, Fig. 1b, Fig. 1c, and Fig. 1e). No significant differences were found in initial body weight, final body weight, ADG, ADFI, and feed conversion rate of lambs in the CKT group compared with those in the HC group ($P > 0.05$, Fig. 1a-e), indicating that dietary 2 g/kg CKT had no negative effect on the growth performance of lambs. To evaluate the effect of CKT on serum biochemical profile of lambs, serum biochemical indicators were measured. Compared with the CON group, lambs in the HC group showed significantly higher serum ALB and CHOL contents, as well as increased AST and ALT activities ($P < 0.05$, Fig. 1g-1i, and Fig. 1k). While the serum CHOL content and AST, ALT activities were significantly decreased compared with the HC group ($P < 0.05$, Fig. 1h-1i, Fig. 1k), suggesting that CKT supplementation may partially alleviate high-concentrate diet-induced disturbances in liver-related biochemical parameters. Because cholesterol is the precursor for bile acid synthesis, the decreased serum cholesterol concentration in the CKT group may indicate altered cholesterol utilization, possibly related to bile acid metabolism.

In the following analyses, we first evaluated whether dietary supplementation with 2 g/kg CKT could alleviate high-concentrate diet-induced colonic barrier dysfunction in lambs. Consistently, CKT alleviated the damage to the colonic barrier function in high-concentrate-treated lambs, as indicated by the significantly increased mRNA expression (Fig. 1o) and protein expression (Fig. 1p) of claudin-1 and occludin ($P < 0.05$). Histological examination further supported the protective effect of CKT on colonic

barrier structure. HE staining results showed that in the HC group, lambs' colonic epithelial disorder, severe surface indentations, and cell damage; while in the CON and CKT groups, lambs' colonic orderly arranged epithelial cells (Fig. 1q). TEM results revealed that in the HC group, the colonic epithelial tight junctions of lambs had decreased electron density and widened intercellular spaces, with partial fracture or disappearance of mitochondrial cristae; in the CON and CKT groups, the colonic epithelial tight junctions were intact with narrow ridges (Fig. 1q). The contents and activities of several intestinal barrier function markers in serum were also detected. Apparently, CKT alleviated the intestinal barrier function damage in lambs induced by a high-concentrate diet, as indicated by the significantly reduced DAO activity, D-LA concentration and LPS content ($P < 0.05$, Fig. 1r-1t). Collectively, these findings demonstrate that CKT mitigates high-concentrate diet-induced disruption of colonic physical barrier integrity in lambs.

CKT attenuates colonic oxidative stress and inflammatory responses in lambs fed a high-concentrate diet

Dietary CKT supplementation restored the serum levels of antioxidant enzymes, T-AOC, GSH-Px, CAT that were significantly reduced by high-concentrate diet (Fig. 2a, 2b, 2d). MDA in the serum was significantly increased by high-concentrate, but restored toward control levels after CKT supplementation ($P < 0.05$, Fig. 2e). Similarly, the colonic levels of T-AOC (Fig. 2a) and CAT (Fig. 2d) were significantly reduced by high-concentrate diet ($P < 0.05$), but were largely attenuated to the normal levels by CKT. The colonic level of MDA was significantly increased by the high-concentrate diet but was markedly reduced after supplementation with 2 g/kg CKT (Fig. 2e). Furthermore, the relative mRNA expression levels of *CAT* and *GSH-Px* in peripheral blood lymphocytes and colonic tissue, were significantly downregulated in HC group ($P < 0.05$); however, the relative mRNA expression levels of the antioxidant enzyme genes in the CKT group were significantly upregulated ($P < 0.05$, Fig. 2f).

The high-concentrate diet significantly increased the serum contents of the pro-inflammatory factors IL-1 β and TNF- α , while dietary 2 g/kg CKT supplementation largely attenuated these elevated levels ($P < 0.05$, Fig. 2g and Fig. 2i). Consistently, IgM in the serum was significantly decreased by high-concentrate diet, but increased to normal in response to CKT ($P < 0.05$, Fig. 2m). The contents of IL-4, IgA, and IgG in the serum of lambs in the CKT group also significantly increased ($P < 0.05$, Fig. 2j and Fig. 2l, Fig. 2n). Similarly, the colonic levels of IL-10 (Fig. 2k) and IgA (Fig. 2l) were significantly reduced by high-concentrate diet ($P < 0.05$), but were largely attenuated to the normal levels by CKT. The colonic level of IL-1 β , IL-6, TNF- α was significantly increased by high-concentrate diet, but returned below normal in response after supplementation with 2 g/kg CKT ($P < 0.05$, Fig. 2g-2i). Furthermore, compared with the CON, the relative mRNA expression levels of *IL-1 β* and *TNF- α* in serum, as well as those of *IL-1 β* , *IL-6* and *TNF- α* in colonic tissue, were significantly upregulated in the HC group; however, compared with the HC group, the relative mRNA expression levels of the aforementioned cytokines in the serum and colon of lambs in the CKT group were significantly downregulated ($P < 0.05$, Fig. 2o). Notably, the relative mRNA expression levels of the anti-inflammatory factors *IL-4* and *IL-10* in peripheral blood lymphocytes of

lambs in the CKT group were significantly higher than those in the HC group ($P < 0.05$, Fig. 2o). The number of colonic goblet cells was also detected, which similarly confirmed that CKT alleviated the intestinal immune response in lambs induced by a high-concentrate diet ($P < 0.05$, Fig. 2p). The results indicate CKT alleviated high-concentrate diet-induced colonic immune dysfunction and contributed to the maintenance of mucosal immune homeostasis in lambs.

CKT alters colonic SCFA profiles and microbial composition in lambs fed a high-concentrate diet

We next investigated whether CKT supplementation altered short-chain fatty acids (SCFA) production and the colonic microbiota in lambs fed a high-concentrate diet. The results showed that, compared with the CON group, both the HC and CKT groups exhibited a significant decrease in acetic acid content and a significant increase in propionic acid content in lamb colonic contents ($P < 0.05$, Fig. S2b, S2c), which is presumably attributed to dietary factors. Notably, the addition of CKT led to a significant increase in butyric acid content in lamb colonic contents ($P < 0.05$, Fig. S2e).

We then explored the impact of CKT on the colonic microbiota composition of lambs via 16S rRNA gene sequencing. The bacterial diversity in the colon of lambs in both the HC and CKT groups was significantly reduced, however, the relative abundance of certain specific bacteria was significantly increased after CKT supplementation (Fig. S2i). The Bray-Curtis distance-based principal co-ordinates analysis (PCoA) plots showed that the three groups were separated from each other ($R^2 = 0.064$, $P = 0.002$, Fig. S2j). At the phylum level, Firmicutes, Bacteroidota, Spirochaetota, Verrucomicrobiota were predominant phyla in the colonic microbiota (Fig. S2k). At the genus level, the colonic microbiota was dominated by *unclassified_f_Lachnospiraceae*, *Christensenellaceae_R-7 group*, *norank_f_Eubacterium_coprostanolig*, *Rikenellaceae_RC9_gut_group*, *norank_f_UCG-010*, *Monoglobus*, *unclassified_c_Clostridia*, *Alistipes*, *Bacteroides* (Fig. S2l). LEfSe analysis revealed that CKT supplementation altered the abundance of several colonic bacterial taxa in lambs, with the most pronounced change observed in *Alistipes* (Fig. 3f).

