



Original Research Article

Probiotics alleviate heat stress-induced uterine inflammation, reproductive hormone disruption and fetal growth restriction in mice

Hongda Zhou, Mengwei Chen, Xueqin Wang, Ying Pan, Xinyu Liu, Jinbo An, Zhipeng Liu, Baohua Ma^{*}, Sha Peng^{**}

College of Veterinary Medicine, Northwest A&F University, No. 22 Xinong Road, Yangling District, Xianyang City, Shaanxi Province, 712100, China

ARTICLE INFO

Keywords:

Heat stress
Uterus
Inflammation
Fetal growth
Reproductive hormone
Probiotics

ABSTRACT

With rising global temperatures, the detrimental effects of heat stress on the reproductive performance of female animals have received increasing attention. In this study, we investigated the effects of heat stress on reproductive function in female mice and explored the therapeutic potential of probiotics in alleviating uterine damage through repair of the intestinal barrier. Seven-week-old female ICR mice were used to establish a systemic heat stress model. Mice in the treatment groups received *Enterococcus faecium* or *Clostridium butyricum* during heat stress. The results showed that heat stress induced systemic inflammation, oxidative stress, and elevated endotoxin (LPS) levels, leading to downregulation of tight junction proteins (ZO-1, occludin, and claudin-1) in the colon and uterine tissues. Systemic inflammation increased the levels of the pro-inflammatory cytokines IL-1 β and IL-2 while decreasing the levels of the anti-inflammatory cytokines IL-4 and IL-10 in uterine tissue, resulting in uterine inflammatory damage and reproductive hormone dysregulation, thereby exacerbating placental injury and increasing the risk of fetal growth restriction. Treatment with *E. faecium* or *C. butyricum* repaired the intestinal barrier and inhibited the TLR4-MyD88 signaling pathway, thereby protecting the uterus from heat stress-induced injury and effectively alleviating uterine inflammation and fetal growth restriction. The 16S rRNA sequencing results demonstrate the regulatory effects of *Enterococcus faecium* and *Clostridium butyricum* on intestinal microbiota. Fecal microbiota transplantation (FMT) experiments further demonstrated that oxidative stress and uterine inflammatory damage in heat-stressed mice were alleviated following transplantation of intestinal microbiota from mice treated with *E. faecium* or *C. butyricum*. These findings suggest that *E. faecium* and *C. butyricum* have potential applications in reducing heat stress-induced reproductive damage in female animals.

1. Introduction

With the intensification of global climate change, heat stress caused by high temperatures has become an increasingly prominent issue, posing a serious threat to the reproductive function of livestock and the health of their offspring. In mammals, the uterus is particularly sensitive to elevated temperatures [1]. Heat stress exacerbates uterine damage by inducing reproductive hormone disorders, oxidative stress, and inflammation in the ovaries [2,3]. Adverse intrauterine conditions during gestation can impair fetal growth and adversely affect placental development and function, leading to fetal growth restriction, low birth weight, and poor postnatal performance [4–6].

The intestinal barrier, a critical component of the immune system,

plays a key role in regulating systemic inflammation [7]. It consists of an epithelial cell layer sealed by tight junction proteins (occludin, claudins, and ZO-1), a mucus layer rich in MUC2, and a mucosal immune system that secretes immunoglobulin A (IgA) and antimicrobial peptides [8,9]. Disruption of the intestinal barrier increases intestinal permeability, allowing LPS and inflammatory factors to enter the bloodstream [10]. By activating Toll-like receptors (TLR4 and TLR5) on neutrophils and endothelial cells, these molecules trigger the NF- κ B-dependent inflammatory cascade and aggravate organ damage through the gut-organ axis [11,12].

Probiotics are a class of live microorganisms with physiological regulatory functions that can improve host health through various mechanisms. *Enterococcus faecium* is a common commensal bacterium in

* Corresponding author.

** Corresponding author.

E-mail addresses: mabh@nwsuaf.edu.cn (B. Ma), pengshacxh@nwsuaf.edu.cn (S. Peng).

<https://doi.org/10.1016/j.theriogenology.2026.118192>

Received 3 March 2026; Received in revised form 3 August 2026; Accepted 16 September 2026

Available online 16 September 2026

0093-691X/© 2026 Elsevier Inc. All rights are reserved, including those for text and data mining, AI training, and similar technologies.

the gastrointestinal tract of humans and animals. It is a Gram-positive member of the genus *Enterococcus* and exhibits heat resistance, acid resistance, and bile tolerance [13]. *E. faecium* synthesizes and secretes digestive enzymes that degrade proteins into polypeptides [14]. A previous study reported that *E. faecium* can prevent intestinal mucosal damage, reduce systemic inflammation and decrease intestinal permeability caused by enteritis [15]. *Clostridium butyricum* is an anaerobic Gram-positive bacterium that colonizes the gastrointestinal tract of animals and produces butyrate, which protects against intestinal pathogenic bacterial infection [16]. *C. butyricum* primarily colonizes the colon, enhances colonic microbial diversity, produces short-chain fatty acids, and promotes vitamin E absorption [17,18]. *C. butyricum* also promotes intestinal mucosa repair and alleviates enteritis [19]. In addition, studies have reported that oral administration of butyrate enhances dietary fiber fermentation, upregulates epithelial AMPK and HIF-1 α expression, increases tight junction protein expression, and reduces plasma LPS levels and pulmonary neutrophil infiltration [20]. However, the mechanisms underlying heat stress-induced female reproductive dysfunction and their association with the intestinal barrier and intestinal microbiota remain to be fully elucidated. Therefore, in this study, female mice subjected to heat stress were orally administered *E. faecium* or *C. butyricum* to repair the intestinal barrier, thereby reducing heat stress-induced systemic inflammation and protecting uterine function and offspring health.

2. Materials and methods

2.1. Animals and experimental designs

All experimental protocols were reviewed and approved by the Animal Ethical and Welfare Committee of Northwest A&F University (Approval No. 201902A299), and all procedures were conducted in accordance with institutional guidelines for animal care and use. Female ICR mice aged 7 weeks were used in this experiment. After a week of acclimatization before the experiment, the mice were randomly divided into two groups ($n = 10$): the control group (CON) and the heat stress group (HS). The female mice were weighed and randomly assigned to the experimental groups, with comparable initial body weights across groups. The mice in the CON group were housed under conditions of 22 ~ 25°C and 30 ~ 50 % humidity. The mice in the heat stress model group were exposed to an environment with temperatures ranging from 37 to 39°C and humidity levels of 60 ~ 80 % for a total of 8 h, from 8:00 a.m. to 12:00 p.m. and from 2:00 p.m. to 6:00 p.m. For the remaining 16 h, they were transferred to an environment with temperatures ranging from 22 to 25°C and humidity levels of 30 ~ 50 %. The heat stress experiment lasted for 14 days to establish a systemic heat stress model in mice. All mice underwent a 12-h light-dark cycle, with free access to food and water during the experiment.

The probiotic treatment experiment divided 7-week-old ICR mice into four groups ($n = 30$): the control group (CON), the heat stress group (HS), the *Enterococcus faecium* treatment group (HS + EF), and the *Clostridium butyricum* treatment group (HS + CB). Simultaneously with the establishment of the heat stress model, mice in the HS + EF and HS + CB groups were fed with the corresponding probiotics. To eliminate the stress response to gavage, the probiotic solution was aspirated using a gavage needle, and mice were trained to learn to independently suck the droplets suspended by the needle, thereby orally feeding the probiotics. Probiotics were administered to mice every day at 8:00 a.m. before heat stress treatment. The *Enterococcus faecium* and *Clostridium butyricum* used in the experiment were provided by Beijing Xindayang Biotechnology Co., Ltd. 9×10^8 CFU/g of *Clostridium butyricum* was diluted to 5×10^7 CFU/mL with PBS, and 20×10^8 CFU/g of *Enterococcus faecium* was diluted to 5×10^7 CFU/mL. The solutions were freshly prepared daily during the experiment, thoroughly mixed, and used to ensure that each mouse received 200 μ L of diluted solution containing 1×10^7 CFU/mL of probiotics.

2.2. Fecal microbiota transplantation

Female ICR mice aged 7 weeks were used in this experiment ($n = 30$). After a week of acclimation before the experiment, all mice underwent a 1-week intestinal microbiota clearance treatment by adding the mixed Antibiotic cocktails (Abx) to drinking water. The composition of the antibiotic mixture was as follows: neomycin 100 mg, streptomycin 50 mg, penicillin 100 mg, vancomycin 50 mg, and metronidazole 100 mg, dissolved in 1L of distilled water. The feces for fecal microbiota transplantation came from mice in the HS group, HS + EF group, and HS + CB group after two weeks of treatment. Fecal samples were collected from mice in the HS, HS + EF, and HS + CB groups. The bacterial suspension was prepared according to the proportion of 200 μ L LB medium + 15 % glycerol + 1.8 g feces. Each prepared donor-specific suspension was intragastrically administered to a distinct group of recipient mice whose native intestinal flora had been depleted. Mice in each group were gavaged with fecal bacterial suspension from the corresponding group, once every two days, with 200 μ L each time. The three recipient groups were named HS + FMT-HS, HS + FMT-(HS + EF) and HS + FMT-(HS + CB) according to the source of the transplanted fecal material. After 14 days of FMT, mice in each group underwent heat stress treatment using the same method. Blood samples, colon samples, and tissue samples were collected after the experiment.

