

Goat bile acid and *Clostridium butyricum* exert a synergistic effect in enhancing the therapeutic efficacy against diarrhea induced by *Escherichia coli* infection

Zhiyu Zhu

2071585202@qq.com

Qingyun Bu

Chen Li

Haoyu Sun

Yue Li

Yunfei Deng

Feier Liu

Ning Jia

kai Wei

Zhenhong Sun

Research Article

Keywords: Goat bile acid, *Clostridium butyricum*, *Escherichia coli*, Lamb, Intestine

Posted Date: March 24th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-9061762/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Probiotics and Antimicrobial Proteins on September 23rd, 2026. See the published version at <https://doi.org/10.1007/s12602-026-11196-3>.

Abstract

The goat industry has emerged as one of the major livestock sectors in China, with an annual production exceeding 300 million head. Bacterial diseases in goat pose significant threats to the sustainability and productivity of goat farming. Clinically, antibiotic treatments in ruminants are associated with considerable side effects, raising concerns about food safety. Therefore, the development of antibacterial functional feed additives has become an urgent priority for the industry. Bile acids are endogenous molecules synthesized in the liver through cholesterol metabolism and serve critical physiological roles, including hepatobiliary protection, reduction of oxidative stress, and enhancement of intestinal barrier function. They exert antimicrobial effects against pathogenic *Escherichia coli* (*E. coli*) primarily through direct mechanisms—such as disruption of the phospholipid bilayer of the bacterial cell membrane, leading to intracellular content leakage and subsequent bacterial cell death. Indirectly, bile acids promote the secretion of water and electrolytes in the intestine and modulate the gut microbiota composition, thereby creating an intestinal environment unfavorable for pathogenic bacterial survival. *Clostridium butyricum* (*C. butyricum*) contributes to intestinal health by maintaining gut microecological stability and reinforcing the intestinal barrier. Upon entering the intestinal tract, *C. butyricum* produces the antimicrobial agent butyric acid, which lowers the intestinal pH and thereby inhibits the growth of pathogenic *E. coli*. One of its core functions is to promote the proliferation and repair of intestinal epithelial cells and enhance the expression of tight junction proteins, facilitating the restoration of the intestinal barrier damaged by *E. coli* infection, and ultimately alleviating diarrhea and systemic inflammation at their source. Goat bile acid and *C. butyricum* exhibit strong potential for the treatment of *E. coli*-induced diarrhea. While goat bile acid excels in direct pathogen suppression, *C. butyricum* specializes in repairing and restoring intestinal homeostasis. Their combination offers a comprehensive therapeutic strategy that spans acute pathogen control to long-term mucosal recovery, presenting a highly promising biological alternative for reducing or replacing antibiotic use—particularly in cases involving antibiotic-associated dysbiosis and antimicrobial resistance. In this study, a mouse model of *E. coli* infection was first established to investigate the protective effects of dietary supplementation with goat bile acid and *C. butyricum* in infected mice, aiming to determine the optimal infection dose for the *E. coli*-induced diarrhea model and to identify effective therapeutic doses of goat bile acid and *C. butyricum*. The results demonstrated that concurrent supplementation with 200 mg/kg goat bile acid and 0.1 g/mouse of *C. butyricum* freeze-dried powder significantly alleviated infection-related damage and conferred protective benefits. Based on these findings, a diarrheal lamb model was further developed to evaluate the protective efficacy of the same interventions against *E. coli* infection in a clinically relevant large animal model. Dietary co-administration of 50 mg/kg goat bile acid and 5 g/lamb of *C. butyricum* freeze-dried powder was shown to enhance antioxidant capacity, reduce intestinal inflammatory responses, improve jejunal architecture, strengthen intestinal barrier function, and increase gut microbiota diversity in infected lambs. Notably, the combination regimen outperformed goat bile acid monotherapy. This study demonstrates that dietary supplementation with both goat bile acid and *C. butyricum* effectively protects lambs against *E. coli* infection and holds promise for practical application in ruminant health management.

Introduction

China is the world's largest country in terms of goat breeding, mutton production, and mutton consumption. The goat population has remained consistently above 300 million, with annual slaughter numbers also reaching approximately 300 million, ensuring a stable supply for the domestic mutton market (Amoah et al., 2024). The primary goat farming systems in China include traditional grazing, pen feeding, semi-grazing and semi-pen feeding, and modern intensive farming. Currently, pen feeding and the semi-grazing/semi-pen feeding system represent the dominant production models, while intensive farming is emerging as the principal direction for future development (Hume et al., 2011). Pathogenic *E. coli* in goat is a common opportunistic pathogen, and in intensive farming systems, it ranks among the most severe bacterial infectious agents. It primarily induces diarrhea and high mortality in neonatal lambs, which are critical to flock productivity and farm profitability. The death of lambs, as key biological assets, can result in substantial economic losses for goat operations (Gozi et al., 2021). Farmers commonly rely on antibiotics for the prevention and treatment of colibacillosis in goat. However, due to prolonged and inappropriate antibiotic use in farming practices, bacterial resistance has become increasingly prevalent, resulting in high rates of clinical treatment failure. Furthermore, antibiotics often fail to completely eradicate pathogenic bacteria, making the disease prone to recurrence following treatment (Zhao et al., 2022). Moreover, ruminants are less amenable to repeated antibiotic treatments compared to monogastric animals due to their complex gastrointestinal physiology and susceptibility to microbial imbalance (Tardiolo et al., 2025). Therefore, the development of novel biological products capable of reducing or replacing antibiotic use has become an urgent priority in the livestock industry.

Bile acids, as key components of bile, are endogenous molecules synthesized in the liver through cholesterol metabolism. Owing to their safety, environmental compatibility, and high efficacy, bile acids have been widely adopted in the livestock and aquaculture industries as a state-recognized novel feed additive (Li et al., 2019). Our studies have demonstrated that bile acids exert protective effects on the hepatobiliary system, reduce oxidative stress, and enhance intestinal barrier function. Dietary supplementation with bile acids has been shown to significantly increase the abundance of beneficial bacteria such as *Lactobacillus reuteri* in the ileum and *Clostridium leptum* in the colon, elevate butyric acid levels, and promote the expression of its receptors, thereby contributing to the maintenance of intestinal microecological stability (Yang et al., 2017). Some studies have demonstrated that bile acids can directly kill bacteria and inhibit the activity of *Clostridium difficile* toxin B, indicating their capacity to counteract bacterial toxins. By independently suppressing bacterial virulence—without direct killing—they significantly reduce pathogenicity while minimizing selective pressure for the development of antimicrobial resistance (Icho et al., 2023). goat-derived bile acids provide a multi-pronged strategy: they not only exert direct bactericidal effects but also "disarm" pathogens by neutralizing toxins and suppressing virulence factor expression, which is particularly advantageous in managing drug-resistant *E. coli* infections.

Clostridium butyricum (*C. butyricum*) is a Gram-positive, anaerobic bacterium isolated from the intestines of healthy humans and animals, known for its beneficial probiotic properties. It exhibits high tolerance to

heat, acid, and multiple antibiotics, and has the capacity to produce butyric acid. *C. butyricum* can modulate host intestinal microecological balance, restore intestinal barrier function, and enhance systemic immune responses (Stoeva et al., 2021). *C. butyricum* inhibits the growth of pathogenic *E. coli* by producing the antibacterial metabolite butyric acid and bacteriocin, a protein with antibiotic-like activity. It also promotes the repair of intestinal epithelial cells and enhances the expression of tight junction proteins, thereby restoring the intestinal barrier integrity compromised by *E. coli* infection. This effectively prevents bacterial and toxin translocation into the bloodstream, contributing to the fundamental alleviation of diarrhea and systemic inflammation (Fu et al., 2020). The core functions of *C. butyricum* include repairing the intestinal barrier, modulating immune responses, and exerting competitive exclusion. These biological processes are time-dependent and typically require several h to several days to achieve significant effects. Therefore, it is not suitable as a monotherapy for acute conditions and should be used in combination with other therapeutic agents (Xie et al., 2025). In clinical practice, *C. butyricum* is primarily used as a feed additive for preventive purposes. Long-term supplementation with a low dose of *C. butyricum* enables the establishment of a healthy intestinal microecological environment in advance. It can also serve as an adjuvant therapy, acting synergistically with other therapeutic agents (Wu et al., 2025). The combined use of goat bile acids and *C. butyricum* theoretically enables a synergistic effect, forming a highly effective therapeutic strategy. goat bile acids can rapidly reduce the load of pathogenic *E. coli* by leveraging their antibacterial and antitoxin properties. Subsequently, *C. butyricum* not only suppresses residual pathogenic bacteria but also repairs the intestinal mucosa damaged by *E. coli* infection. Some probiotics are inhibited or inactivated by bile acids, whereas *C. butyricum* can form spores and exhibits exceptional tolerance to bile acids, enabling it to transit through the upper gastrointestinal tract successfully and survive and colonize in the intestinal environment containing these compounds (Zhou et al., 2021). This enables the combination with goat bile acids. Goat bile acids and *C. butyricum* show considerable potential for treating *E. coli*-induced diarrhea. goat bile acids are effective in directly targeting pathogens, whereas *C. butyricum* excels at repairing and restoring intestinal health. Together, they theoretically constitute a therapeutic strategy that spans acute pathogen control to long-term mucosal recovery, offering a promising biological alternative to reduce or replace antibiotics—particularly in addressing dysbiosis and antimicrobial resistance caused by conventional antibiotic therapy. However, the specific efficacy requires further scientific validation.

Based on previous research, this project selects *C. butyricum* and ovine bile acids as the target agents. By artificially establishing mouse and lamb infection models, it evaluates the therapeutic efficacy of their combined application in *E. coli*-induced diarrheal infections, with systematic investigation conducted from the perspectives of anti-inflammatory effects, antioxidant activity, intestinal barrier repair, and intestinal microecological regulation. The aim is to explore the synergistic potential of combining microecological preparations with animal-derived antibacterial substances, provide a theoretical foundation for the development of green antibacterial additives for ruminants, and ultimately reduce clinical dependence on antibiotics.

Materials and Methods

Ethics Statement

All animal experiments were conducted in strict accordance with relevant ethical guidelines and regulations. The experimental protocol was reviewed and approved by the Animal Care and Use Committee of Shandong Agricultural University (Approval No. 20010510).