The results showing that CKT altered the colonic microbial community structure, we further performed metagenomic sequencing to identify the specific microbial species affected by CKT. The results showed that there were no differences in the Simpson and Shannon diversity index between the CKT and HC samples, however, the Chao index was significantly increased in the CKT group (Fig. 3a), and PCoA plots showed that the three groups were separated from each other ($R^2 = 0.513$, $P = 0.001$, Fig. 3b). At the phylum level, Bacillota, Bacteroidota, Verrucomicrobiota, Spirochaetota, Uroviricota were predominant phyla in the colonic microbiota (Fig. 3c). At the genus level, *Ruminococcus*, *Bacteroides*, *Alistipes*, *Clostridium*, *Candidatus*, *Faecousia* were predominant genus in the colonic microbiota (Fig. 3d). At the species level, *Ruminococcus sp*, *Bacteroides sp*, *Alistipes onderdonkii*, *Clostridium sp*, *Prevotella sp*, and others were predominant species in the colonic microbiota (Fig. 3e), among these, *Alistipes onderdonkii* was the primary differential microorganism enriched in the colonic microbiota of lambs in the CKT

group, followed by *Ruminococcus_lactaris*, *Ventricola_gallistercoris*, *Ventricola_intestnavium*, *Ruminococcus_flavefaciens* and *Methanomethylophilaceae_archaeon* (Fig. 3g). These results indicate that CKT altered the overall structure of the lamb colonic microbiome, with *Alistipes onderdonkii* identified as a key species-level taxon in the CKT group.

CKT reshapes the interaction network and functional potential of the lamb colonic microbiome

To further determine whether CKT affected microbial interaction patterns, we performed co-occurrence network analysis of the lamb colonic microbiota. The HC and CKT networks exhibited distinct topological features, suggesting that CKT supplementation may alter the organization of microbial associations in the colon. (Fig. 3h and 3i). In the HC co-occurrence network, the average path length (APL) between nodes was 3.669, and the average clustering coefficient (ACC) was 0.51; in the CKT co-occurrence network, the APL between nodes was 3.559, and the ACC was 0.52, the modularity of both co-occurrence networks was ≥ 0.56 (Table S6). To characterize the topological features of the microbial networks, taxa were assigned according to their Zi and Pi values [34]. *Siraeum*, *Glycyrrhizinilyticus* and *Massiliensis* were identified as connectors, and *Orotica* were classified as module hubs in the HC network (Fig. 3k). The majority of the nodes in the CKT network were peripherals, with most of their links inside their modules. The species *Alistipes onderdonkii* was identified as the only module hub, and *Lactaris*, *Porcorum*, *Intestinigallinarum* and *Communis* were connectors in the CKT network (Fig. 3j).

We compared the differences in colonic microbial metabolic pathways and metabolic enzymes between the HC and CKT groups. Functional prediction of the gut microbial metagenomic data based on the KEGG database revealed that a total of 10 metabolic pathways were seen to differ significantly between the HC and CKT groups ($P < 0.05$). In the CKT group, there was an up-regulation in 4 pathways (primary bile acid biosynthesis, butanoate metabolism, staphylococcus aureus infection, ribosome biogenesis in eukaryotes), while 6 pathways (glycosaminoglycan degradation, valine, leucine and isoleucine degradation, tryptophan metabolism, lysine degradation, lipoic acid metabolism, bacterial invasion of epithelial cells) were significantly increased in the HC group ($P < 0.05$, Fig. 3l). We further explored and found 10 enzymes that differ significantly between the HC and CKT groups ($P < 0.05$, Fig. 3m). Of note, the four most abundant enzymes (3.1.2.27, chologyl-CoA hydrolase, 6.2.1.7, cholate:CoA ligase, 2.3.1.65, bile acid CoA:amino acid N-acyltransferase, 3.5.1.24, urocanase) are involved in the primary bile acid biosynthesis (Fig. S3). Together, these results indicate that CKT reshaped both the ecological organization and functional potential of the lamb colonic microbiome, with *Alistipes onderdonkii* identified as a key network species and primary bile acid- and butyrate-related pathways representing important functional features of the CKT-associated microbiome.

CKT enriches *Alistipes onderdonkii* and promotes taurocholic acid production

To further investigate the role of CKT on colonic microbial metabolism, we performed untargeted metabolomic analysis on colonic content samples from lambs in the HC and CKT groups. A total of 1422 compounds were identified across all samples. OPLS-DA analysis revealed that there were significant

differences in metabolite composition between the CKT and HC groups ($P < 0.05$, Fig. 4a). Among the 292 differential metabolites identified between the HC and CKT groups, 197 were upregulated and 95 were downregulated in the CKT group. ($P < 0.05$, Fig. 4b, Table S7). Differential metabolites up-regulated in the CKT group were mainly enriched in the primary bile acid biosynthesis, arachidonic acid metabolism, tyrosine and tryptophan biosynthesis, phenylalanine metabolism, and porphyrin metabolism pathways (Fig. 4c). Among the enriched pathways, primary bile acid biosynthesis pathway showed the highest significance, which was consistent with the KEGG functional prediction based on metagenomic sequencing. CKT-enriched metabolites involved in primary bile acid biosynthesis mainly included cholic acid, TCA, and chenodeoxycholic acid (Fig. 4d). Comparison of metabolite relative abundances showed that the relative abundance of TCA exhibited the most significant difference between the two groups ($P < 0.05$, Fig. 4f-4h). We further performed correlation analysis between differential metabolites and gut microbes. Results showed that the CKT-enriched *Alistipes onderdonkii* was positively associated with cholic acid and TCA ($P < 0.05$, Fig. 4e), suggesting a potential association between *Alistipes onderdonkii* enrichment and the accumulation of cholic acid and TCA.

To further verify whether CKT directly promotes *Alistipes onderdonkii* growth and bile acid metabolism, we cultured *Alistipes onderdonkii* with CKT *in vitro* and performed targeted bile acid metabolomic analysis of the culture supernatant (Fig. 4i). Compared with the control group, 0.2% CKT significantly promoted the growth of *Alistipes onderdonkii* ($P < 0.05$, Fig. 4j). Then, we detected the bile acid levels in the supernatant of *Alistipes onderdonkii* fermentation broth from both the CON group and 0.2% CKT group using targeted bile acid metabolomics technology. PCA analysis showed that the samples were relatively concentrated in distribution, and the samples between the two groups were well separated, with almost all distributed within the 95% confidence ellipse (Fig. 4k). In addition, based on the threshold of $P < 0.05$ and $FC \geq 1$ in the t-test, a total of 10 differential metabolites were detected between the two groups. As shown in Table S8 and Fig. 4l, compared with the CON group, the addition of 0.2% CKT to the bacterial broth significantly up-regulated 10 bile acids. These bile acids were TCA, GUDCA-3S, LCA-3G, THCA, GCDCA-3S, 23-DCA, CA-3S, 3-oxo-DCA, TLCA-3S, and GHCA. Together, these findings indicate that CKT directly enhances *Alistipes onderdonkii* growth and affect bile acid metabolic production.