2.3. Tissue and serum sample collection

During the experiment, the mice were allowed to eat and drink normally. Perform HE staining on vaginal exfoliated cells of mice using the smear method from 8:00 to 9:00 every morning. After the completion of the entire experimental process, the mice were sacrificed and retro-orbital blood was collected from mice in each group. Through three independent experimental repetitions, we collected at least six serum samples from each estrus period. After retro-orbital blood was kept at room temperature for 0.5 h, the serum was collected after centrifugation at 8000 rpm for 20 min. Mice identified as being in the proestrus phase were selected for collecting colon and uterus tissue samples. One part of the samples was fixed in PFA solution and processed into paraffin sections, while the other part was stored in a -80°C refrigerator for Western blot and RT-qPCR experiments.

2.4. 16S rRNA gene sequencing

Fecal samples were subjected to 16S rRNA gene sequencing. DADA2 within QIIME processed raw sequences for quality filtering, denoising, merging and chimera elimination. ASV taxonomy was assigned using the sklearn classifier. α -diversity was calculated from ASV distributions. ASV-based distance matrices were constructed for unsupervised ordination, clustering and statistical comparison of β -diversity. LEfSe analysis identified differentially abundant taxa as potential microbial biomarkers. Functional prediction based on 16S rRNA gene sequencing was conducted to reveal altered metabolic pathways and their contributing microbial species.

2.5. Enzyme-Linked Immunosorbent Assay (ELISA)

The concentrations of inflammatory factors including IL-1 β , IL-2, IL-4, IL-10, and TNF- α , LPS, (IL-1 β : F2040-A, IL-2: F2698-B, IL-4: F2165-B, IL-10: F2176-A, TNF- α : F2132-A, LPS: F30715-A, Shanghai Kexing, China) as well as reproductive hormones including FSH, LH, 17- β E $_2$, and P $_4$ (FSH: F2555-A, LH: F2582-A, 17- β E $_2$: F9396-A, P $_4$: F30188-A, Shanghai Kexing, China) in the serum of mice were measured using ELISA kits. The absorbance of the sample wells was measured at a wavelength of 450 nm using a microplate reader. Based on the standard curve and the absorbance of each sample well, the concentration of each sample was calculated.

2.6. Oxidative stress index detection

Serum levels of MDA, SOD, GSH, and T-AOC (MDA: A003-1-2, SOD: A001-3-2, GSH: A006-2-1, T-AOC: A015-2-1, Nanjing Jiancheng, China) were determined using assay kits from the Nanjing Jiancheng Bioengineering Institute according to the manufacturer's protocols.

2.7. Hematoxylin and Eosin (H&E) staining

The tissues were fixed in a 4 % PFA solution, washed, and then embedded in paraffin. The paraffin blocks were cut at a thickness of 5 μ m. The paraffin sections underwent dewaxing, rinsing with running water, and then staining. After being stained with hematoxylin for 5 min, the sections were stained with eosin for 30 s. Subsequently, the sections were dehydrated using ethanol of gradient concentrations, and then cleared using xylene. Neutral gum was evenly applied to the surface of the paraffin sections. The slides were sealed and observed under a microscope.

2.8. Immunofluorescence staining (IF) staining

Deparaffinize the paraffin sections by thoroughly dehydrating them in 95 %, 80 %, and 75 % ethanol. Soak them in distilled water to ensure rehydration. Heat them to boiling in a citrate antigen retrieval solution, maintain for 5 min, and allow to cool naturally. Apply 10 % fetal bovine serum to the paraffin sections and block for 1 h at room temperature. Dilute the primary antibody at a ratio of 1:300 and add it to the tissue surface, leaving it overnight. Wash the sections thoroughly with PBS solution. Dilute the fluorescently labeled secondary antibody at a ratio of 1:300 and incubate it for 2 h at 4°C. Stain the cell nuclei with a mounting medium containing DAPI to prevent fluorescence quenching. Take photos of the paraffin section test results using a laser confocal microscope. (ZO-1: ab221547, Occludin: ab216327, Claudin1: ab307692, Goat Anti-Rabbit IgG H&L-Alexa Fluor® 488: ab150077, Abcam, England).

2.9. Western blot

Tissues were added to an appropriate amount of RIPA at a ratio of 1 mg:10 μ L. DTT and protease and phosphatase inhibitors were mixed thoroughly with the RIPA at a ratio of 1:100. After grinding, the mixture was left to stand for 30 min, vortexed every 10 min, and the supernatant was collected. The supernatant was then centrifuged at 12,000 g for 15 min, and the supernatant was further processed by adding a reducing SDS protein loading buffer in a volume of 1/4 of the supernatant. The mixture was thoroughly mixed and placed in a water bath at 100°C for 6–8 min. Proteins were separated via SDS-PAGE and transferred onto PVDF membranes (Millipore, USA). The PVDF membranes were blocked in a skim milk powder blocking solution for more than 2 h and incubated overnight with primary antibodies. The next day, they were incubated with secondary antibodies at 25°C for 2 h. Protein bands were obtained using an enhanced chemiluminescence (ECL) detection kit (Mishu, MI00607B) and captured using a multifunctional imaging system. (SHENHUA, Hangzhou, China). (β -Actin, 81115-1-RR, Proteintech, China; IL-1R2, PA6439, Abmart, China; TLR4, RT1666, HUABIO, China; TRAF6, T55175, Abmart, China; MyD88, PA1779, Abmart, China; HRP-conjugated goat anti-rabbit secondary antibody, BA1054, BOSTER, America).

2.10. RNA extraction and real-time quantitative PCR (RT-qPCR)

Total protein from uterine tissue was extracted using TRIzol reagent (TAKARA, Japan), and complementary DNA (cDNA) was synthesized using the FastKing RT Kit (Tiangen Biotech, China). Quantitative PCR was performed using SYBR Green Master Mix (Vazyme, China) on a BIO-RAD CFX Connect Real-Time PCR Detection System (Serial No.

788BR08727, Singapore). Gene-specific primers are listed in Table 1.

2.11. TUNEL staining

The one-step TUNEL in situ apoptosis detection kit (E-CK-A322, Wuhan Elaborate Biotechnology Co., Ltd.) was used to detect apoptosis in colon and uterine tissues.

2.12. Mating and gestation

After establishing the systemic heat stress model and probiotic intervention model in female mice, each female mouse was mated with 12-week-old male ICR mice. Male and female mice were co-housed at a 1:1 ratio. Vaginal plugs were examined at 8:00 a.m. on the following day, and female mice with visible vaginal plugs were separated and housed individually. Non-pregnant female mice were excluded during the experiment until 10 pregnant dams were acquired in each group. Blood, placenta, and fetal mouse samples were collected on day 16.5 of gestation.

2.13. Statistical analysis

Data are presented as means $\pm$ standard deviation (SD). Comparisons between two groups were analyzed using Student's t-test, while differences among multiple groups were assessed by one-way analysis of variance (ANOVA) followed by Tukey HSD test. Statistical analyses were performed using SPSS software version 26.0 (IBM, USA), and graphical representations were generated with GraphPad Prism 10 (GraphPad Software, USA). In bar charts, different superscript letters indicate significant differences ($P < 0.05$), while “ns” indicates no significant difference.

3. Results

3.1. Heat stress induces uterine inflammatory injury and intestinal barrier dysfunction

To investigate the effects of heat stress on the uterus of female mice and its association with the intestinal barrier, a systemic heat stress model was established (Fig. 1A). Because the colonic barrier is particularly susceptible to oxidative stress-induced damage, we focused on the pathological changes in the colon. Compared with the CON group, the HS group exhibited a shorter colon ($P < 0.05$) (Fig. 1B and C). Histological examination of the colonic tissue of CON mice showed normal architecture, characterized by intact and continuous intestinal wall layers with uniform thickness and straight, regularly arranged crypts of uniform depth. In contrast, the colonic tissue of the HS group exhibited marked pathological changes, including disruption of intestinal wall integrity, significant thinning of the basal layer of the intestinal wall, and irregular, overall shallower mucosal crypts (Fig. 1F).

Uterine tissue was collected from mice identified as being in proestrus based on vaginal smear H.E. staining. Compared with the CON group, the HS group exhibited uterine redness and swelling, accompanied by an increased uterine organ index ($P < 0.05$) (Fig. 1D and E). In mice of the CON group, all layers of the uterine wall maintained intact structure. Endometrial epithelial cells were tightly arranged with

Table 1

Primers of the genes used in the study.