Extraction of bile acids (BAs)

Fresh goat gallbladders were purchased from a local farmers' market in Tai'an. Bile acid (BAs) extraction was performed according to the method described by (Huang et al., 2025). Briefly, 500 mL of fresh goat bile was measured, filtered, and mixed with 95% ethanol, followed by heating to boiling. After cooling, the ethanol was recovered, and dilute hydrochloric acid was added to adjust the pH to ≥ 3.0 . A viscous precipitate subsequently formed, which was re-dissolved in ethanol, decolorized with activated carbon, and filtered; the ethanol in the filtrate was then recovered. The residue was dried to yield a white or light yellow solid. This solid was redissolved in ethanol and decolorized again, with the purification cycle repeated three times. Finally, the purified material was ground to obtain pure goat bile acid powder.

Animals and Experimental Design

The *C. butyricum* strain used in this study was provided by Shandong Fanyin Biotechnology Co., Ltd., with a concentration of 1×10^9 CFU/g. All animal experiments were conducted in strict accordance with applicable ethical guidelines and regulations.

Mice experiment: Ninety six-week-old Kunming (KM) mice were selected and, after a three-day acclimatization period, randomly assigned to five groups, with three replicates per group and six mice per replicate. The groups were as follows: the control group (Control), *E. coli* infection group (*E. coli*), goat BAs group (BAs), *C. butyricum* group (*C. B*), and *C. butyricum* + goat BAs group (*C. B* + BAs). The Control and *E. coli* groups received the basal diet only; the BAs group received the basal diet supplemented with 200 mg/kg goat bile acids; the *C. B* group received the basal diet supplemented with 0.1 g/kg *C. butyricum* freeze-dried powder; and the BAs + *C. B* group received the basal diet supplemented with both 200 mg/kg goat bile acids and 0.1 g/kg *C. butyricum* freeze-dried powder. All treatments were administered once daily for seven consecutive days. Body weights were measured at 1, 4, 7, and 10 days post-treatment (dpt) by randomly selecting three mice from each group. At 7 dpt, 1.0 mL of an *E. coli* bacterial suspension (1.0×10^8 CFU/mL) was intraperitoneally injected into the *E. coli*, BAs, *C. B*, and *C. B* + BAs groups. The *E. coli* strain was isolated from fecal samples of clinically infected lambs in the laboratory and has been preserved for experimental use. The challenge dose was determined based on preliminary trials. Clinical signs, incidence, and mortality were recorded following infection, and tissue and biological samples were collected at 10 dpt.

Goat experiment: Twelve 6-week-old Bordeaux goats were selected and acclimatized for 3 days before being randomly assigned to four groups, with three animals per group. The groups were as follows: Control, *E. coli* infection group, goat BAs group (BAs), and *Clostridium butyricum* + goat BAs group (C. B + BAs). The Control and *E. coli* groups received the basal diet only; the BAs group received the basal diet supplemented with 50 mg/kg goat bile acids; and the C. B + BAs group received the basal diet supplemented with 50 mg/kg goat bile acids and 5 g/kg *C. butyricum* freeze-dried powder. All treatments were administered once daily for 14 consecutive days. The dosages were determined based on standard feeding practices observed on commercial goat farms. At 14 dpt, 1.0 mL of an *E. coli* bacterial suspension (5×10^9 CFU/mL) was administered orally to the *E. coli*, BAs, and C. B + BAs groups. The *E. coli* strain was previously isolated from fecal samples of clinically infected lambs in the laboratory and has been preserved for experimental use. The challenge dose was established through preliminary trials. Tissue and biological samples were collected at 21 dpt.

Sample collection

Ten days after the mice were challenged, their body weights were measured. They were then anesthetized with ether and decapitated. The blood was collected and left to stand at 37°C for 2 h. After centrifugation at 3000 r/min for 15 min, the upper serum layer was taken and stored at -80°C for future use. The intestinal samples were taken after dissection and stored at -80°C or fixed in 4% paraformaldehyde. The lambs were sent to the slaughterhouse 21 days after challenge, where they were stunned and bled from the jugular vein. Blood, liver and intestinal samples were collected and stored at -80°C. Cecal content samples were collected and frozen in liquid nitrogen for 16S rRNA sequencing.

Detection of blood biochemical indicators

The serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) were measured according to the manufacturer's instructions using assay kits from Nanjing Jiancheng Bioengineering Institute (Batch No.: 20250508).

Detection of antioxidant capacity in serum, liver and jejunum

The activities of superoxide dismutase (SOD) and reduced glutathione (GSH), as well as the level of malondialdehyde (MDA), in serum, liver tissue, and jejunum tissue were measured using commercial assay kits from Nanjing Jiancheng Bioengineering Institute (Batch No.: 20250429). To quantitatively assess the antioxidant capacity of liver and jejunum tissues, 10% tissue homogenates were prepared in physiological saline, centrifuged at 2500×g for 10 min, and the supernatants were collected for measurement of oxidative stress markers.

Real-time fluorescence quantitative PCR (qRT-PCR) was employed to quantify the mRNA expression levels in tissues.

The mRNA expression levels of the target genes in jejunum tissue were quantified by qRT-PCR. Total RNA was extracted from liver and jejunum tissues using TRIzol reagent (CW BIO, China) according to the manufacturer's instructions for the Ultrapure RNA Kit (CW BIO, China). The concentration and purity of the extracted RNA were assessed using a micro UV spectrophotometer and adjusted to a uniform concentration. RNA samples were reverse transcribed into cDNA using the HiScript QRT SuperMix for qPCR (+ gDNA wiper) (Vazyme, China). The primer sequences for the target genes are listed in Table 1 and were synthesized by Beijing Qikexin Biotechnology Co., Ltd. qPCR amplification was performed using the SYBR Green method with β -actin as the internal reference gene. The relative mRNA expression levels of the target genes were calculated using the comparative CT method ($2^{-\Delta\Delta C_T}$).

Table 1
Primer sequences

Primer name	Forward(5'→3')	Reverse(5'→3')
β -actin	GAGAAGAGCTACGAGCTGCC	CGCAGGATTCCATGCCCAG
TNF- α	AGGGAAGAGCAGTCCCCAG	CGCTGATGTTGGCTACAACG
IL-1 β	TGCTGGATAGCCCATGTGTG	TGCAGAACACCACTTCTCGG
IL-6	AATCTGGGTTCAATCAGGCGA	GCTCTGCAACTCCATGACAG
IL-4	TTGGCAAGCAAGACCTGTTC	TTTCCAAGAGGTCTCTCAGCG
IL-10	TCAAGGAGCACGTGAACTCG	CAGAAAACGATGACAGCGCC
ZO-1	CTCCTCGTCGGGTGATCCT	ACAGAAACACAGTTTGCGCC
Occludin	CCATACCACTCCTCCTCCGTA	GTAGTGATTAGGTTTGCTGCGG
Claudin-1	GAGTAGGGTGCTGTGGTTTGA	TATCACCTGCACACGCCCAT

Pathological histological analysis

After jejunal tissues from each group were collected and fixed in 4% paraformaldehyde for 24 h, they were sequentially dehydrated through a graded ethanol series (70%, 80%, 95%, and 100%) with 40 min at each concentration, followed by clearing in xylene. The tissues were then embedded and sectioned into 5 μ m-thick slices using a microtome, baked at 60°C for 1 hour, dewaxed in xylene, rehydrated through a descending alcohol series, and stained with hematoxylin and eosin (H&E). The sections were mounted with neutral balsam and examined under a light microscope to capture clear and complete images. Histological architecture, pathological changes, and goblet cell numbers in the jejunal tissues were evaluated.

Statistical analysis

In this study, Graphpad Prism 8.0 statistical analysis software was used for graphing and calculating the means and standard deviations (SD) of each group. The data of each group were expressed as Mean $\pm$ SD. Among them, “*” indicated $P < 0.05$, with significant differences; “**” indicated $P < 0.01$, with extremely significant differences; and “ns” indicated no significant difference, with $0.05 < P \leq 0.10$, showing a significant trend.

Results and analysis

The impact of ovine bile acids on body weight dynamics and mortality in mice infected with *E. coli*

To investigate the effects of *E. coli* infection on body weight dynamics and mortality in mice, we monitored weight changes and survival outcomes in each experimental group and presented the data using kinetic curves (Fig. 1). Within 7 dpi, mice in the *E. coli*, C. B, C. B + BAs, and BAs groups exhibited clinical signs including depression, reduced appetite, and decreased activity, with the first deaths occurring within 12 h after infection. Significant weight loss was observed in all infected groups, with the most pronounced reduction in the *E. coli* group and the least in the C. B + BAs group. The survival rates were 100% in the Control group, 40% in the *E. coli* group, 55% in the C. B group, 70% in the C. B + BAs group, and 60% in the BAs group. These findings suggest that the combined administration of ovine bile acids and *C. butyricum* mitigates *E. coli*-induced weight loss and enhances survival in infected mice.

The effects of ovine bile acids and *C. butyricum* on jejunal histopathology in mice infected with *E. coli*

To investigate the effects of ovine bile acids and *C. butyricum* on jejunal morphology in mice following *E. coli* infection, H&E staining was performed on jejunal tissue sections (Fig. 2). Histopathological examination revealed that *E. coli* infection induced significant structural damage to the jejunum. However, this damage was ameliorated following treatment with ovine bile acids and *C. butyricum*. In the *E. coli* group, H&E staining showed extensive disruption of the mucosal epithelium, exposure of the lamina propria, and widespread necrosis and dissolution of intestinal gland tissues within the lamina propria, which were replaced by proliferative connective tissue. In contrast, the C. B, C. B + BAs, and BAs groups exhibited only mild connective tissue proliferation and focal areas of glandular necrosis, indicating partial preservation of jejunal architecture.

The effects of ovine bile acids and *C. butyricum* on the expression of tight junction proteins in the mouse jejunum following *E. coli* infection

To investigate the effects of ovine bile acids and *C. butyricum* on tight junction protein expression in the jejunum of mice infected with *E. coli*, we analyzed the mRNA levels of ZO-1, Occludin, and Claudin-1 in jejunal tissues (Fig. 3). Compared with the Control group, the *E. coli* group exhibited significantly reduced mRNA expression of ZO-1, Occludin, and Claudin-1 in the jejunum ($P < 0.01$). Relative to the *E. coli* group, the C. B group showed significant upregulation of ZO-1 and Occludin mRNA ($P < 0.05$), while the C. B +

BAs group demonstrated marked increases in the mRNA expression of all three proteins ($P < 0.01$). The BAs group significantly elevated ZO-1 mRNA levels ($P < 0.05$) and Occludin mRNA levels ($P < 0.01$). These results indicate that *E. coli* infection downregulates the expression of tight junction proteins ZO-1, Claudin-1, and Occludin in the mouse jejunum, and treatment with ovine bile acids and *C. butyricum* can partially restore their expression.