Transcriptomic profiling the intestinal gene expressions in the HC and CKT lambs

A total of 142 differentially expressed genes (DEGs) were identified in the HC and CKT lambs, among which 72 and 70 genes were up and down-regulated in the CKT lambs (Fig. 5a, Table S9). GO functional analysis indicated that the up-regulated gene mainly associated with Catalytic activity, Cell part, Immune system process, Biological regulation and Cellular process (Fig. 5b). KEGG analysis identified 20 significantly enriched pathways among the upregulated genes, including the Antifolate resistance, PPAR signaling pathway, Bile secretion, Tryptophan metabolism, Biosynthesis of cofactors, cAMP signaling pathway, Antigen processing and presentation and Retinol metabolism ($P < 0.05$, Fig. 5c). GO functional analysis indicated that the down-regulated gene mainly associated with Molecular transducer activity,

Molecular function regulator, Structural molecule activity, Catalytic activity, Binding, Protein-containing complex (Fig. S4a). KEGG enrichment analysis showed that the down-regulated genes mainly associated with Autoimmune thyroid disease, EGFR tyrosine kinase inhibitor resistance, Glycerophospholipid metabolism, Gap junction, Legionellosis, Protein digestion and absorption ($P < 0.05$, Fig. S4b). We then investigated the correlations between the primary bile acid biosynthesis - regulated metabolites and DEGs ($P < 0.05$, $\text{Log}_2\text{FC} > 2$). We found that the levels of TCA, which were enriched in the CKT group, were positively correlated with the expression of *FABP*, *SCD*, and *PPAR α* ($P < 0.05$, Fig. 4d). Interestingly, all three genes were enriched in the PPAR signaling pathway, as previously identified by KEGG enrichment analysis. PPARs have been increasingly recognized as pivotal regulators of intestinal homeostasis, orchestrating epithelial barrier integrity, immune cell polarization, and mucosal repair processes. Furthermore, bile acids are known to regulate intestinal stem cell renewal, tight junction protein expression, the mucus layer, and immune cell function, with PPARs serving as one of the key nuclear receptor families mediating these effects [35, 36]. These results suggest that CKT may ameliorate colonic barrier function in lambs partly through a TCA-associated modulation of PPAR signaling.

To determine whether TCA could directly modulate the PPAR pathway via FABP, we conducted molecular docking and dynamics simulations. The results showed that TCA directly bound to FABP through key residues including Gly100, Asp99, Glu102, and Trp98, with a binding energy of -6.6 kcal/mol (Fig. 5e). During the subsequent molecular dynamics simulation, the protein and ligand reached a stable state after 20 ns without significant fluctuations, indicating that TCA binding induced conformational tightening of FABP and facilitated the formation of multiple hydrogen bonds with the ligand (Fig. 5f). The root-mean-square fluctuation (RMSF) analysis revealed that the flexibility of amino acid residues remained below 0.5 nm, suggesting conformational stability in the core functional regions (Fig. 5g). The radius of gyration (R_g) and solvent-accessible surface area (SASA) of the complex system exhibited minimal fluctuations during the simulation, indicating the compactness and stability of the overall protein-ligand complex structure (Fig. 5h, i). Notably, FABP adopted a more tightly folded conformation after TCA binding, accompanied by increased structural stability. Hydrogen bonds are the primary stabilizing forces in protein-ligand interactions. During the simulation, the number of hydrogen bonds between TCA and FABP fluctuated between 0 and 6, with an average of approximately one persistent bond throughout the trajectory, contributing to the stability of the complex (Fig. 5j). MM-PBSA calculations showed that the binding free energy over 0-100 ns was -23.68 kcal/mol, indicating a highly stable interaction (Fig. 5k). Free energy landscape analysis further confirmed that the system predominantly occupied a thermodynamically stable low-energy conformational state (Fig. 5l, m). Collectively, these results suggest that TCA exhibits a favorable binding affinity for FABP, supporting a potential direct interaction between TCA and this PPAR pathway-related protein.

Then, we incubated OCMECs in media supplemented with 20, 40, or 80 $\mu\text{g/mL}$ TCA for 24 h. The results showed that treatment with 20 and 40 $\mu\text{g/mL}$ TCA significantly increased OCMECs viability at 24 hours (Fig. S5). And the 40 $\mu\text{g/mL}$ TCA treatment significantly up-regulated the expression of PPAR signaling pathway-related genes, including *PPAR α* , *FABP*, and *SCD*, as well as tight junction protein-related genes in OCMECs ($P < 0.05$, Fig. 5n, o). Consistently, Western blot analysis revealed that 40 $\mu\text{g/mL}$ TCA treatment

significantly increased the protein expression of PPAR α , FABP, SCD, and tight junction proteins (Claudin-1, Occludin, and ZO-1) compared with the control group (Fig. 5p, q). Notably, retinoid X receptor (RXR) protein expression was also significantly upregulated upon TCA treatment (Fig. 5q). As an obligatory heterodimer partner of PPAR α , RXR forms transcriptionally active PPAR/RXR heterodimers that bind to peroxisome proliferator response elements (PPREs) in the promoter regions of target genes, thereby coordinating the expression of genes involved in lipid metabolism and barrier function. The TCA-induced upregulation of RXR, together with PPAR α , may thus potentiate the transcriptional activity of the PPAR signaling pathway, contributing to the enhanced expression of downstream target genes and ultimately the improvement of colonic epithelial barrier function.

***Alistipes onderdonkii* and TCA mitigates DSS-induced intestinal barrier disruption through PPAR signaling activation**

To further confirm the protective effects of *Alistipes onderdonkii* and TCA on intestinal inflammation and barrier integrity, a DSS-induced colitis model was established in mice (Fig. 6a). Compared with the DSS group, administration of *Alistipes onderdonkii* or TCA alleviated colitis severity, as indicated by reduced DAI scores, improved body weight changes, decreased colon shortening, and preservation of colonic mucosal integrity (Fig. 6b–f). Both treatments improved intestinal barrier function, with decreased DAO, D-lactic acid, and LPS levels and restoration of tight junction proteins Claudin-1, Occludin, and ZO-1 (Fig. 6g–k). In addition, *Alistipes onderdonkii* and 50 mg/kg TCA increased the expression of PPAR signaling-related genes, including PPAR α , FABP2, RXR, and SCD, at both mRNA and protein levels in colonic tissues (Fig. 6j and Fig. 6l). Collectively, these results suggest that *Alistipes onderdonkii* and TCA protect against DSS-induced colitis, potentially through activation of PPAR signaling and restoration of epithelial barrier function.

Discussion

Long-term feeding of high-concentrate diets disrupts the microecological balance in the rumen and hindgut of lambs as reflected by, decreased digesta pH and elevated LPS, ultimately compromising intestinal barrier integrity [37]. In this study, lambs fed the high-concentrate diet showed clear evidence of colonic barrier dysfunction, including impaired mucosal morphology, reduced expression of tight junction proteins, widened intercellular spaces, increased serum DAO, D-LA, and LPS levels, reduced antioxidant capacity, and an exaggerated mucosal inflammatory response. These results are consistent with previous studies showing that feeding induces intestinal fermentation disorders, promotes LPS translocation, and aggravates epithelial injury and barrier disruption [38]. In addition, the reduced antioxidant enzyme activities observed in the HC group suggest that oxidative stress may also contribute to epithelial damage and increased intestinal permeability. The HC group also showed increased pro-inflammatory cytokine levels, and fewer goblet cells in the colonic mucosa, indicating impaired mucosal immune homeostasis. These results are consistent with the study by Li et al., who confirmed that LPS translocation induced by high-concentrate diets promote the transcription of pro-inflammatory cytokines, and simultaneously inhibit the synthesis of anti-inflammatory cytokines and

immunoglobulins [39]. Collectively, these results demonstrate that high-concentrate feeding damages colonic barrier function through coordinated alterations in epithelial integrity, oxidative status, and inflammatory responses.