Genes	Forward	Reverse
<i>Gapdh</i>	TGGCCTCCGTGTTCTCTAC	GAGTTGCTGTTGAAGTCGCA
<i>Tlr4</i>	CAGCAAAGTCCCTGATGACATTC	ATTTTGTCTCCACAGCCACCA
<i>MyD88</i>	CCACCTGTAAGGCTTCTCG	CTAGAGCTGCTGGCCTTGTT
<i>p-NF-κB p65</i>	TGTATTTCACGGGACCTGGC	CAGGCTAGGGTCAGCGTATG
<i>TRAF6</i>	GCGCTGTGCAAACTACATTT	AAGGATCGTGAGGCGTATTG

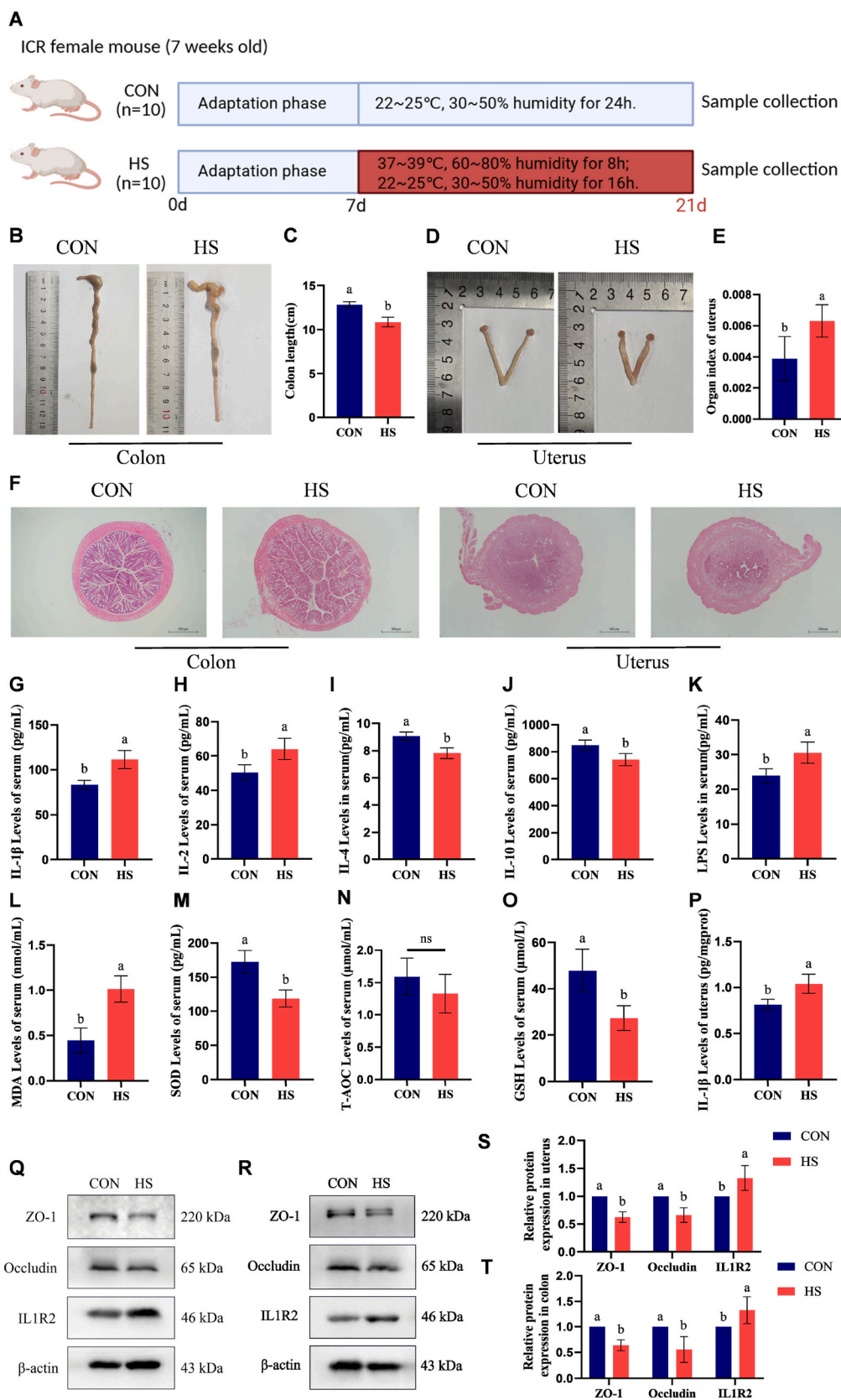


Fig. 1. Effects of heat stress on the uterus and colon. (n = 10) (A) Method for establishing a heat stress model. (B) Colon appearance. (C) Colon length. (D) Uterus appearance. (E) Uterine organ index. (F) H&E staining images of colon and uterus tissues. (G) Serum IL-1 β level. (H) Serum IL-2 level. (I) Serum IL-4 level. (J) Serum IL-10 level. (K) Serum LPS level. (L) Serum MDA level. (M) Serum SOD level. (N) Serum T-AOC level. (O) Serum GSH level. (P) Uterine tissue IL-1 β level. (Q) Western blot results of ZO-1, Occludin, and IL-1R2 in colon tissue. (R) Western blot results of ZO-1, Occludin, and IL-1R2 in uterine tissue. (S) Western blot quantification of colon tissue. (T) Western blot quantification of uterine tissue. Statistical significance was as follows: Different letters above the bar graph indicate significant differences ($P < 0.05$), while "ns" indicates no statistical significance.

continuous integrity. Endometrial glands displayed branched distribution with uniformly shaped lumens, and only a small number of scattered lymphocytes were observed in the stroma, indicating normal physiological status. In contrast, the continuity of the uterine wall structure was disrupted in the HS group, accompanied by abnormal hyperplasia of endometrial glands (Fig. 1F).

Heat stress significantly increased the levels of pro-inflammatory cytokines IL-1 β and IL-2 while significantly decreasing IL-4 and IL-10 in serum, accompanied by elevated serum LPS levels ($P < 0.05$) (Fig. 1G–K). Simultaneously, systemic inflammation also induced inflammatory responses in the uterine tissue (Fig. 1P). Heat stress significantly increased the level of the oxidative stress marker MDA while significantly decreasing SOD and GSH levels in serum ($P < 0.05$), whereas no significant difference was observed in T-AOC ($P > 0.05$) (Fig. 1L–O). Western blot analysis showed that heat stress significantly reduced the expression of the tight junction proteins occludin, ZO-1, and claudin-1 in colon and uterus tissues, while significantly increasing the expression of the inflammation-related receptor IL1R2 ($P < 0.05$) (Fig. 1G–K). Overall, heat stress induced pathological damage and barrier disruption in the colon and uterus, thereby promoting oxidative stress and inflammatory responses.

3.2. *Enterococcus faecium* and *Clostridium butyricum* alleviate heat stress-induced oxidative stress and systemic and uterine inflammation

To investigate the protective effects of probiotics against uterine inflammation in heat-stressed mice, corresponding probiotics were administered to mice in the HS + EF and HS + CB groups concurrently with the induction of heat stress (Fig. 2A). Heat exposure markedly increased water intake, while reducing feed intake and body weight. In the probiotic treatment group, feed intake was restored and body weight partially recovered (Fig. S1). Treatment with *E. faecium* and *C. butyricum* effectively reduced the heat-stress induced increase in pro-inflammatory cytokines and decrease in anti-inflammatory cytokines in serum (Fig. 2B–E) and alleviated heat stress-induced oxidative stress (Fig. 2F–I). Heat stress significantly increased systemic LPS levels, whereas oral administration of *E. faecium* and *C. butyricum* effectively reduced serum LPS levels (Fig. 2J). Analysis of uterine tissue homogenates showed that *E. faecium* and *C. butyricum* also effectively reduced the heat stress-induced increase in pro-inflammatory cytokines and decrease in anti-inflammatory cytokines in the uterus (Fig. 2K–M).

3.3. *Enterococcus faecium* and *Clostridium butyricum* alleviate reproductive hormone disruption induced by heat stress

The uterus and reproductive hormones undergo reciprocal regulation, and uterine health is closely related to reproductive hormone levels. The estrous cycle stage of the mice was identified using HE staining of vaginal smears (Fig. 3A). Measurements of serum reproductive hormone levels in mice at different stages of the estrous cycle revealed that heat stress induced an abnormal increase in FSH levels during proestrus, a decrease in LH levels during estrus and diestrus, a decrease in 17- β E₂ levels during proestrus and estrus, and a decrease in P₄ levels during estrus and diestrus. *E. faecium* and *C. butyricum* effectively alleviated these heat-stress induced reproductive hormone disorders (Fig. 3B–E). The results of ovulation counts in mouse oviducts revealed that heat stress significantly increased ovulation number, which was associated with elevated FSH concentrations during proestrus. Supplementation with probiotics alleviated this abnormal rise in ovulation number (Fig. S2).