The effects of ovine bile acids and *C. butyricum* on jejunal inflammatory responses in mice infected with *E. coli*

E. coli infection typically induces an inflammatory response characterized by elevated levels of pro-inflammatory cytokines and reduced levels of anti-inflammatory cytokines in tissues and organs. To evaluate the effects of ovine bile acids and *C. butyricum* on *E. coli*-induced inflammation, we measured the mRNA expression levels of pro-inflammatory cytokines IL-1 β and TNF- α , as well as anti-inflammatory cytokines IL-4 and IL-10, in jejunal tissues of mice from each group using qRT-PCR (Fig. 4). As shown in the figure, compared with the Control group, the *E. coli* group exhibited significantly higher expression of IL-1 β and TNF- α ($P < 0.01$) and significantly lower expression of IL-4 and IL-10 ($P < 0.01$). Relative to the *E. coli* group, both the C. B and C. B + BAs groups significantly attenuated the upregulation of IL-1 β and TNF- α ($P < 0.01$). The C. B group significantly mitigated the downregulation of IL-4 and IL-10 ($P < 0.05$), and the C. B + BAs group showed a more pronounced effect with significant restoration of these anti-inflammatory factors ($P < 0.01$). The BAs group significantly reduced the expression of IL-1 β ($P < 0.01$) and TNF- α ($P < 0.01$), and also partially reversed the suppression of IL-4 and IL-10 ($P < 0.05$). These findings indicate that *E. coli* infection triggers a robust inflammatory response in the mouse jejunum, and dietary supplementation with ovine bile acids and *C. butyricum* can effectively alleviate this inflammatory reaction.

The effects of ovine bile acids and *C. butyricum* on the antioxidant capacity in mice infected with *E. coli*

Studies have shown that *E. coli* infection can lead to an increase in ROS content in the animal body, causing oxidative stress and damaging the animal's immune system. Therefore, we detected the changes in the oxidative indicators MDA and SOD in the liver tissue, jejunum tissue, and serum of each group of mice to evaluate the effects of goat bile acid and *C. butyricum* on antioxidant capacity (Fig. 5). As shown in the figure, the MDA levels in the liver tissue, jejunum tissue, and serum of the *E. coli* group were significantly and extremely significantly increased compared to the Control group after *E. coli* infection. Adding goat bile acid and *C. butyricum* to the diet could significantly alleviate the increase in MDA levels in the jejunum ($P < 0.05$) and extremely significantly alleviate the increase in MDA levels in the serum and liver tissue ($P < 0.01$) caused by *E. coli*. Compared to the *E. coli* group, the C. B group could extremely significantly alleviate the increase in MDA levels in the liver ($P < 0.01$) and significantly alleviate the increase in MDA levels in the serum ($P < 0.05$), with no significant difference in MDA levels in the jejunum. The BAs group could significantly alleviate the increase in MDA levels in the liver ($P < 0.05$), with no significant difference in MDA levels in the jejunum tissue and serum. Compared to the Control group, the SOD content in the liver tissue, jejunum tissue, and serum of the *E. coli* group decreased

extremely significantly. Compared to the *E. coli* group, the C. B group could extremely significantly alleviate the decrease in SOD levels in the jejunum tissue ($P < 0.01$) and significantly alleviate the decrease in SOD levels in the liver tissue and serum ($P < 0.05$); the C. B + BAs group could extremely significantly alleviate the decrease in SOD levels in the liver tissue, jejunum tissue, and serum. This indicates that *E. coli* infection can cause oxidative stress in mice, and goat bile acid and *C. butyricum* have strong antioxidant capacity.

The effects of ovine bile acids and *C. butyricum* on jejunal histology in goats infected with *E. coli*

To investigate the effects of ovine bile acids and *C. butyricum* on jejunal morphology in goats following *E. coli* infection, H&E staining was performed on jejunal tissue sections (Fig. 6). Histopathological examination revealed that *E. coli* infection induced structural damage in the goat jejunum. However, this damage was ameliorated by treatment with ovine bile acids and *C. butyricum*. In the *E. coli* group, the jejunal architecture was severely disrupted, with extensive necrosis, accumulation of necrotic cellular debris and amorphous eosinophilic material, and widespread loss of intestinal gland epithelial cells due to necrosis and sloughing. In contrast, the C. B + BAs group exhibited numerous leaf-like villi of reduced length and minimal epithelial cell shedding. The BAs group displayed abundant finger-like villi that were elongated, although accompanied by moderate epithelial cell shedding and loss. The submucosa consisted of loose connective tissue with mild granulocyte infiltration. These findings suggest a partial protective effect of the interventions on jejunal integrity.

The effects of ovine bile acids and *C. butyricum* on the expression of tight junction proteins in the jejunal tissue of goats infected with *E. coli*

To investigate the effects of ovine bile acids and *C. butyricum* on jejunal barrier function in goats infected with *E. coli*, we analyzed the mRNA expression levels of tight junction proteins ZO-1, Occludin, and Claudin-1 in jejunal tissues (Fig. 7). Compared with the Control group, the *E. coli* group exhibited significantly reduced mRNA expression of ZO-1 and Occludin ($P < 0.01$) and a highly significant decrease in Claudin-1 expression ($P < 0.001$). Relative to the *E. coli* group, the C. B + BAs group showed significant upregulation of ZO-1 and Claudin-1 ($P < 0.01$) and a moderate increase in Occludin expression ($P < 0.05$). The BAs group significantly increased the mRNA levels of ZO-1 and Claudin-1 ($P < 0.05$), but no significant change was observed in Occludin expression compared to the *E. coli* group. These results indicate that *E. coli* infection downregulates the expression of key tight junction proteins—ZO-1, Occludin, and Claudin-1 in the goat jejunum, and supplementation with ovine bile acids and *C. butyricum* can partially restore their expression. Notably, the combination treatment exerted a more pronounced protective effect than either agent alone.

The effects of ovine bile acids and *C. butyricum* on jejunal inflammatory responses in goats infected with *E. coli*

E. coli infection typically induces an inflammatory response characterized by elevated levels of pro-inflammatory cytokines and reduced levels of anti-inflammatory cytokines in tissues and organs. To

evaluate the effects of caprine bile acids and *C. butyricum* on *E. coli*-induced inflammation, we measured the mRNA expression levels of pro-inflammatory cytokines IL-1 β , TNF- α , and IL-6, as well as anti-inflammatory cytokines IL-4 and IL-10, in jejunal tissues of mice from each group using qRT-PCR (Fig. 8). Compared with the Control group, the *E. coli* group exhibited significantly increased mRNA expression of IL-1 β , TNF- α , and IL-6 ($P < 0.01$), while mRNA levels of IL-4 and IL-10 were significantly decreased ($P < 0.01$). Relative to the *E. coli* group, the C. B + BAs group significantly downregulated TNF- α and IL-6 expression ($P < 0.01$), moderately reduced IL-1 β expression ($P < 0.05$), and significantly upregulated IL-4 expression, with no significant change in IL-10. The BAs group significantly suppressed IL-1 β and TNF- α expression ($P < 0.05$) and markedly reduced IL-6 levels ($P < 0.01$), but showed no significant effect on IL-4 or IL-10 expression. These findings indicate that *E. coli* infection triggers a robust inflammatory response in the jejunum, and dietary supplementation with caprine bile acids and *C. butyricum* can mitigate this inflammatory reaction, with the combination treatment showing greater efficacy.

The effects of ovine bile acids and *C. butyricum* on antioxidant capacity in goats infected with *E. coli*

E. coli infection increases reactive oxygen species (ROS) levels in animals, leading to oxidative stress and impaired immune function. To evaluate the effects of caprine bile acids and *C. butyricum* on antioxidant capacity, we measured glutathione (GSH) and superoxide dismutase (SOD) levels in liver tissue, jejunal tissue, and serum from each group of goats using assays corresponding to Fig. 9. Compared with the Control group, the *E. coli* group exhibited a highly significant decrease in GSH levels in the jejunum and serum ($P < 0.01$) and a significant reduction in hepatic GSH ($P < 0.05$). SOD levels in the liver, jejunum, and serum were also profoundly reduced ($P < 0.01$). Relative to the *E. coli* group, the C. B + BAs group significantly increased GSH levels in the liver and jejunum ($P < 0.01$), as well as in the serum ($P < 0.05$), and showed a highly significant restoration of SOD activity across all three compartments ($P < 0.01$). The BAs group significantly elevated hepatic GSH ($P < 0.01$) and serum GSH ($P < 0.05$), but no significant change was observed in jejunal GSH. Additionally, the BAs group significantly enhanced SOD levels in the liver and serum ($P < 0.01$), with no significant improvement in jejunal SOD. These findings indicate that *E. coli* infection induces systemic oxidative stress in goats, and supplementation with caprine bile acids and *C. butyricum* enhances antioxidant defenses, with the combination treatment demonstrating superior efficacy.

The effects of ovine bile acids and *C. butyricum* on serum biochemical indicators in goats infected with *E. coli*

E. coli infection is likely to impair liver function. To assess the impact of *E. coli* infection on hepatic status and the effects of dietary supplementation with ovine bile acids and *C. butyricum*, we measured serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) in goats from each group (Fig. 10). Compared with the Control group, the *E. coli* group exhibited significantly elevated serum AST and ALT levels ($P < 0.001$) and a marked increase in LDH levels ($P < 0.01$). Relative to the *E. coli* group, both the C. B + BAs and BAs groups significantly reduced serum AST, ALT, and LDH levels ($P < 0.01$). These results indicate that *E. coli* infection induces

hepatocellular injury, as reflected by increased serum transaminase and LDH activities, and that dietary supplementation with ovine bile acids and *C. butyricum* effectively mitigates this enzyme elevation, suggesting a protective effect on liver function.