Supplementation of 2 g/kg CKT in high-concentrate diets effectively alleviates high-concentrate-induced intestinal damage, as reflected by improved mucosal morphology, enhanced tight junction integrity, reduced serum DAO, D-LA, and LPS levels, and attenuated mucosal inflammation. This effect is closely associated with the antioxidant, anti-inflammatory, and barrier-repairing activities of tannins. Moreover, CKT increased IL-10 levels, decreased IL-1 β and TNF- α levels, and promoted goblet cell abundance, suggesting improved colonic mucosal immune homeostasis. The elevated sIgA and IgG levels further indicate enhanced intestinal immune defense. Overall, these results support a protective role of CKT in maintaining intestinal barrier function in lambs fed a high-concentrate diet. Given the central role of gut microbiota in regulating intestinal barrier homeostasis, these beneficial effects of CKT may be associated, with alterations in gut microbial composition and microbiota-derived metabolites.

To further explore the mechanisms underlying the barrier-protective effects of CKT, we next examined whether CKT supplementation altered the colonic microbial community and microbial metabolite profiles in lambs fed a high-concentrate diet. Intestinal microorganisms and their metabolites act as the core hub connecting plant active substances and host intestinal health. By combining microbiomics, metabolomics, and in vitro experiments, this study is the first to clarify that *Alistipes onderdonkii* and TCA are the key flora and core metabolites involved in the regulation of lamb intestinal health by CKT. CKT can reshape the colonic flora structure of lambs, enrich the key beneficial bacterium *Alistipes onderdonkii*, and improve the homeostasis of the microbial barrier. The core function of the intestinal microbial barrier depends on flora diversity and the abundance of key species. As a representative commensal bacterium of the phylum Bacteroidota, the genus *Alistipes* is characterized by bile tolerance and involvement in organic matter metabolism, making it an important indicator of intestinal health. At the genus level, the abundance of *Alistipes* was significantly elevated. Metagenomic analysis further confirmed that *Alistipes onderdonkii* was the main differential species enriched in the CKT group and served as a module hub in the microbial co-occurrence network. These results are consistent with the study by Zhong et al., who found that Phellinus igniarius polyphenols could specifically enrich *Alistipes onderdonkii* in the intestine of mice. This strain alleviates colitis by synthesizing 5-hydroxyindole-3-acetic acid to activate the aryl hydrocarbon receptor pathway [40]. Mechanistically, as a condensed tannin, the catechol groups in the molecular structure of CKT can be catalytically degraded by polyphenol oxidase secreted by *Alistipes onderdonkii*, generating small-molecule fragments such as gallic acid and catechin that provide carbon sources and energy for bacterial growth.

CKT regulates *Alistipes onderdonkii*-mediated bile acid metabolism, increasing the content of primary bile acids such as TCA and providing a metabolic basis for intestinal barrier repair. As core signaling molecules in the interaction between intestinal microorganisms and the host, bile acid metabolism disorders are closely associated with intestinal damage. Primary bile acids can directly regulate barrier function by activating epithelial cell receptors such as farnesoid X receptor and G protein-coupled bile

acid receptor 1 [41]. Metabolomic results showed that the primary bile acid biosynthesis pathway was significantly upregulated in the colonic digesta of the CKT group. The contents of TCA, cholic acid, and chenodeoxycholic acid were significantly higher than those in the HC group, with TCA showing the highest VIP value among all differential metabolites, identifying it as the core differential metabolite. Spearman correlation analysis further confirmed a significant positive correlation between the abundance of *Alistipes onderdonkii* and TCA content, suggesting that this strain may be the functional mediator of bile acid metabolism regulation by CKT. Bile acids are important signaling molecules in host–microbiota interactions, and disturbances in bile acid metabolism have been closely associated with intestinal injury. Previous studies have shown that TCA exerts protective effects on the intestinal barrier by enhancing tight junction expression, suppressing pro-inflammatory cytokine production, and helping maintain microbial homeostasis [42–44]. In addition, the elevated levels of cholic acid and chenodeoxycholic acid in the CKT group may further contribute to epithelial repair and anti-inflammatory regulation [45]. Together, these findings suggest that the altered bile acid profile in the CKT group may represent an important mechanism underlying the barrier-protective effects of CKT. To further explore the relationship among CKT, *A. onderdonkii*, and bile acid metabolism, in vitro bacterial culture experiments were performed. The results showed that supplementation with 0.1%, 0.2%, and 0.4% CKT all promoted the growth of *Alistipes onderdonkii*, with the 0.2% CKT concentration exerting the optimal effect. Targeted bile acid metabolomic analysis confirmed that the TCA content in the bacterial broth of the 0.2% CKT group was significantly higher than that in the control group. Collectively, these results suggest that CKT may promote the growth of *A. onderdonkii* and modulate bile acid metabolism, thereby contributing, at least in part, to intestinal barrier protection.

Given that CKT supplementation was associated with enrichment of *Alistipes onderdonkii* and remodeling of bile acid metabolism, we next performed colonic transcriptomic analysis to determine how these changes might be translated into host barrier-protective responses. KEGG enrichment analysis identified the PPAR signaling pathway among the pathways associated with CKT group. Notably, TCA, which was enriched in the CKT group, was positively correlated with the expression of *PPARα*, *FABP2*, and *SCD*, and all three genes were enriched in the PPAR signaling pathway. As *PPARα* plays an important role in lipid metabolism, inflammation, and intestinal homeostasis [46,47], these findings suggest that bile acid remodeling induced by CKT may be linked to activation of the PPAR signaling pathway. To verify whether TCA directly regulates PPAR signaling, in vitro experiments were performed using OCMECs. Consistent with this, TCA treatment upregulated the expression of PPAR signaling pathway genes and tight junction proteins in OCMECs. These results provide direct evidence that TCA acts as an activator of the PPAR signaling pathway, supporting the barrier-protective effect observed in vivo. A parallel study on DSS-induced colitis also reported that activation of PPAR-γ signaling enhances intestinal epithelial energy metabolism and restricts the expansion of pathogenic Enterobacteriaceae, suggesting that PPAR family members may coordinate to maintain colonic homeostasis [48].

We further observed that both TCA and *Alistipes onderdonkii* interventions activated PPAR signaling in mouse colonic tissues, restored tight junction protein expression, and reduced intestinal permeability

indicators. These findings are consistent with our transcriptomic analysis in lambs and in vitro cell experiments. A recent work has shown that bile acid profiles are significantly altered in DSS-induced colitis, with specific secondary bile acids such as lithocholic acid promoting colonic inflammation via activation of the S1PR2/NF- κ B pathway [49]. In contrast, our results position TCA as a protective bile acid in this context, further supporting the concept that individual bile acid species have distinct functional roles in intestinal inflammation. The direct link between PPAR α activation and tight junction protein expression was not experimentally validated. Future studies using PPAR α agonists or gene knockout approaches are needed to establish this causal relationship.