3.4. *Enterococcus faecium* and *Clostridium butyricum* alleviate fetal growth restriction caused by uterine inflammatory damage resulting from preconception heat stress

To investigate whether inflammatory damage to the uterine tissue induced by preconception heat stress affects fetal growth and development, female and male mice from each group were mated after the experiment, and fetal and placental tissues were collected on gestation day 16.5. The results showed that preconception heat stress had no significant effect on litter size ($P > 0.05$) (Fig. 4B). Although no significant changes in serum oxidative stress markers were observed among the groups during pregnancy ($P > 0.05$) (Fig. 4E and F), uterine damage caused by preconception heat stress significantly reduced fetal weight (Fig. 4C), placental efficiency (Fig. 4D), and the expression of placental tight junction proteins (Fig. 4I and J) ($P < 0.05$). The protective effects of *E. faecium* and *C. butyricum* were significant ($P < 0.05$) (Fig. 4C, D, I, J). Because steroid hormone synthesis during late pregnancy primarily occurs in the placenta, placental damage can affect progesterone and estrogen levels. We measured serum 17- β E₂ and P₄ levels in pregnant mice and found that *E. faecium* and *C. butyricum* alleviated the heat stress-induced decreases in both hormones ($P < 0.05$) (Fig. 4G and H).

3.5. *Enterococcus faecium* and *Clostridium butyricum* repair heat stress-induced intestinal barrier damage and prevent uterine damage

To investigate whether probiotics alleviate uterine inflammation by repairing gut-uterine barrier damage in heat-stressed mice, the gut-uterine barrier was examined in each group. The results indicated that *E. faecium* and *C. butyricum* alleviated heat stress-induced shortening of the colon ($P < 0.05$) (Fig. 5A and B), histopathological damage (Fig. 5C), and cell apoptosis (Fig. 5E–H). Immunofluorescence staining showed that Occludin and ZO-1 were localized between adjacent cells. The fluorescence intensities of Occludin and ZO-1 in the perimetrium of HS group mice were significantly lower than those in the control group. The expression of Occludin and ZO-1 in the perimetrium was reduced in HS group mice. The fluorescence intensities of Occludin and ZO-1 in the colons of mice in the HS + EF and HS + CB groups were significantly higher than those in the HS group ($P < 0.05$) (Fig. 5D–J). Western blot results indicated that *E. faecium* and *C. butyricum* alleviated the heat stress-induced decrease in the expression of tight junction proteins Occludin, ZO-1, and claudin-1 in the colon ($P < 0.05$) (Fig. 5F–I).

Probiotics also effectively repaired the damaged uterine barrier. *E. faecium* and *C. butyricum* alleviated the heat stress-induced increase in the uterine organ index ($P < 0.05$) (Fig. 6B), as well as uterine inflammation, pathological damage (Fig. 6A–C), and apoptosis (Fig. 6E–G). Immunofluorescence staining and Western blot results indicated that *E. faecium* and *C. butyricum* mitigated the heat stress-induced decrease in the expression of tight junction proteins Occludin, ZO-1, and claudin-1 in the uterus ($P < 0.05$) (Fig. 6D–F, H, I).

3.6. *Enterococcus faecium* and *Clostridium butyricum* were associated with reduced activation of the TLR4-MyD88 inflammatory pathway

To explore the molecular mechanism by which probiotics alleviate inflammatory responses following intestinal barrier damage, we investigated the TLR4-MyD88 pathway, which is closely associated with inflammation in the colon ($P < 0.05$) (Fig. 7A–D, I) and uterus ($P < 0.05$) (Fig. 7B–E, H). Immunofluorescence results indicated that *E. faecium* and *C. butyricum* alleviated the heat stress-induced increases in TLR4 and MyD88 expression in the colon and uterus. *E. faecium* and *C. butyricum* also reduced IL-1 β levels in the uterus under heat stress conditions ($P < 0.05$) (Fig. 7C–H). Western blot and RT-qPCR results showed that *E. faecium* and *C. butyricum* effectively inhibited the excessive activation of the TLR4-MyD88 pathway in the uterus under heat stress conditions (Fig. 7F, G, J).

Uterine damage is associated with placental development and

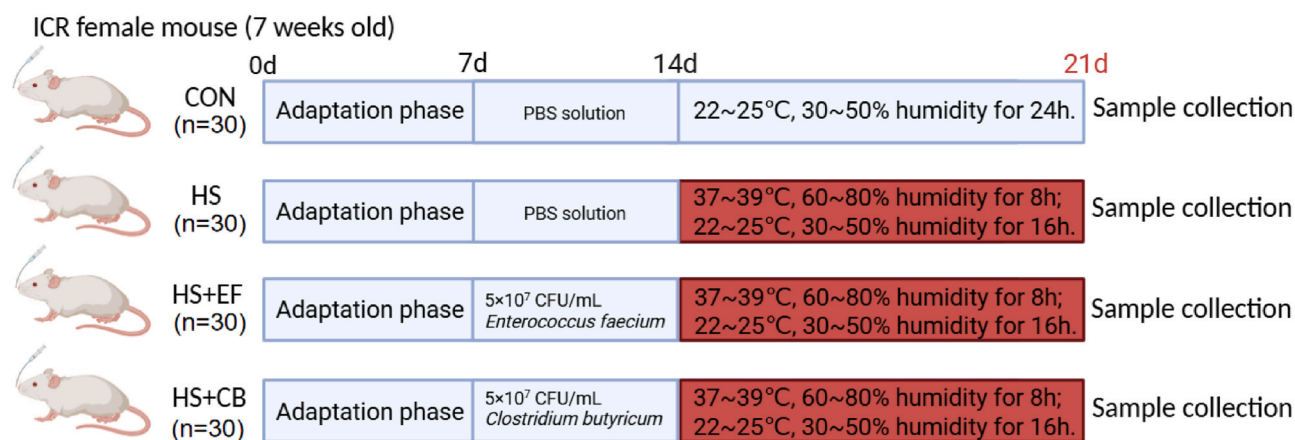
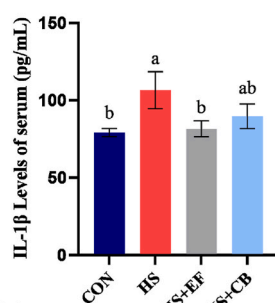
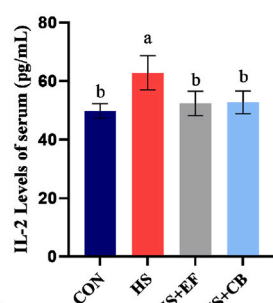
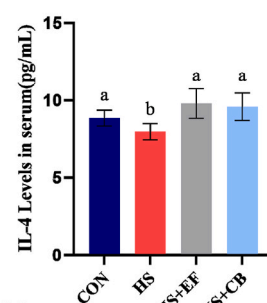
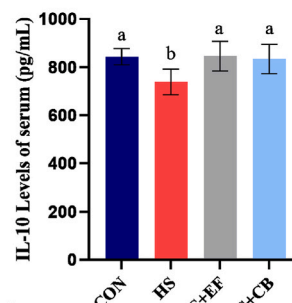
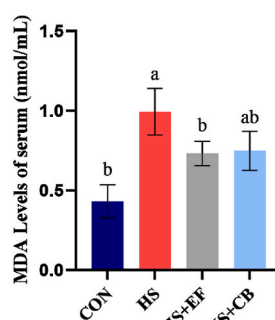
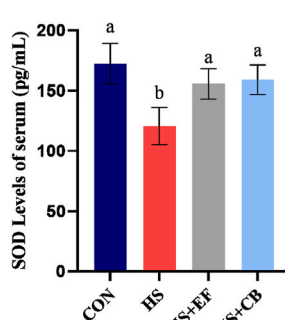
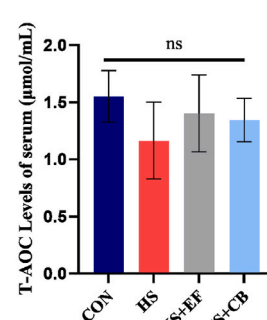
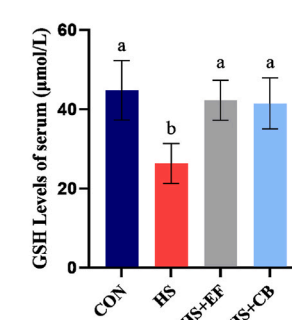
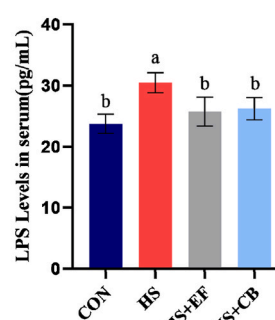
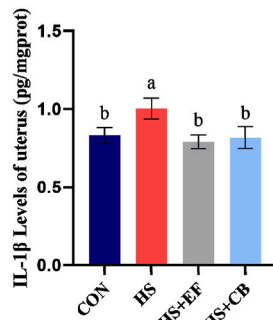
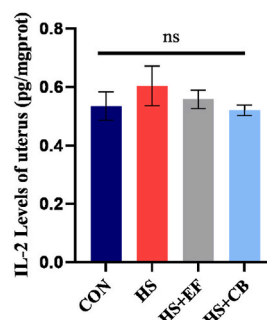
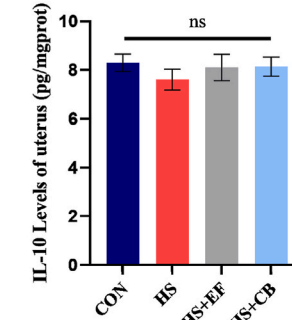
A**B****C****D****E****F****G****H****I****J****K****L****M**

Fig. 2. Probiotics alleviate oxidative stress, systemic and uterine inflammation induced by heat stress. (n = 30)

(A) Establishment methods of heat stress model and probiotic treatment model. (B) Serum IL-1 β level. (C) Serum IL-2 level. (D) Serum IL-4 level. (E) Serum IL-10 level. (F) Serum MDA level. (G) Serum SOD level. (H) Serum T-AOC level. (I) Serum GSH level. (J) Serum LPS level. (K) Uterine tissue IL-1 β level. (L) Uterine tissue IL-2 level. (M) Uterine tissue IL-10 level. (N) Serum FSH level. (O) Serum LH level. (P) Serum 17- β E₂ level. (Q) Serum P₄ level. Statistical significance was as follows: Different letters above the bar graph indicate significant differences ($P < 0.05$), while “ns” indicates no statistical significance.