The effects of ovine bile acids and *C. butyricum* on the intestinal microbiota of goats infected with *E. coli*

To investigate the impact of *E. coli* infection on the intestinal microbiota in goats, 16S rRNA gene sequencing was performed, and effective sequences were clustered into operational taxonomic units (OTUs) using the Vsearch algorithm at a 97% sequence similarity threshold (Fig. 11). The total number of OTUs was 6,491 in the Control group, 6,117 in the *E. coli* group, 6,809 in the C. B + BAs group, and 5,427 in the BAs group. A total of 351 OTUs were shared among all four groups, with unique OTUs numbering 5,066, 4,545, 5,606, and 4,306, respectively. The C. B + BAs group exhibited the highest species richness, as reflected by both the total and unique OTU counts, indicating that the combination of caprine bile acids and *C. butyricum* significantly enhanced microbial diversity. To assess sequencing depth, rarefaction curves were analyzed; as sequencing depth increased, the curves approached saturation, suggesting that the sequencing effort adequately captured the majority of microbial diversity in the samples. Rank-abundance curves, which reflect both species richness and evenness, showed low richness and uneven distribution in the *E. coli* group, whereas the C. B + BAs group displayed the highest richness and improved evenness. Alpha diversity analysis revealed that the C. B + BAs group had a significantly lower Simpson index compared to the *E. coli* group ($P < 0.05$), indicating higher diversity, while no significant differences were observed in the Chao1 or ACE indices. At the phylum level, the dominant bacterial taxa included Bacillota (formerly Firmicutes), Bacteroidota, Pseudomonadota, and Verrucomicrobiota. Notably, the relative abundance of Bacillota was increased in the C. B + BAs group compared to the *E. coli* group, suggesting a beneficial modulation of gut microbiota composition. Furthermore, principal coordinate analysis (PCoA) based on beta diversity revealed distinct clustering patterns, with the *E. coli* group clearly separated from the C. B + BAs and BAs groups, indicating significant structural differences in microbial communities. These findings demonstrate that dietary supplementation with caprine bile acids and *C. butyricum*, particularly in combination, can effectively enrich and reshape the intestinal microbiota in *E. coli*-infected goats.

Discussion

The harm and economic losses caused by pathogenic *E. coli* in goat have become increasingly severe, making it one of the most serious infectious diseases threatening the goat industry today. In China, antibiotics are commonly used in clinical practice to prevent and treat *E. coli* infections in goat; however, excessive and inappropriate use has led to widespread bacterial resistance. Against the current backdrop of antibiotic bans and restrictions in animal production, developing effective alternative antibacterial agents has become particularly urgent (Ren et al., 2023). Bile acids are important components of bile and are endogenous molecules synthesized from cholesterol in the liver. Due to their safety, environmental compatibility, and high efficacy, they have been approved by the national

authorities as a novel feed additive and are widely used in livestock and aquaculture industries (Collins et al., 2023). Previous studies have demonstrated that ovine bile acids possess beneficial properties including hepatobiliary protection, reduction of oxidative stress, and improvement of intestinal barrier function (Xu et al., 2023). *C. butyricum*, a Gram-positive, anaerobic bacterium isolated from the intestines of healthy humans and animals, exhibits strong probiotic characteristics (Chen et al., 2019). *C. butyricum* is resistant to heat, acid, and multiple antibiotics, and produces butyric acid (Liang et al., 2021). It can modulate host intestinal microecological balance, restore intestinal barrier integrity, and enhance immune function, thereby offering broad application potential in animal health and production.

The combined use of goat bile acid and *C. butyricum* theoretically can produce synergistic effect, forming a very ideal treatment strategy. goat bile acid can quickly control the number of pathogenic *E. coli* by taking advantage of its antibacterial and antitoxin capabilities. Subsequently, *C. butyricum*, on the one hand, inhibits the remaining pathogenic bacteria and, on the other hand, repairs the intestinal mucosa damaged by *E. coli*. Some probiotics are inhibited or killed by bile acid, but *C. butyricum* can form spores and has extremely strong tolerance to bile acid, allowing it to smoothly pass through the upper digestive tract and survive and colonize in the intestinal environment containing bile acid (Zhou et al., 2021). This makes the combination of it and goat bile acid possible. Goat bile acid and *C. butyricum* have great potential in treating diarrhea caused by *E. coli*. Here, we evaluated the protective effects of ovine bile acids and *C. butyricum* in mice and goats infected with *E. coli*. We demonstrated that dietary supplementation with ovine bile acids and *C. butyricum* mitigates the adverse effects of *E. coli* infection by enhancing antioxidant capacity and intestinal immune function, reducing inflammatory responses, and improving intestinal barrier integrity and microbiota composition in both animal models.

The jejunum is a key segment of the gastrointestinal tract and serves as the primary site for nutrient digestion and absorption in the body (Koga et al., 2012). In this study, *E. coli* infection was found to induce structural damage in the jejunum of both mice and goats, whereas supplementation with ovine bile acids and *C. butyricum* alleviated such intestinal injury. Tight junction proteins play a critical role in maintaining intestinal barrier function and integrity and constitute a vital defense mechanism against inflammation and dysbiosis. Reduced expression of these proteins has been shown to increase intestinal permeability, compromising gut health (Kuo et al., 2022). Our results demonstrate that ovine bile acids and *C. butyricum* upregulated the mRNA expression of Occludin, Claudin-1, and ZO-1 in the jejunum of *E. coli*-infected mice, a finding consistent with observations in infected goats. This protective effect may be mediated through oxidative stress pathways, as oxidative stress can disrupt tight junctions; ovine bile acids may counteract this disruption by enhancing systemic antioxidant capacity, thereby preserving junctional protein integrity. The intestinal mucosal immune system functions largely through lymphocytes, including intraepithelial lymphocytes, which secrete cytokines such as IL-4 and IL-10, playing essential roles in maintaining mucosal integrity and regulating immune responses. Alterations in cytokine levels and gene expression serve as indirect indicators of host health status (Chen et al., 2021). In *E. coli*-infected mice, jejunal expression of pro-inflammatory cytokines IL-6, IL-1, and TNF- α was significantly elevated, while anti-inflammatory cytokines IL-4 and IL-10 were markedly downregulated. Dietary supplementation with ovine bile acids and *C. butyricum* effectively reversed these

imbalances, and similar trends were observed in infected goats. Notably, the combination of ovine bile acids and *C. butyricum* exerted a more pronounced protective effect than bile acid treatment alone.

E. coli infection induces systemic inflammation and triggers the accumulation of reactive oxygen species (ROS) in organs and tissues. Excessive ROS accumulation can impair cellular activity, leading to apoptosis and necrosis, ultimately compromising normal tissue and organ function (Zeng et al., 2023). In this study, *E. coli* infection in mice significantly increased malondialdehyde (MDA) levels in the liver, jejunum, and serum, while decreasing superoxide dismutase (SOD) activity. Dietary supplementation with ovine bile acids and *C. butyricum* alleviated these oxidative stress markers, indicating a protective effect against *E. coli*-induced oxidative damage. Consistent with findings in mice, *E. coli* infection in goats also altered the levels or activities of MDA, SOD, and glutathione (GSH) in liver, jejunum, and serum, indicative of oxidative injury. The present results demonstrate that ovine bile acids and *C. butyricum* mitigate oxidative damage in both animal models; however, the underlying mechanisms remain to be fully elucidated and warrant further investigation.

Serum biochemical indicators can reflect the health status of animals to some extent (Tang et al., 2022). Consistent with previous findings, this study showed that the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) in the serum of goats infected with *E. coli* were significantly elevated, indicating that *E. coli* infection induced hepatic dysfunction. Dietary supplementation with ovine bile acids and *C. butyricum* alleviated this impairment to varying degrees.

The digestive system microecosystem of ruminants is a complex environment composed of diverse microbial communities, with bacteria playing a dominant role (Asakura et al., 2022). These microbial populations provide the host with a wide range of enzymes and play a crucial role in promoting nutrient digestion and absorption, supporting animal growth and development, and enhancing immune function. *E. coli* infection disrupts intestinal homeostasis in the host, and restoring this balance represents an effective strategy for managing *E. coli* infection. In this study, *E. coli* infection in goats was associated with reduced diversity and richness of intestinal microbiota. However, dietary supplementation with bile acids and *C. butyricum* increased the abundance of Firmicutes in the intestine compared to the *E. coli* group. Most members of the phylum Firmicutes are beneficial bacteria that play a key regulatory role in maintaining intestinal health, suggesting that bile acids and *C. butyricum* can modulate the intestinal microbiota and may contribute significantly to the restoration of microbial homeostasis.

In conclusion, our results demonstrate that *E. coli* infection causes damage to intestinal morphology and structure, impairs intestinal barrier function, induces intestinal inflammation, reduces systemic antioxidant capacity, and disrupts the homeostasis of the gut microbiota in both mice and goats. Dietary supplementation with ovine bile acids and *C. butyricum* effectively improves serum enzyme activity and antioxidant capacity, alleviates intestinal inflammatory responses, enhances intestinal barrier integrity, and promotes microbial richness and diversity in *E. coli*-infected animals. The combined use of ovine bile acids and *C. butyricum* exerts a synergistic protective effect in infected mice and goats. These findings

provide theoretical and experimental support for the joint development of these agents as novel green antibacterial additives in animal production.

Declarations

Author Contribution

Methodology: Z.Y.Z, Q.Y.B. Project administration: K.W, Z.H.S. Resources: Z.H.S, K.W. Software: Z.Y.Z, Q.Y.B. C. L. Supervision: Z.H.S, K.W. Validation: Z.Y.Z, Q.Y.B, Z.H.S, K.W. Visualization: Z.Y.Z, Q.Y.B, Z.H.S, K.W. Writing – original draft: Z.Y.Z, Q.Y.B. Writing – review & editing: Z.Y.Z, Q.Y.B, K.W, H.Y.S, Y.F.D, Y.L, F.E.Y, N, J

Acknowledgments

This work has been provided with technical support by the earmarked fund for Shandong Agriculture Research System (SDARS-10-05), and Shandong science and technology project of traditional chinese medicine (M-2023041).