Conclusion

In conclusion, dietary supplementation with CKT effectively alleviated high-concentrate diet-induced hindgut barrier dysfunction in lambs. Mechanistically, CKT reshaped the colonic microbiota, selectively enriched *Alistipes onderdonkii*, and promoted *Alistipes onderdonkii*-associated TCA accumulation. Increased TCA may subsequently activate PPAR signaling, thereby restoring tight junction protein expression, reducing intestinal permeability, and suppressing inflammatory responses. These findings reveal a microbiota-dependent regulatory mechanism underlying the protective effect of CKT on colonic barrier function and provide a theoretical basis for improving intestinal health in intensively reared lambs.

Declarations

Ethics approval and consent to participate

This study was supported and approved by the Animal Ethics Committee of Inner Mongolia Agricultural University (No.NND2022033).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Author Contribution

D.B.L. and A.L.T.Z.L. conceived and designed the experiments and wrote and revised the manuscript. X.Y.N. and Y.Y.X. performed the experiments, collected the samples, analyzed the data, and wrote the manuscript. Z.Y.C., H.L., S.X.Z., M.S., J.Y. and S.X.L. collected the samples and analyzed the data. D.Y.Y. conducted the mouse experiments, collected samples and performed the tests. All the authors have read and approved the final manuscript.

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Not applicable.

Data Availability

All sequencing data and assembled genomes have been deposited in the NCBI Sequence Read Archive (SRA) database under the accession numbers PRJNA1452600 (16S rRNA raw sequence data), PRJNA1452632 (RNA-seq raw data) and PRJNA1453195 (Metagenomes raw sequence data). All other relevant data are available in the main manuscript and supplementary files.

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Figures

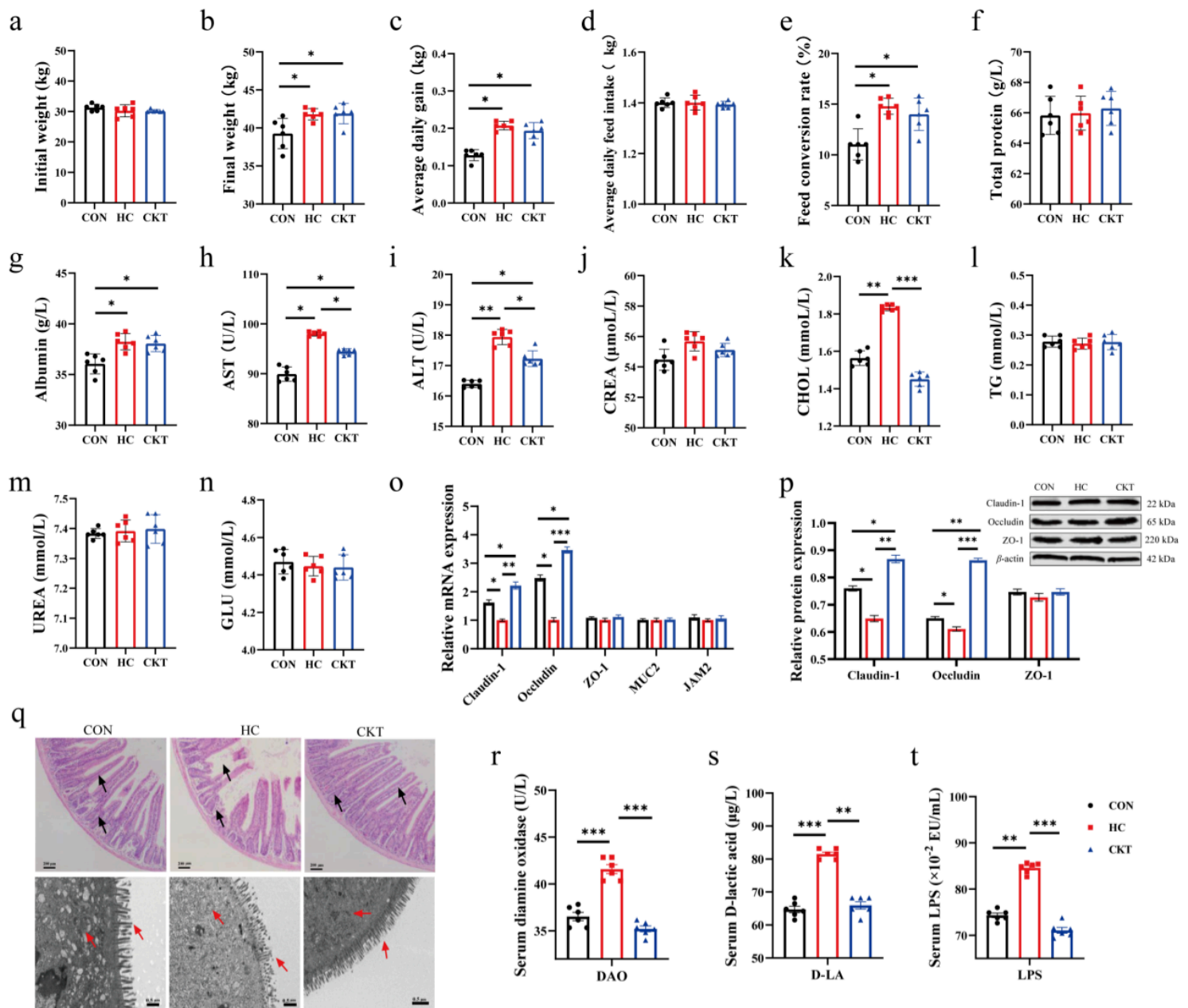


Figure 1

CKT regulates the growth performance and serum biochemical parameters of lambs. (a) IW. (b) FW. (c) ADG. (d) ADFI, and (e) FCR of lambs in the CON, HC, and HC+ 2 g/kg CKT groups. Serum biochemical parameters: (f) total protein. (g) albumin. (h) aspartate transaminase. (i) alanine transaminase. (j) creatinine. (k) cholesterol. (l) triglycerides. (m) urea. (n) glucose. (o, p) relative mRNA and protein expression of tight junction proteins. (q) H&E tissue sections of lamb colon, scale bars represent 200 μm . (r) TEM tissue sections of lamb colon. (E) activity of DAO in serum. (s) Concentration of D-LA in serum. (t) concentration of LPS in serum. Data are expressed as the mean $\pm$ SEM ($n = 6$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