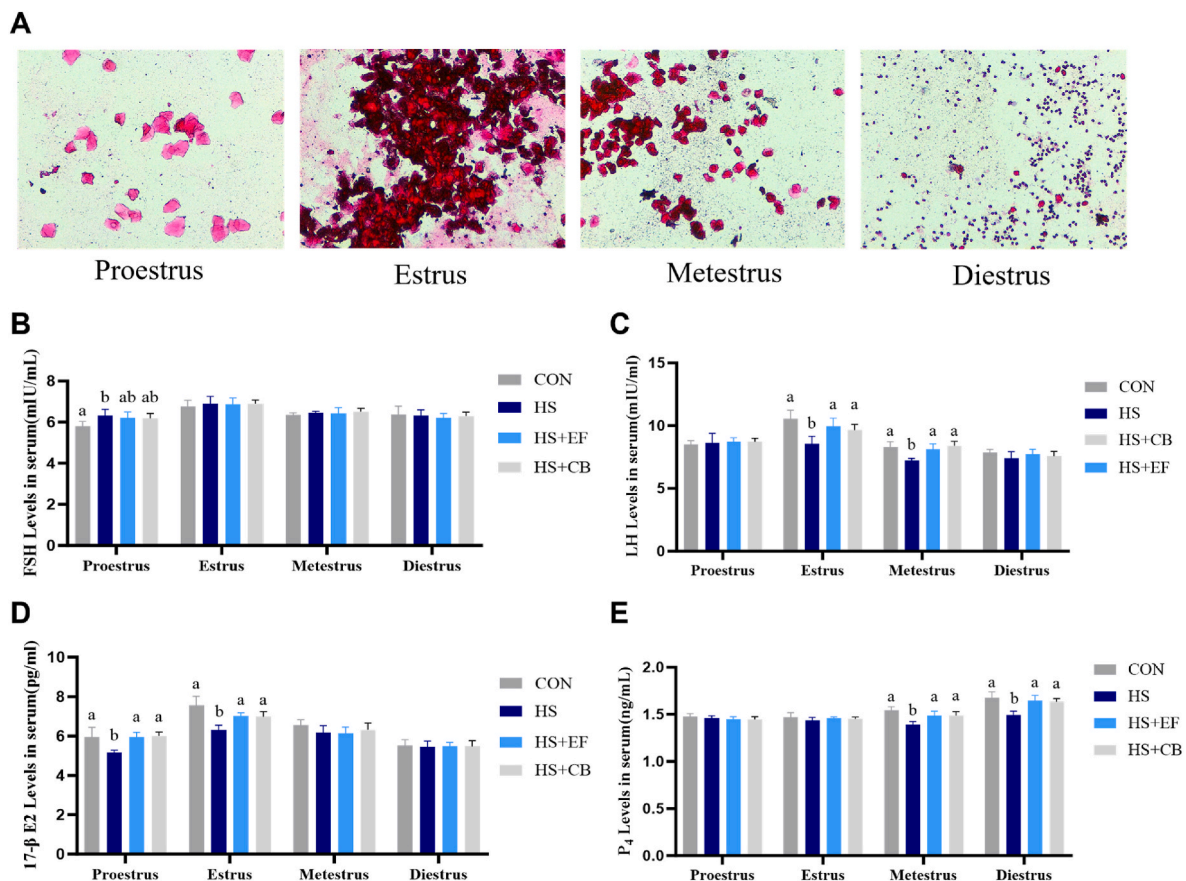


Fig. 3. Probiotics regulate reproductive hormone disorders induced by heat stress. (n = 30)

(A) H&E staining images of vaginal abscission smears at different stages of mouse estrous cycle. (B) Serum FSH level. (C) Serum LH level. (D) Serum 17-β E₂ level. (E) Serum P₄ level. Statistical significance was as follows: Different letters above the bar graph indicate significant differences ($P < 0.05$).

3.7. *Enterococcus faecium* and *Clostridium butyricum* exert functions by modulating the intestinal microbiota

To explore the pivotal role of the intestinal microbiota in probiotic-mediated repair of intestinal barrier damage, we performed 16S rRNA sequencing on fecal samples collected from mice in each group. The sequencing results revealed that *Enterococcus faecium* and *Clostridium butyricum* reshaped the gut microbial composition and partially alleviated the reduction in intestinal microbiota diversity induced by heat stress (Fig. 8A and B). The relative abundances of pathogenic genera including *Helicobacter*, *Desulfovibrio*, and pathogenic *Lactobacillus* were markedly elevated in the intestinal microbiota of heat-stressed mice. *Enterococcus faecium* modulated intestinal microbiota by increasing the relative abundances of commensal genera such as *Ruminococcus* and *Mucispirillum*. *Clostridium butyricum* significantly elevated the abundance of *Bacteroides* (Fig. 8C). Fecal suspensions from mice in the HS, HS + EF, and HS + CB groups were transplanted via gavage into mice with depleted intestinal microbiota, followed by the establishment of a heat stress model. The results indicated that mice receiving intestinal microbiota from the FMT HS + EF and HS + CB groups exhibited less damage to the colon and uterus than mice receiving intestinal microbiota from the FMT HS group (Fig. 8D–F, G). Compared with mice receiving intestinal microbiota from the FMT HS group, mice receiving intestinal microbiota from the FMT HS + EF and HS + CB groups showed significantly higher expression of tight junction proteins in the uterine tissue ($P < 0.05$) (Fig. 8E–H). The improved uterine barrier also reduced systemic inflammation (Fig. 8I, J, K), oxidative stress (Fig. 8O and P), and uterine inflammation (Fig. 8L, M, N) (see Fig. 9).

4. Discussion

Heat stress-induced intestinal barrier damage and the subsequent injury to testicular tissue have been preliminarily demonstrated [21], and studies on heat stress-induced oxidative stress damage to uterine tissue have also increased in recent years [3,22,23]. This study further explored the mechanism underlying inflammatory damage to the female reproductive system induced by heat stress. The results confirmed that heat stress leads to typical inflammatory pathological changes in the uterus of female mice, including obvious macroscopic inflammation, structural damage to the uterine wall, and intrauterine inflammation. These reproductive injuries are closely associated with disruption of the intestinal barrier, as evidenced by reduced expression of intestinal tight junction proteins and increased inflammatory factors. In addition, this study demonstrated the protective effects of two probiotics, *E. faecium* and *C. butyricum*, against heat stress-induced reproductive damage in female mice. These probiotics protected against reproductive damage by repairing the intestinal barrier.