References

1. Amoah EA, Gelaye S (1997) Biotechnological advances in goat reproduction. *J Anim Sci* 75:578–585
2. Hume DA, Whitelaw CBA, Archibald AL (2011) The future of animal production: improving productivity and sustainability[J]. *J Agricultural Sci* 149:9–16
3. Gozi KS, Deus Ajude LPT, Barroso MDV, Silva CRD, Peiró JR, Mendes LCN, Nogueira MCL, Casella T (2021) Potentially Pathogenic Multidrug-Resistant in Lamb Meat. *Microb Drug Resist* 27:1071–1078
4. Zhao X, Zhao H, Zhou Z, Miao Y, Li R, Yang B, Cao C, Xiao S, Wang X, Liu H, Wang J, Yang Z (2022) Characterization of Extended-Spectrum β -Lactamase-Producing *Escherichia coli* Isolates That Cause Diarrhea in Sheep in Northwest China. *Microbiol Spectr* 10:159
5. Tardiolo G, La Fauci D, Riggio V, Daghighi M, Di Salvo E, Zumbo A, Suter AM (2025) Gut Microbiota of Ruminants and Monogastric Livestock: An Overview. *Anim Basel* 15:758
6. Li J, Dawson PA (2019) Animal models to study bile acid metabolism. *Biochim Biophys Acta Mol Basis Dis* 1865:895–911
7. Yang Y, Nirmagustina DE, Kumrungsee T, Okazaki Y, Tomotake H, Kato N (2017) Feeding of the water extract from *Ganoderma lingzhi* to rats modulates secondary bile acids, intestinal microflora, mucins, and propionate important to colon cancer. *Biosci Biotechnol Biochem* 81:1796–1804
8. Icho S, Ward JS, Tam J, Kociolek LK, Theriot CM, Melnyk RA (2023) Intestinal bile acids provide a surmountable barrier against *C. difficile* TcdB-induced disease pathogenesis. *Proc Natl Acad Sci U S A* 120:120

9. Stoeva MK, Garcia-So J, Justice N, Myers J, Tyagi S, Nemchek M, McMurdie PJ, Kolterman O, Eid J (2021) Butyrate-producing human gut symbiont, *Clostridium butyricum*, and its role in health and disease. *Gut Microbes* 13:1–28
10. Xie M, Yuan K, Zhang Y, Zhang Y, Zhang R, Gao J, Wei W, Jiang L, Li T, Ding Y, Wang L, Lin Y, Wong CC, Yu J (2025) Tumor-resident probiotic *Clostridium butyricum* improves aPD-1 efficacy in colorectal cancer models by inhibiting IL-6-mediated immunosuppression. *Cancer Cell* 43:1885–1901
11. Wu Minyan H, Min C, Weidong (2025) Effects of a combination of *Clostridium butyricum* and *Lactobacillus acidophilus* on production performance, immune function, antioxidant capacity and meat quality of broilers [J]. *Chin J Anim Sci* 61:300–307
12. Zhou H, Quanxi X, Xiaohui L (2021) Performance Study of Several *Clostridium butyricum* Strains [J]. *China Brew* 40:120–123
13. Ren Z, Chen W, Getachew T, Mwacharo JM, Haile A, Sun W (2023) Expression analysis of TLR signaling pathway genes under lipopolysaccharide-induced and *E. coli* F17-infected sheep intestinal epithelial cells. *Anim Biotechnol* 34:1815–1821
14. Collins SL, Stine JG, Bisanz JE, Okafor CD, Patterson AD (2023) Bile acids and the gut microbiota: metabolic interactions and impacts on disease. *Nat Rev Microbiol* 21:236–247
15. Xu H, Luo H, Zhang J, Li K, Lee MH (2023) Therapeutic potential of *Clostridium butyricum* anticancer effects in colorectal cancer. *Gut Microbes* 15:114
16. Chen D, Jin D, Huang S, Wu J, Xu M, Liu T, Dong W, Liu X, Wang S, Zhong W, Liu Y, Jiang R, Piao M, Wang B, Cao H (2020) *Clostridium butyricum*, a butyrate-producing probiotic, inhibits intestinal tumor development through modulating Wnt signaling and gut microbiota. *Cancer Lett* 469:456–467
17. Liang J, Kou S, Chen C, Raza SHA, Wang S, Ma X, Zhang WJ, Nie C (2021) Effects of *Clostridium butyricum* on growth performance, metabonomics and intestinal microbial differences of weaned piglets. *BMC Microbiol* 21:85
18. Liu H, Xu X, Liang J, Wang J, Li Y (2022) The relationship between *Clostridium butyricum* and colorectal cancer. *J Cancer Res Ther* 18:1855–1859
19. Kuo WT, Odenwald MA, Turner JR, Zuo L (2022) Tight junction proteins occludin and ZO-1 as regulators of epithelial proliferation and survival. *Ann N Y Acad Sci* 1514:21–33
20. Chen Y, Cui W, Li X, Yang H (2021) Interaction Between Commensal Bacteria, Immune Response and the Intestinal Barrier in Inflammatory Bowel Disease. *Front Immunol* 12:761981
21. Zeng Y, Yang Q, Ouyang Y, Lou Y, Cui H, Deng H, Zhu Y, Geng Y, Ouyang P, Chen L, Zuo Z, Fang J, Guo H (2023) Nickel induces blood-testis barrier damage through ROS-mediated p38 MAPK pathways in mice. *Redox Biol* 67:102886
22. Tang H, Zhang H, Liu D, Wang Z, Yu D, Fan W, Guo Z, Huang W, Hou S, Zhou Z (2022) Genome-wide association study reveals the genetic determinism of serum biochemical indicators in ducks. *BMC Genomics* 23:856
23. Yamamoto S, Sasaki Y, Okada Y, Katabami S, Fujimori A, Munakata K, Shiraki Y, Nishibu H, Hisamoto C, Kawase J, Ojima Y, Kiyoshima A, Shiroma K (2022) Bacterial Distribution and Community

24. Nesta B, Pizza M (2018) Vaccines Against *Escherichia coli*. Curr Top Microbiol Immunol 416:213–242

Figures

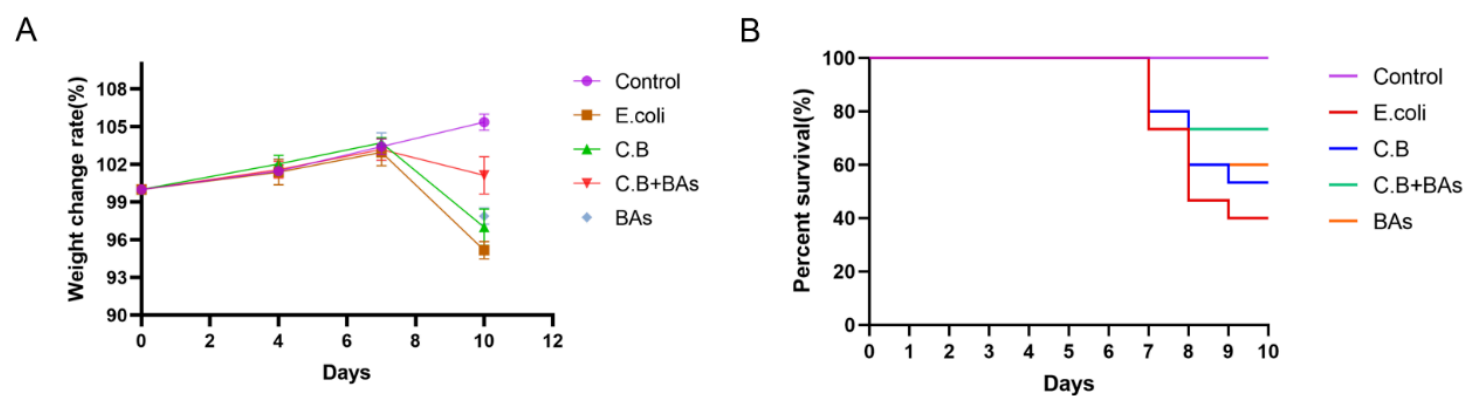


Figure 1
The effects of ovine bile acid and *Clostridium butyricum* on the weight change rate and mortality of infected mice.

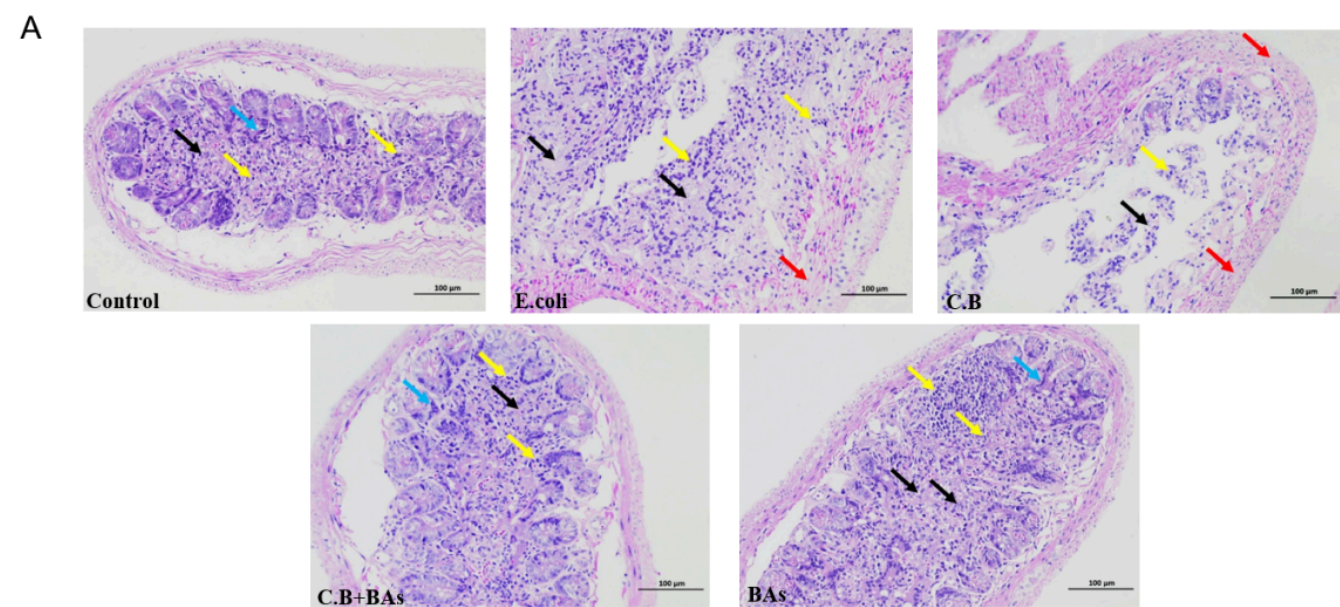


Figure 2
Effects of ovine bile acids and *C. butyricum* jejunal structural changes in mice infected with *E. coli*. Histopathological observations show connective tissue hyperplasia (black arrow), necrosis of intestinal

gland cells (blue arrow), lymphocyte infiltration (yellow arrow), and reduced staining intensity of muscle fibers in the muscular layer (red arrow)