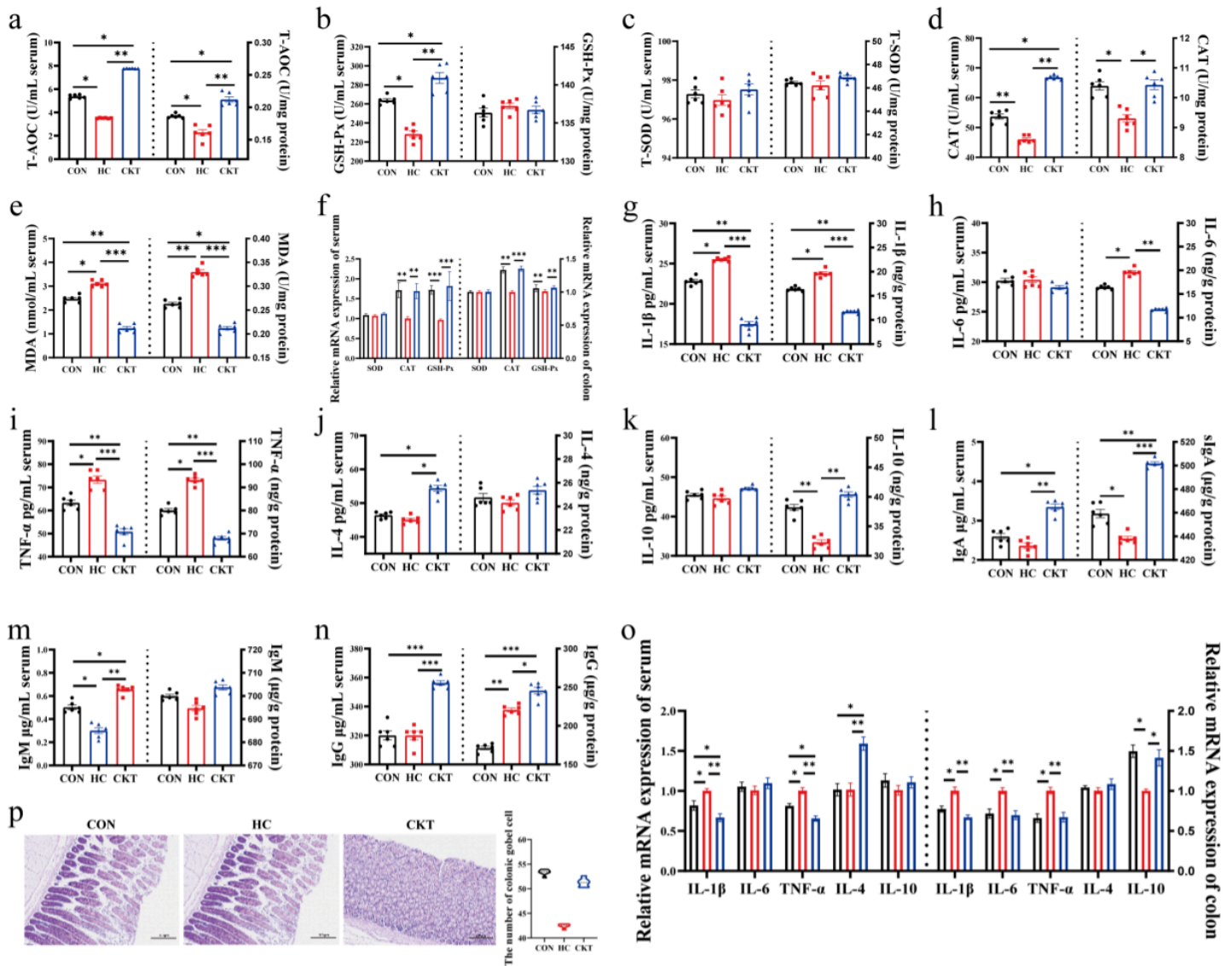


Figure 2

CKT attenuates colonic oxidative stress and inflammatory responses in lambs fed a high-concentrate diet. (a–e) Levels of T-AOC, GSH-Px, T-SOD, CAT, and MDA in serum and colon. (f) Relative expression of antioxidant-related genes. (g–k) Levels of inflammatory cytokines, including IL-1 β , IL-6, TNF- α , IL-4, and IL-10. (l–n) Serum and colonic immunoglobulin levels (IgA, IgM, and IgG). (o) Relative expression of inflammatory-related genes. (p) PAS staining of colon sections and quantification of goblet cells (scale bar = 200 μ m). Data are presented as mean $\pm$ SEM (n = 6). * P < 0.05, ** P < 0.01, *** P < 0.001.

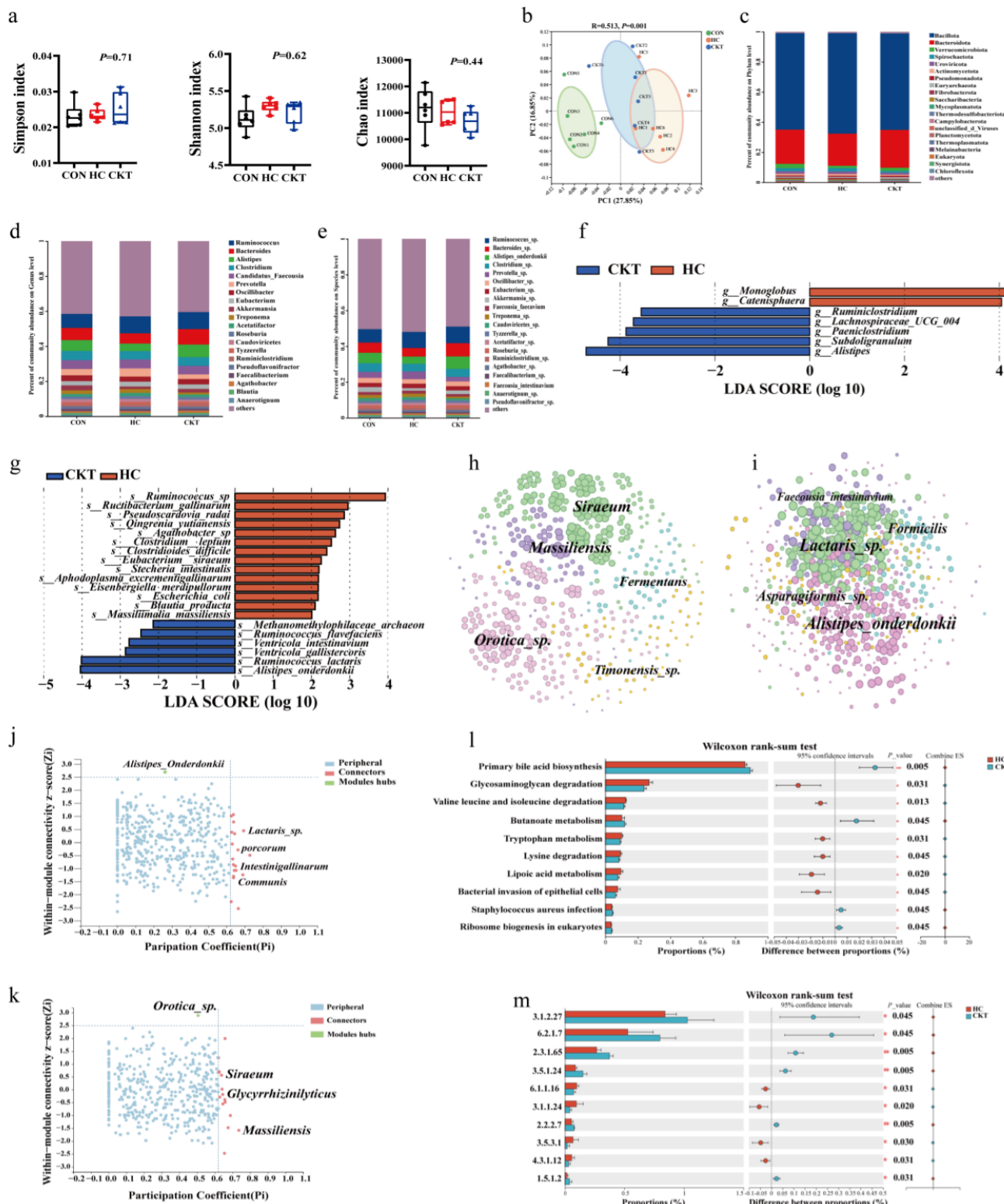


Figure 3

CKT regulated the lamb colonic microbial community structure. (a) Species-level α -diversity of microbiota among groups. (b) PCoA based on Bray–Curtis distance showing microbial community differences. (c–e) Relative abundance of dominant taxa at phylum, genus, and species levels. (f,g) LEfSe analysis identifying differential taxa based on 16S rRNA sequencing (genus level) and metagenomic sequencing (species level). (h,i) Microbial co-occurrence networks in HC and CKT groups. (j,k) Network

topology classification for identification of potential keystone taxa. (l) Enriched differential metabolic pathways. (m) Enriched differential enzymes, 3.1.2.27 (chologyl-CoA hydrolase), 6.2.1.7 (cholate:CoA ligase), 2.3.1.65 (bile acid CoA:amino acid N-acyltransferase), 3.5.1.24 (urocanase). *P*-values were calculated using the two-tailed Wilcoxon rank-sum test. Data are expressed as the mean $\pm$ SEM (*n* = 6). **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