Heat stress, as a major inducer of oxidative stress [24], can disrupt the body's oxidant-antioxidant balance, leading to the abnormal accumulation of reactive oxygen species and subsequent oxidative damage to key biological macromolecules, including nucleic acids, proteins, and lipids [25–27]. Simultaneously, heat stress induces ovarian hormone dysregulation, oxidative stress, and inflammation, exacerbating uterine damage and reducing endometrial function [1,28]. Consistent with these findings, the present study also demonstrated that heat stress-induced oxidative stress leads to intestinal barrier damage, which in turn induces uterine inflammation. Furthermore, we found that heat stress reduced the expression of tight junction proteins Occludin, ZO-1,

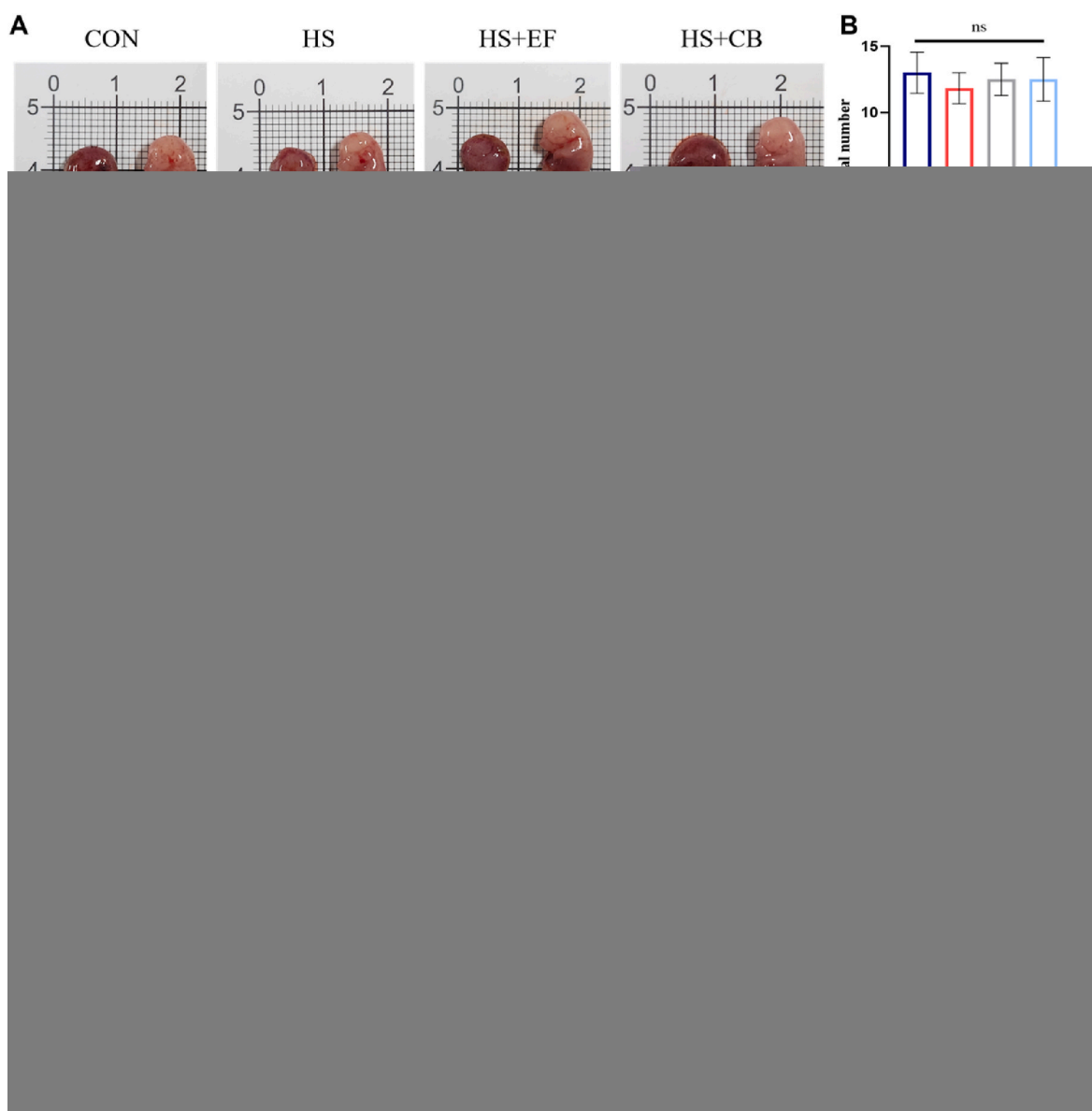


Fig. 4. Probiotics alleviate heat stress-induced fetal growth restriction. (n = 10) (A) Appearance of placentas and fetuses in each group of mice. (B) Number of fetuses. (C) Fetal weight. (D) Placental efficiency. (E) Serum MDA level in pregnant mice. (F) Serum SOD level in pregnant mice. (G) Serum P_4 level in pregnant mice. (H) Serum $17-\beta E_2$ level in pregnant mice. (I) Western blot results of tight junction proteins in placentas of pregnant mice. (J) Western blot quantification. Statistical significance was as follows: Different letters above the bar graph indicate significant differences ($P < 0.05$), ns indicating no statistical significance.

and claudin-1, resulting in intestinal barrier damage. We also found that heat stress impaired steroid hormone synthesis, leading to decreased levels of $17-\beta E_2$ during proestrus and estrus and reduced P_4 levels during estrus and post-estrus. Given that granulosa cells synthesize steroid hormones, these changes may be associated with damage to ovarian granulosa cells. In addition to oxidative stress and inflammatory response, circadian rhythm represents a non-negligible factor in heat stress-induced reproductive damage. Studies have demonstrated that heat stress-triggered circadian disruption can independently aggravate reproductive dysfunction. Since heat exposure was conducted during the light phase in this work, the disturbance of circadian rhythm induced by heat stress may also contribute to ovarian and uterine injury [29].

The placenta is formed from embryonic trophoblast cells, the extraembryonic mesoderm, and the maternal decidual endometrium. Uterine inflammation and oxidative stress before or during pregnancy can impair placental structure and function [30,31]. Placental dysfunction can lead to impaired fetal nutrient supply, ultimately

resulting in fetal growth restriction [32–34]. Previous studies have demonstrated that placental hypoxia, structural destruction, and impaired transplacental nutrient transport are classic mechanisms triggering fetal growth restriction under adverse gestational conditions [35, 36]. Our results indicated that uterine damage caused by pre-pregnancy heat stress can impair fetal growth and development. Probiotics may serve as a potential strategy to mitigate the adverse effects of pre-conception heat stress.

As a key regulatory pathway of the inflammatory response, TLR4 is activated upon recognition of exogenous stimuli such as LPS, triggering NF- κ B nuclear translocation through MyD88-dependent signaling and thereby regulating the expression of downstream inflammatory genes [37]. Inhibiting activation of the TLR4-MyD88 pathway can effectively alleviate inflammatory responses [38]. The TLR4/NF- κ B signaling pathway interacts with the mitochondrial apoptosis pathway. Inhibition of TLR4 and NF- κ B expression can effectively suppress apoptosis by upregulating BCL-2 and downregulating Bax [39]. The results of this



Fig. 5. Probiotics repair intestinal barrier damage induced by heat stress (A) Colon appearance images of mice in each group. (B) Colon length. (C) H&E staining of colon tissue. (D) Immunofluorescence staining of tight junction proteins in the colon. (E) TUNEL staining of colon tissue. (F) Western blot of tight junction proteins and inflammation-related receptor IL1R2 in the colon. (G) Quantification of fluorescence intensity. (H) Quantification of TUNEL positive signals. (I) Western blot quantification. Statistical significance was as follows: Different letters above the bar graph indicate significant differences ($P < 0.05$), while “ns” indicates no statistical significance.