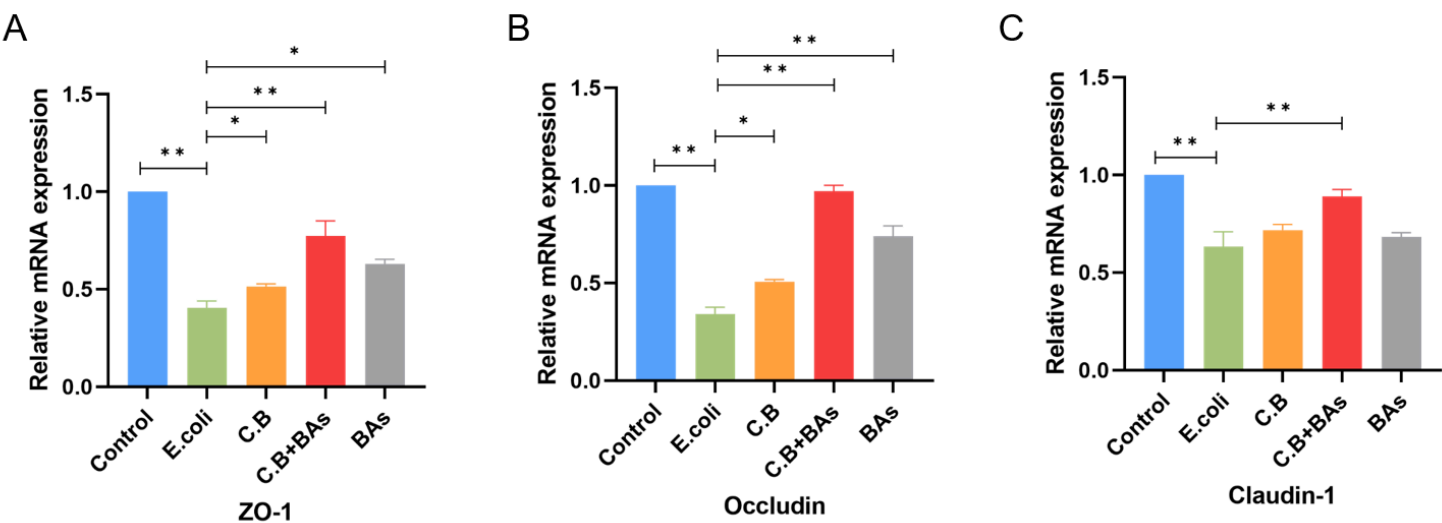


Figure 3

Effects of ovine bile acids and *C. butyricum* on the mRNA expression levels of tight junction proteins in the jejunum of mice infected with *E. coli*. “*” denotes $P < 0.05$; “**”denotes $P < 0.01$.

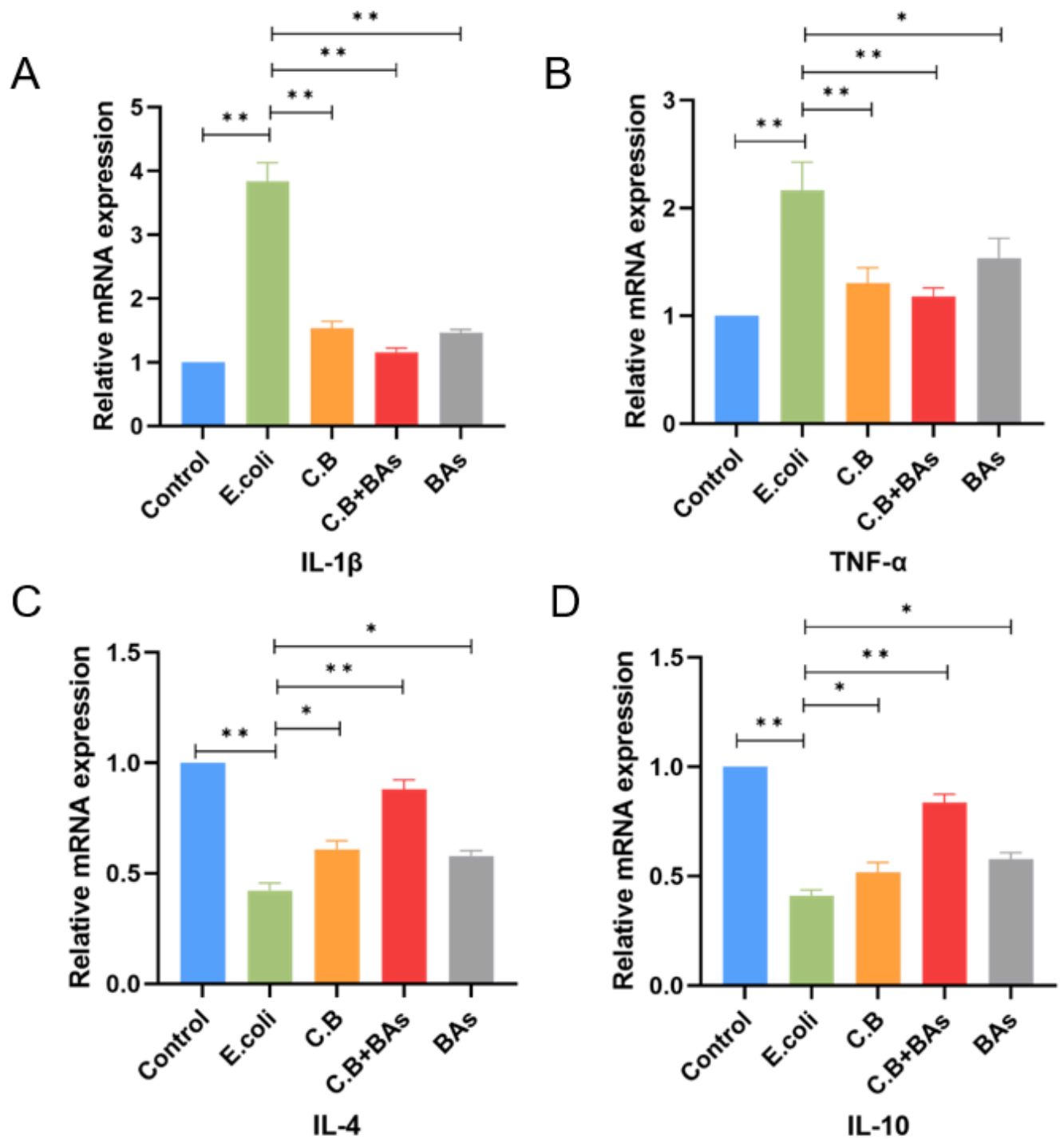


Figure 4

Effects of ovine bile acids and *C. butyricum* on the expression levels of inflammatory cytokines in the jejunum of mice infected with *E. coli*. The mRNA expression levels of IL-1 β (A), TNF- α (B), IL-4 (C), and IL-10 (D) in jejunal tissue were determined by qRT-PCR. "*"denotes $P < 0.05$; "***"denotes $P < 0.01$. IL-1 β : interleukin-1 β ; TNF- α : tumor necrosis factor- α ; IL-4: interleukin-4; IL-10: interleukin-10.

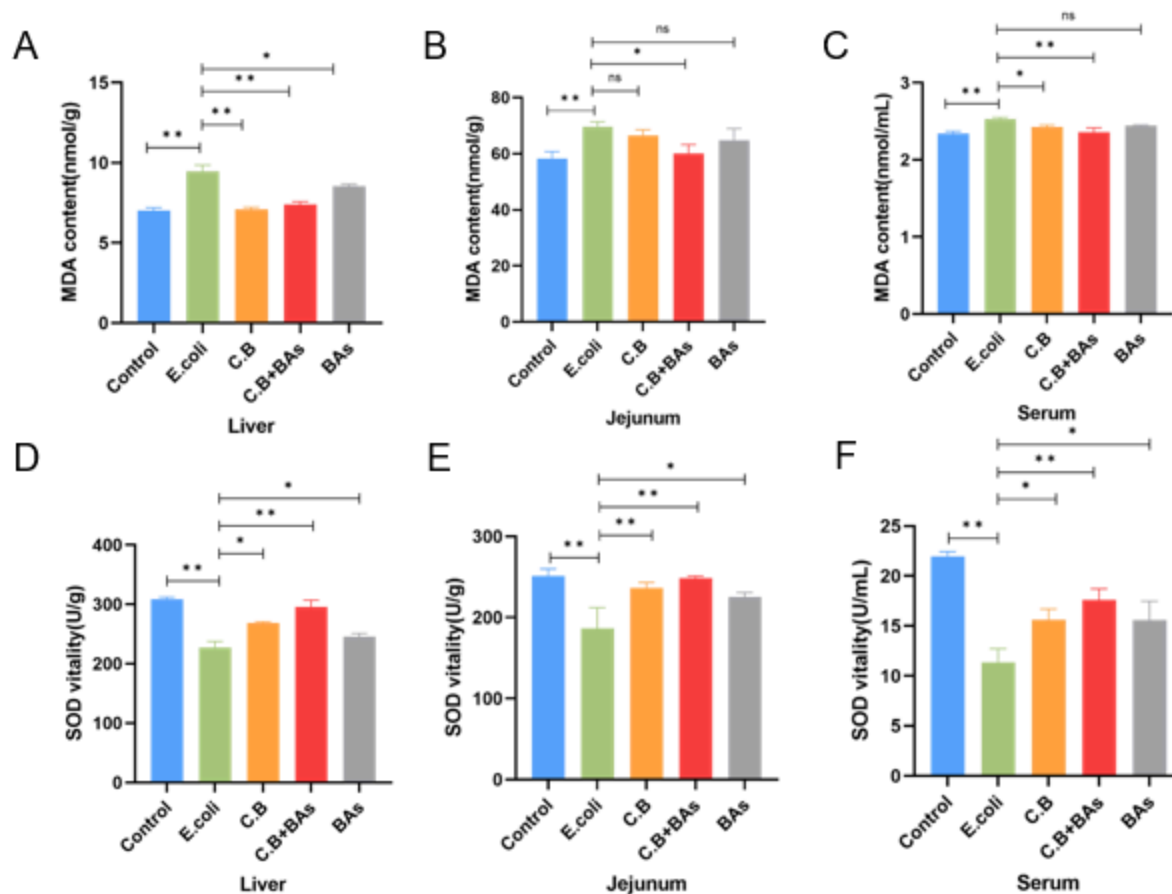


Figure 5

Effects of ovine bile acids and *C. butyricum* on oxidative stress markers in mice infected with *E. coli*.

The levels or activities of malondialdehyde (MDA) in liver tissue (A), jejunum (B), and serum (C), as well as superoxide dismutase (SOD) in liver tissue (D), jejunum (E), and serum (F), were measured by appropriate assays. "*"denotes $P < 0.05$; "**"denotes $P < 0.01$. MDA: malondialdehyde; SOD: superoxide dismutase.