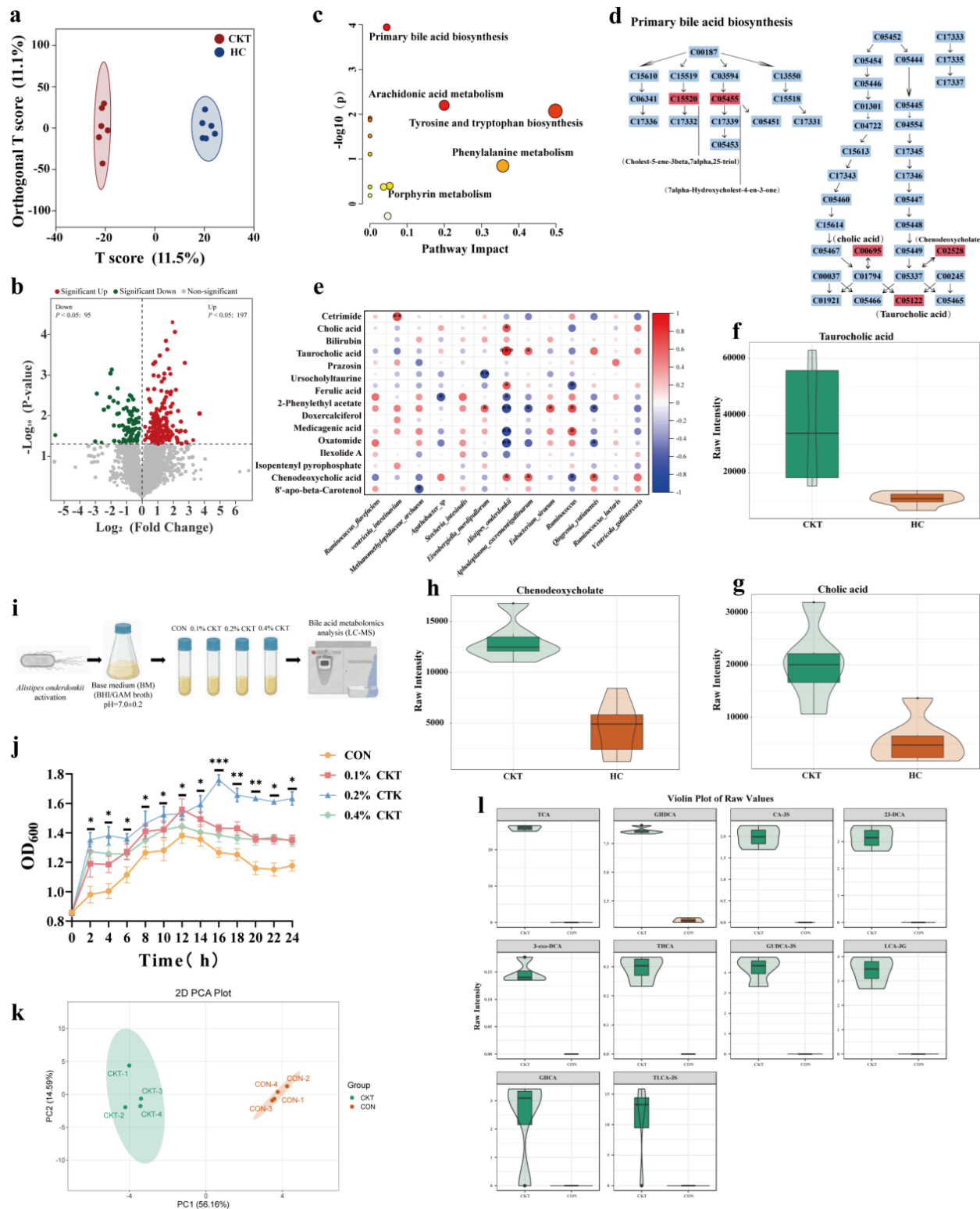


Figure 4

CKT enriches *Alistipes onderdonkii* and promotes taurocholic acid production. (a) OPLS-DA of colonic metabolites between the HC and CKT lamb. (b) Significantly up-regulated and down-regulated metabolites are represented as 'red' and 'blue' dots in volcano plot, respectively. (c) Significantly changed metabolites in the two groups. (d) Primary bile acid biosynthesis. (e) Spearman's correlation analysis between differential metabolites and the differential abundant colonic microbes. (f-h) TCA, Cholic acid and chenodeoxycholic acid levels. (i) Culture of *Alistipes onderdonkii*. (j) In vitro growth of *Alistipes onderdonkii* on graded concentrations of CKT. (k) PCA of bile acid metabolites between the CON and CKT groups bacterial broth. (l) Differential metabolites of bile acids. *P*-values were calculated using the two-tailed Wilcoxon rank-sum test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