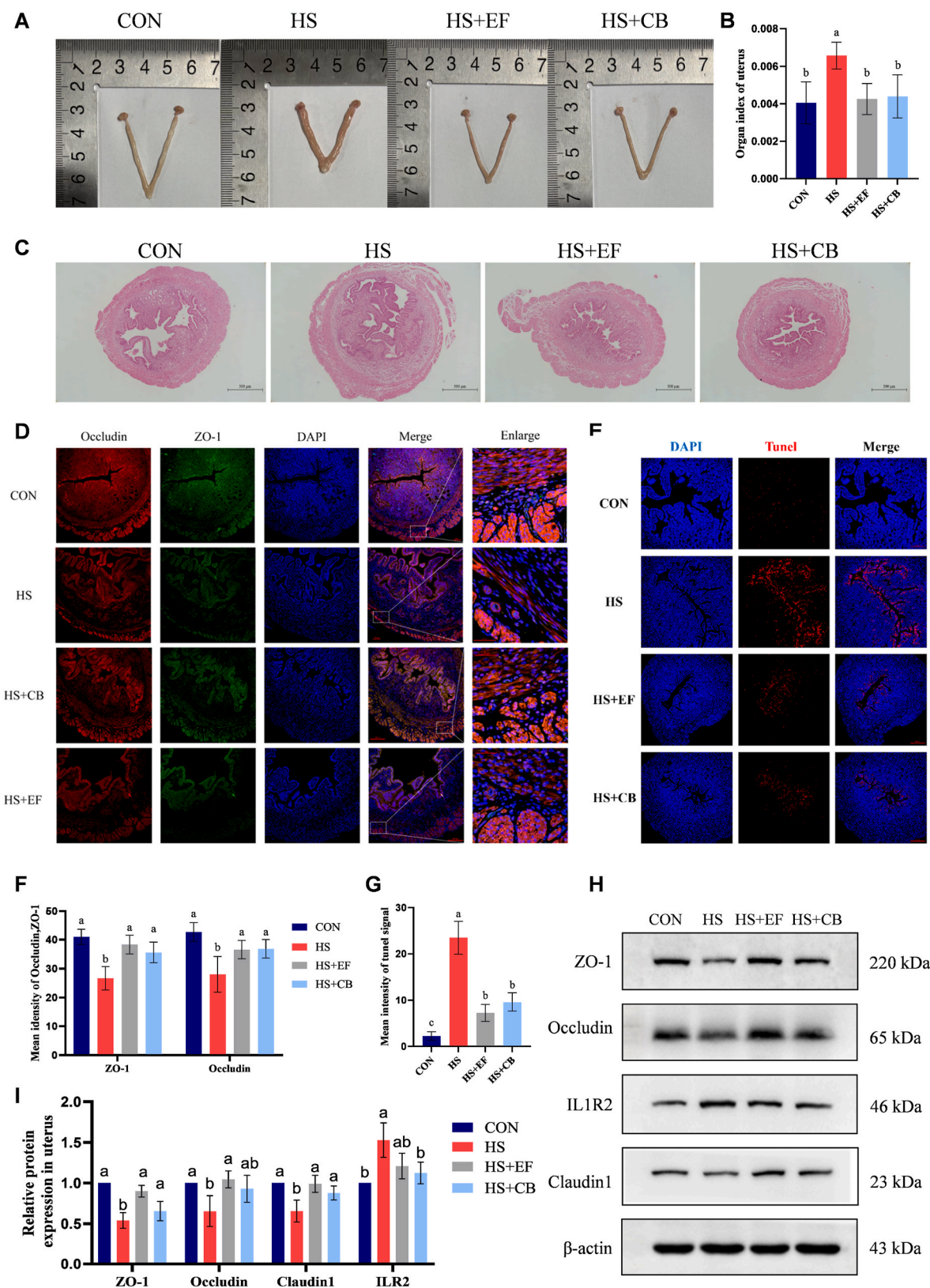


Fig. 6. Probiotics repair heat stress-induced damage to the uterus. (A) Visual appearance of uterine tissue in mice of each group. (B) Uterine organ index. (C) H&E staining of uterine tissue. (D) Immunofluorescence staining of tight junction proteins in uterine tissue. (E) TUNEL staining of uterine tissue. (F) Quantification of fluorescence intensity. (G) Quantification of TUNEL signal. (H) Western blot results for uterine tight junction proteins and inflammation-related receptor IL1R2. (I) Quantification by Western blot. Statistical significance was as follows: Different letters above the bar graph indicate significant differences ($P < 0.05$), while ns indicates no statistical significance.

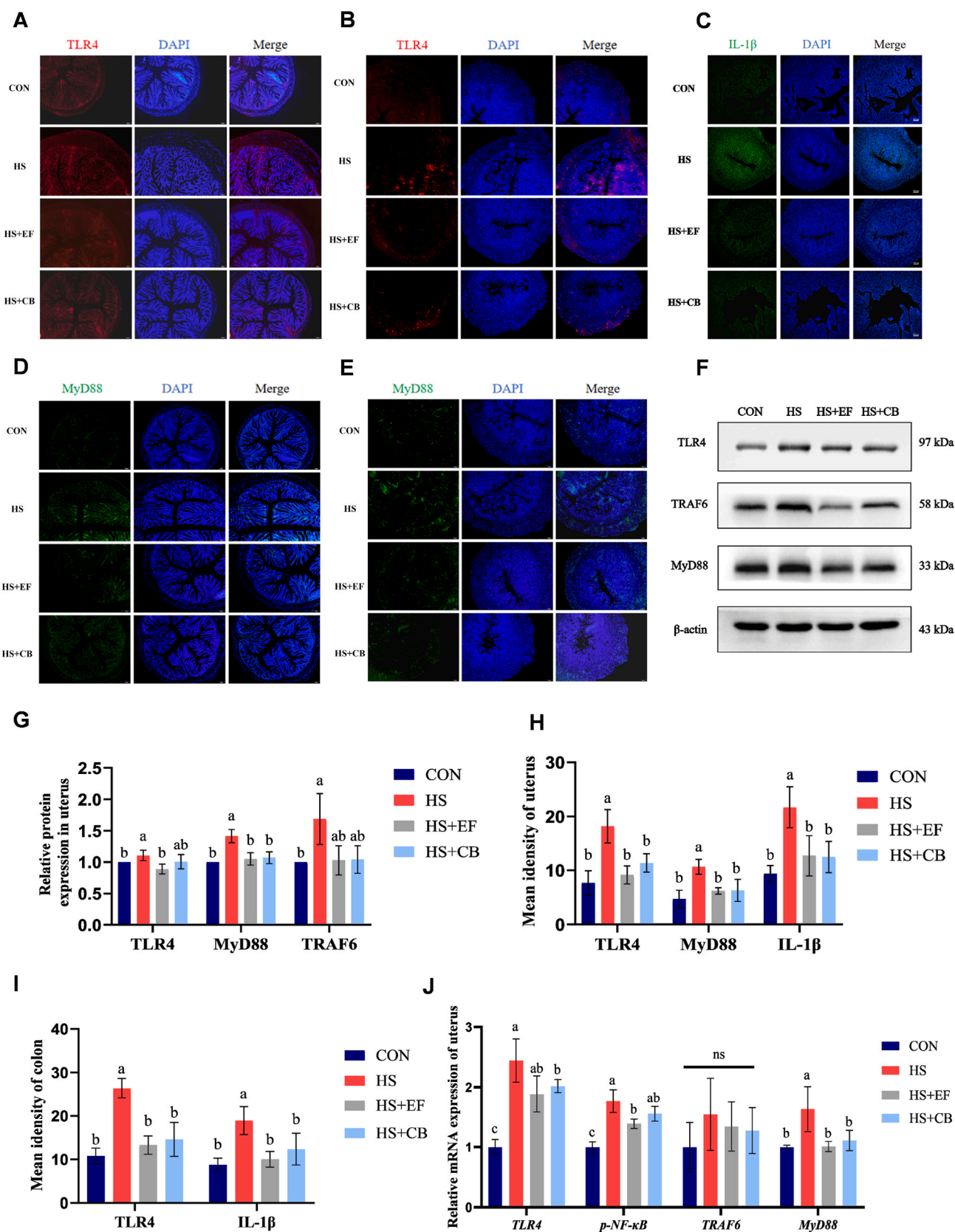


Fig. 7. Probiotics alleviate inflammatory responses by inhibiting the TLR4-MyD88 pathway. (A) Immunofluorescence staining of TLR4 in colonic tissue. (B) Immunofluorescence staining of TLR4 in uterine tissue. (C) Immunofluorescence staining of IL-1 β in uterine tissue. (D) Immunofluorescence staining of MyD88 in colonic tissue. (E) Immunofluorescence staining of MyD88 in uterine tissue. (F) Western blot results of proteins in the TLR4-MyD88 pathway in the uterus. (G) RT-qPCR results of proteins in the TLR4-MyD88 pathway in the uterus. (H) Quantification of immunofluorescence in uterine tissue. (I) Quantification of immunofluorescence in colonic tissue. (J) Western blot quantification. Statistical significance was as follows: Different letters above the bar graph indicate significant differences ($P < 0.05$), ns indicating no statistical significance.