A

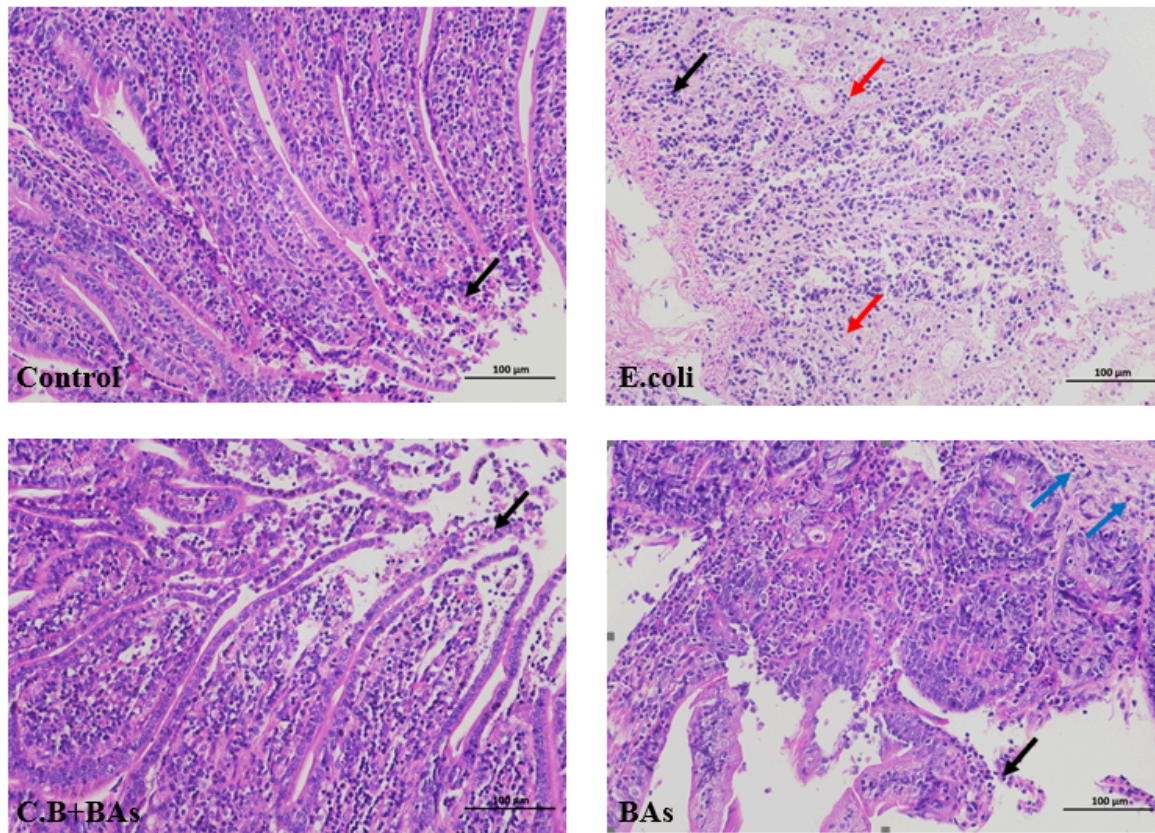


Figure 6

Effects of ovine bile acids and *C. butyricum* jejunal structural changes in goats infected with *E. coli*.

Histopathological observations reveal intestinal villus epithelial shedding (black arrow), disorganized jejunal architecture, and extensive necrosis (red arrow)

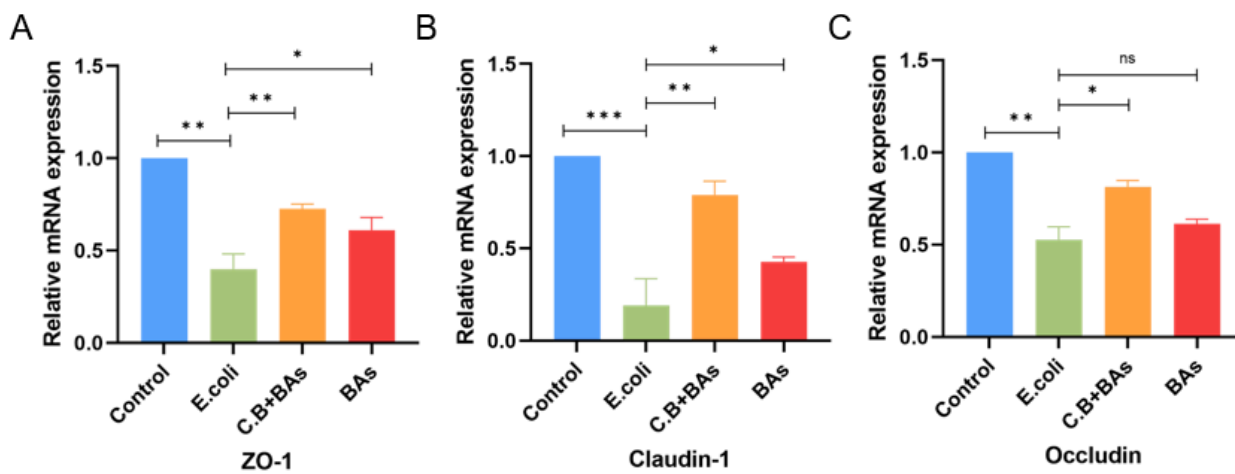


Figure 7

Effects of ovine bile acids and *C. butyricum* on the mRNA expression levels of tight junction proteins in the jejunum of goats infected with *E. coli*. “*” denotes $P < 0.05$; “**” denotes $P < 0.01$.

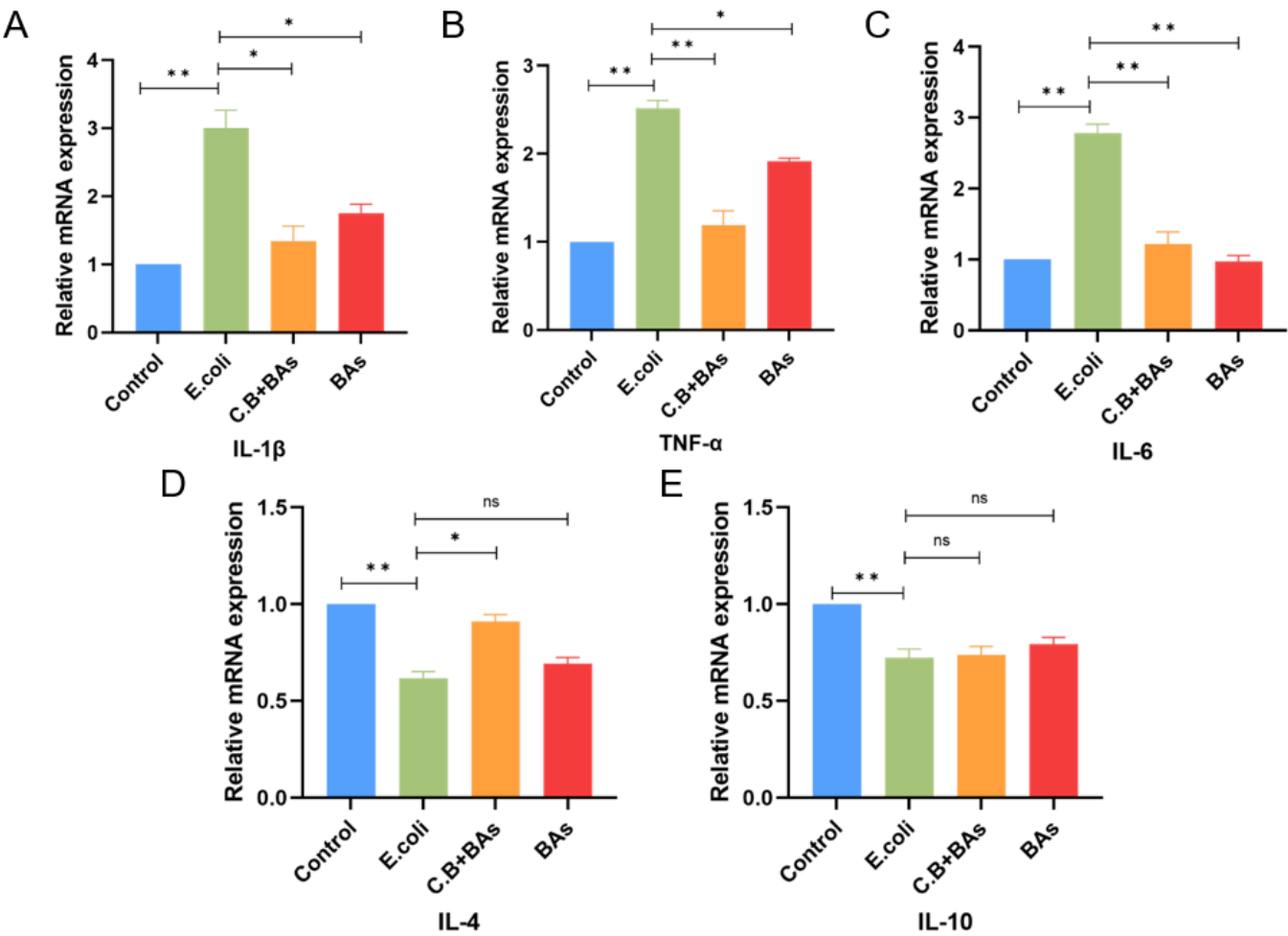


Figure 8

Effects of ovine bile acids and *C. butyricum* on the expression levels of inflammatory cytokines in the jejunum of goats infected with *E. coli*. The mRNA expression levels of IL-1 β (A), TNF- α (B), IL-6 (C), IL-4 (D), and IL-10 (E) in jejunal tissue were determined by qRT-PCR. “*” denotes $P < 0.05$; “**” denotes $P < 0.01$. IL-1 β : interleukin-1 β ; TNF- α : tumor necrosis factor- α ; IL-6: interleukin-6; IL-4: interleukin-4; IL-10: interleukin-10.