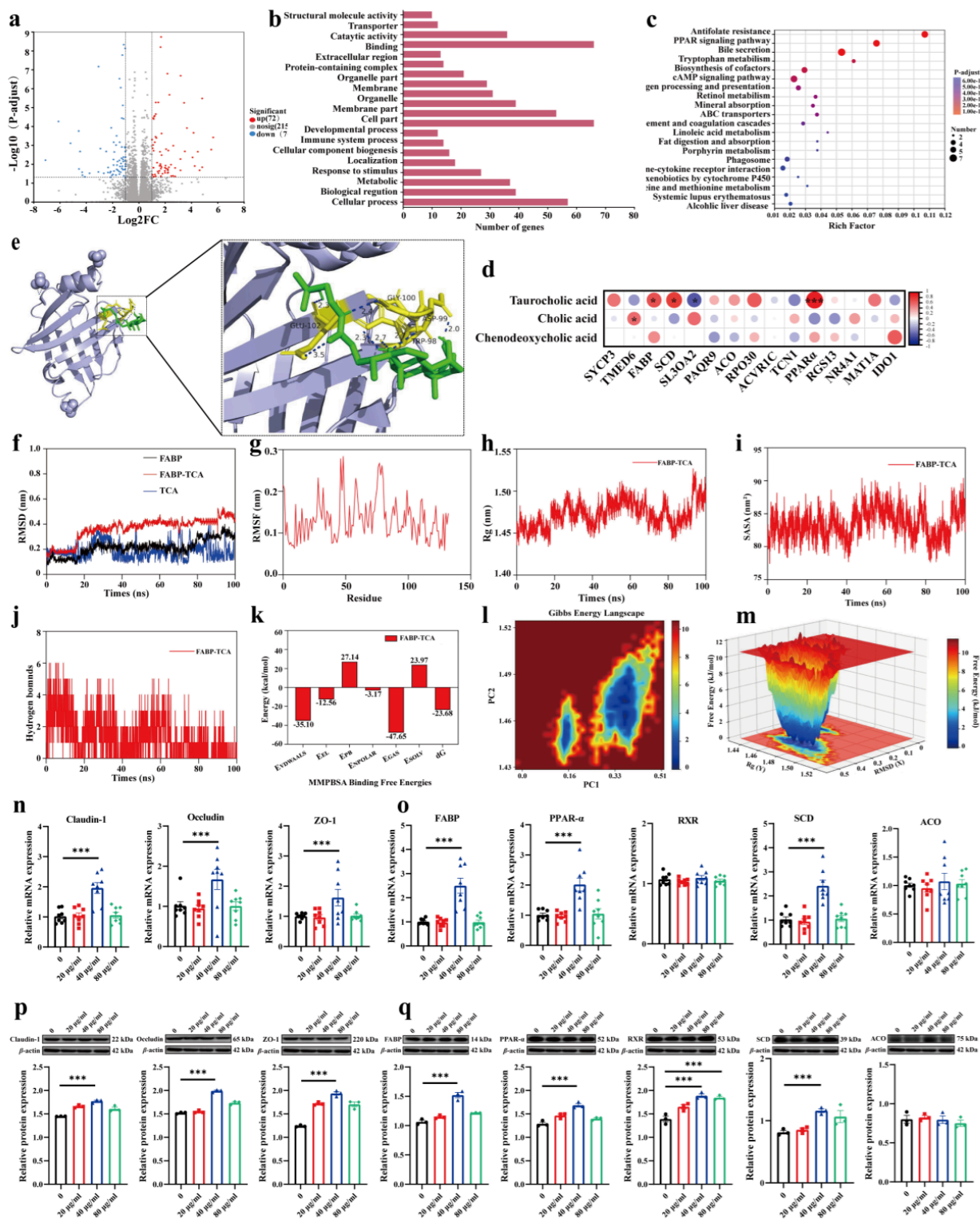


Figure 5

Colon tissue transcriptomic profiles of the CKT and HC groups (n=6). (a) Volcano plot of differentially expressed genes (DEGs). (b,c) GO and KEGG enrichment analyses of DEGs. (d) Spearman correlation between differential metabolites and DEGs. (e) Molecular docking model showing TCA binding with FABP. (f–m) Molecular dynamics analyses, including RMSD, RMSF, Rg, SASA, hydrogen bonds, binding free energy, and Gibbs energy landscapes. (n–q) Effects of TCA treatment on tight junction proteins and

PPAR signaling-related genes at mRNA and protein levels in OCMECs. *P*-values were calculated using the two-tailed Wilcoxon rank-sum test. *** *P* < 0.001.

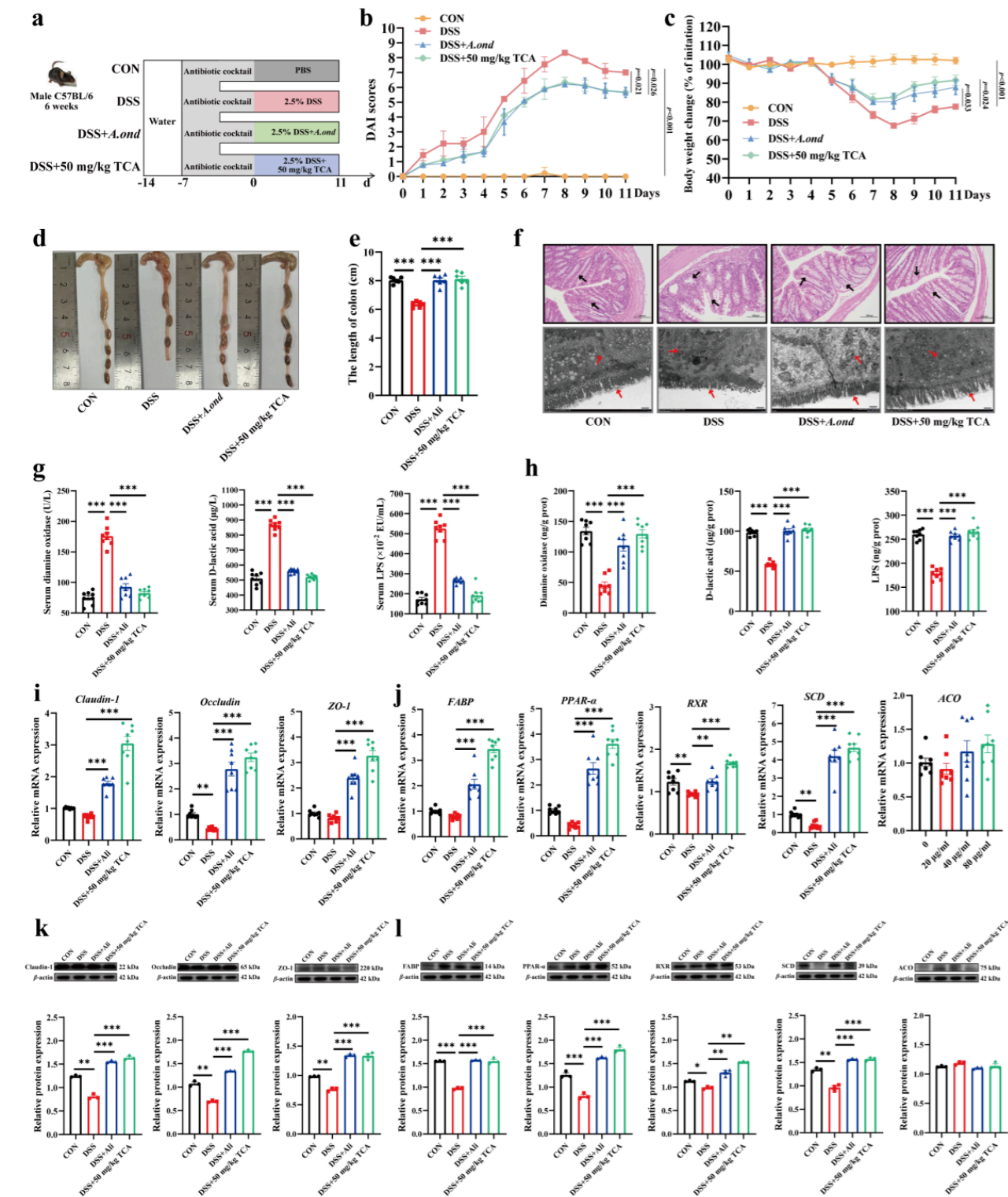


Figure 6

TCA and *Alistipes onderdonkii* are highly effective in ameliorating intestinal barrier dysfunction in mice (n=8). (a) Schematic diagram of experimental animal grouping and treatment. (b) DAI score in each

group. (c) Body weight change in each group. (d-e) Representative colon images and colon length. (f) H&E and TEM tissue sections of mice colon, scale bars represent 200 μm . (g, h) Serum and colon activity of DAO, concentrations of D-lactic acid, and concentrations of LPS. (i-l) mRNA and protein expression of tight junction proteins and PPAR signaling-related genes in colon tissues. Data are expressed as the mean $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Supplementary Files

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