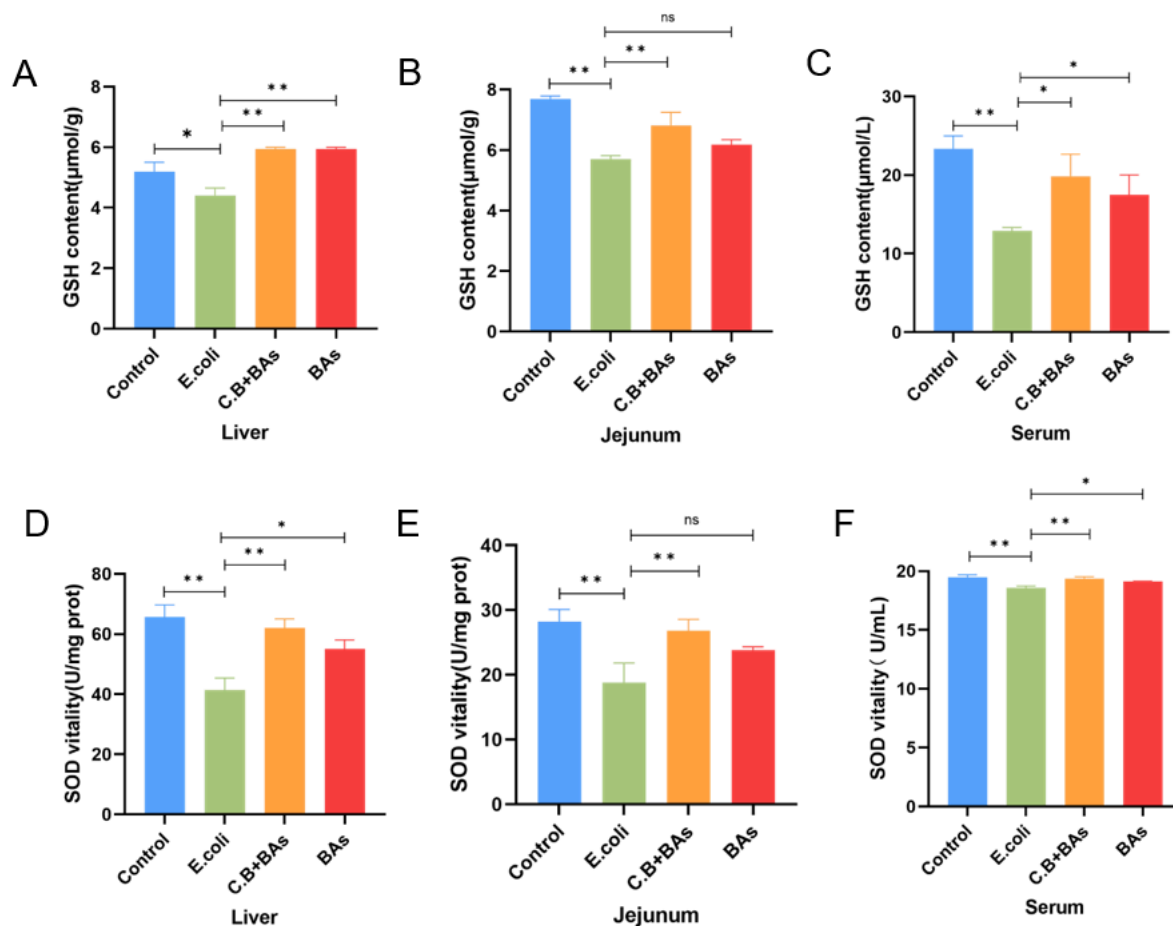


Figure 9

Effects of ovine bile acids and *C. butyricum* on oxidative stress markers in goats infected with *E. coli*.

The levels or activities of reduced glutathione (GSH) in liver tissue (A), jejunum (B), and serum (C), as well as superoxide dismutase (SOD) in liver tissue (D), jejunum (E), and serum (F), were measured using appropriate assays. “*”denotes $P < 0.05$; “**”denotes $P < 0.01$. GSH: reduced glutathione; SOD: superoxide dismutase.

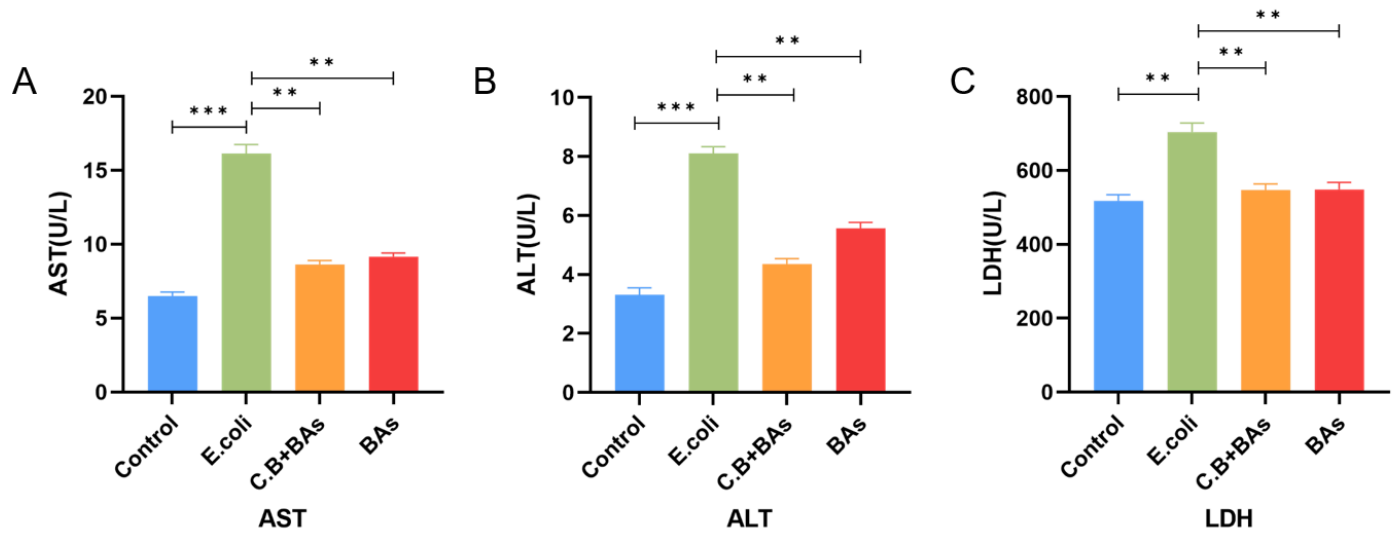


Figure 10

Effects of ovine bile acids and *C. butyricum* serum levels of AST (A), ALT (B), and LDH (C) in goats infected with *E. coli*. “*” denotes $P < 0.05$; “**” denotes $P < 0.01$. AST: aspartate aminotransferase; ALT: alanine aminotransferase; LDH: lactate dehydrogenase. Values are expressed in U/L, representing enzyme activity per liter of serum.

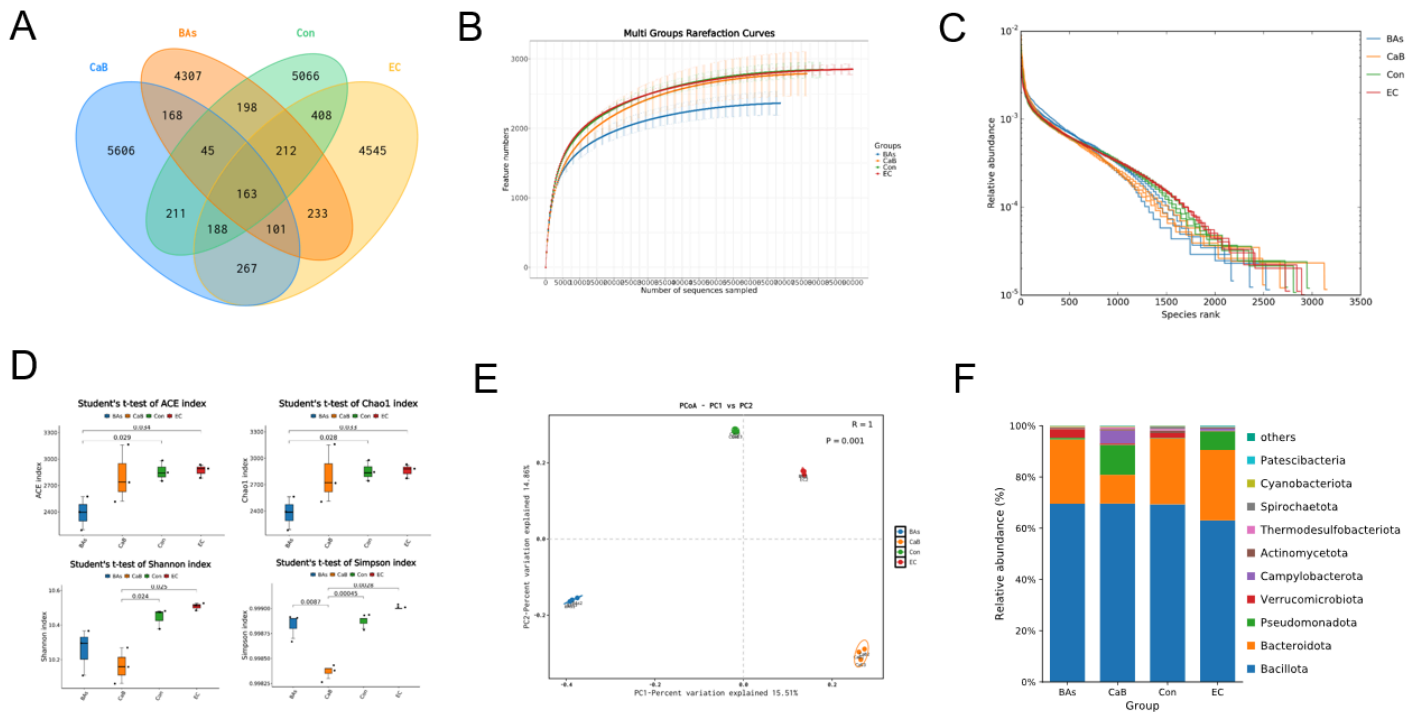


Figure 11

Effects of ovine bile acids and *C. butyricum* on the intestinal microbiota of goats infected with *E. coli*.

(A) Venn diagram showing shared operational taxonomic units (OTUs) among groups. (B) Sample dilution curves reflecting sequencing depth. (C) Rank abundance curves illustrating species richness and evenness. (D) Alpha diversity indices (Chao1, Ace, Shannon, Simpson) across groups. “*”denotes $P < 0.05$; “**”denotes $P < 0.01$. (E) Principal component analysis (PCA) of bacterial β -diversity in the intestinal microbiota. (F) Relative abundance of bacterial taxa at the phylum level among experimental groups. Con: control group; EC: *E. coli*-infected group; CaB: C. B+ BAs group; BAs: bile acids group.