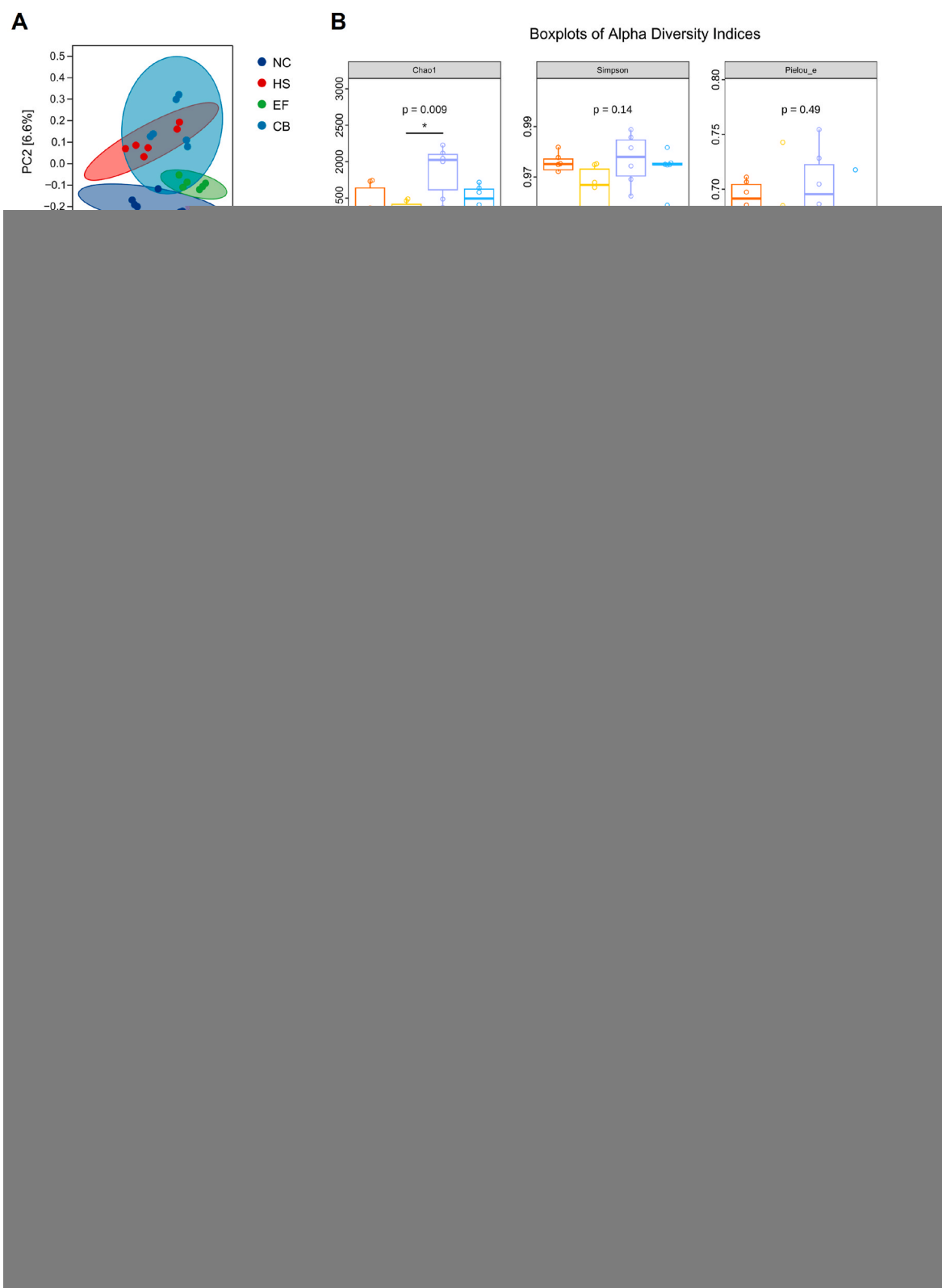


Fig. 8. *Enterococcus faecium* and *Clostridium butyricum* exert functions by modulating the intestinal microbiota. (A) PCA based on 16S rRNA sequencing results. EF represents the HS + EF group, CB represents the HS + CB group (B) Alpha diversity analysis. (C) Lefse analysis. (D) H&E staining of colon and uterus tissues. (E) Western blot of tight junction proteins in uterus tissues. (F) Colon length. (G) Uterus organ index. (H) Western blot quantification. (I) Serum IL-1 β level. (J) Serum IL-2 level. (K) Serum IL-4 level. (L) Serum IL-10 level. (M) IL-2 level in uterus tissues. (N) IL-4 level in uterus tissues. (O) Serum MDA level. (P) Serum SOD level. Statistical significance is as follows: Different letters above the bar graph indicate significant differences ($P < 0.05$), while ns indicates no statistical significance.

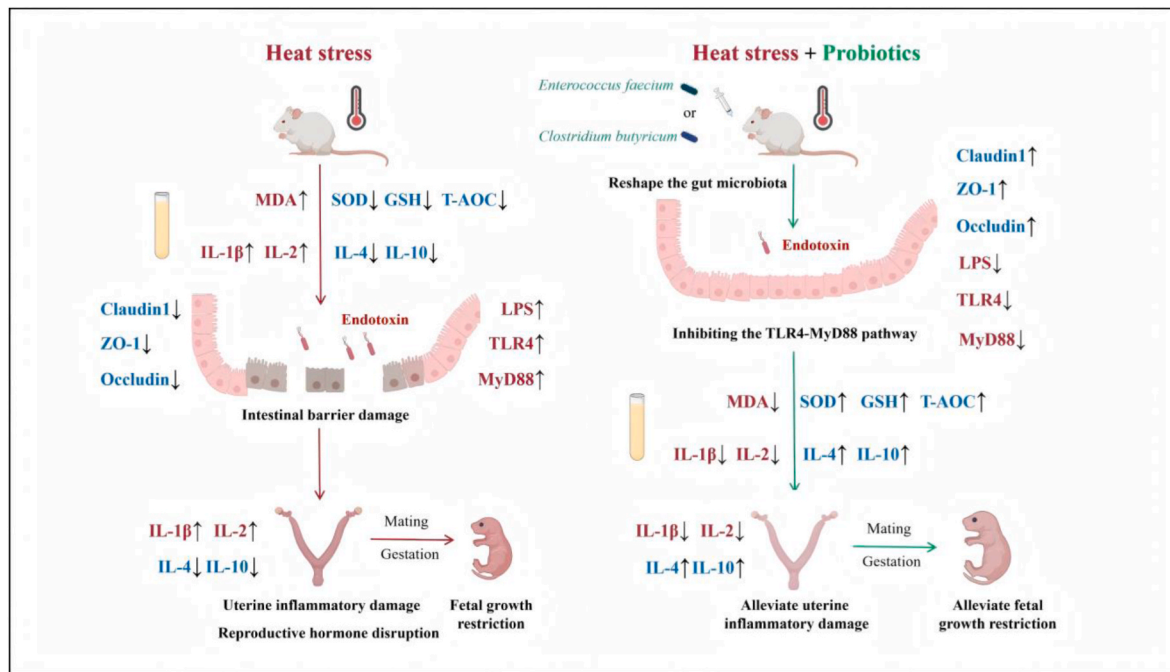


Fig. 9. *Enterococcus faecium* and *Clostridium butyricum* alleviate heat stress-induced uterine inflammatory damage and fetal growth restriction in mice by repairing the intestinal barrier and inhibiting the TLR4-MyD88 pathway.

study showed that, under heat stress conditions, the expression levels of key signaling molecules NF- κ B and TRAF6 in the TLR4-MyD88 pathway were significantly upregulated in uterine tissue, confirming the activation of this pathway is an important mechanism underlying heat stress-induced uterine inflammation. Probiotics effectively inhibited the TLR4-MyD88 pathway by remodeling the intestinal barrier, thereby reducing uterine inflammation.

The intestinal microbiota is altered by the colonization and proliferation of pathogenic microorganisms induced by heat stress. Following disruption of the intestinal microbiota, it no longer provides adequate nutrients and support to the host but instead promotes the production of pro-inflammatory factors and oxidative stress inducers, which damage the intestinal barrier [40–42]. Harmful substances produced by pathogenic microorganisms are then transported to target organs through the “gut-target organ axis” [21]. The intestinal barrier is a complex physical and biochemical defense system composed of the mucus layer, epithelial cell layer, tight junctions, and other components. Its core function is to selectively allow nutrients to pass through while preventing the entry of pathogens and toxins [43–45]. When the intestinal barrier is damaged and its permeability is abnormally increased, bacteria, endotoxins, and other harmful substances can enter the bloodstream, triggering local or systemic inflammation [46–48]. Probiotics and their metabolites can directly upregulate the expression and promote the proper assembly of tight junction proteins. For example, *Lactobacillus plantarum* HGD228 has been reported to upregulate the expression of ZO-1 and Occludin, thereby maintaining intestinal barrier integrity and repairing epithelial damage [49,50]. This study confirmed that two probiotics, *E. faecium* and *C. butyricum*, enhanced the expression of the tight junction proteins Occludin, ZO-1, and claudin-1, thereby repairing the intestinal and uterine barriers and ultimately alleviating oxidative stress and inflammation. In addition, probiotics ferment dietary fiber to produce short-chain fatty acids, including butyric acid and acetic acid. Butyric acid is the main energy source for intestinal epithelial cells, directly enhancing barrier function and exerting antioxidant and anti-inflammatory effects. *C. butyricum* is an important producer of butyrate [51,52]. Therefore, butyrate production is also a key factor in the ability of *C. butyricum* to repair epithelial damage and alleviate oxidative stress and inflammation.

The intestinal microbiota and the intestinal barrier have a symbiotic and interdependent relationship that jointly maintains gut homeostasis. The intestinal barrier provides a niche for microbial colonization and helps maintain the stability of the intestinal microbiota, whereas the intestinal microbiota regulates and strengthens intestinal barrier function [53]. Colonization by probiotics restores the intestinal barrier, and this effect is associated with the intestinal microbiota. 16S rRNA sequencing results indicate that heat stress elevates the abundances of *Helicobacter*, *Desulfovibrio*, and pathogenic *Lactobacillus*, which aggravate oxidative stress and inflammatory injury of the intestinal barrier. *Ruminococcus* degrades insoluble fibers to produce short-chain fatty acids. *Enterococcus faecium* improves intestinal fiber degradation capacity by facilitating the proliferation of *Ruminococcus*. *Mucispirillum* represents commensal bacteria colonizing the intestinal mucus layer. *Enterococcus faecium* boosts the abundance of *Mucispirillum* to stimulate intestinal mucus secretion, thereby repairing intestinal mucosa damaged by heat stress. *Bacteroides* efficiently degrades polysaccharides and facilitates bile acid metabolism, and exerts beneficial effects on enhancing intestinal immunity. *Clostridium butyricum* modulates intestinal barrier function by elevating the abundance of *Bacteroides*.

Although fecal microbiota transplantation (FMT) without subsequent heat exposure can reflect microbiome effects, recipient mice in our study received heat stress after FMT. This design aimed to explore whether probiotic-modulated intestinal microbiota confers resistance against heat stress. Consistent with published work transferring fecal microbiota from heat-stressed pigs to mice, all recipients underwent uniform heat exposure post-colonization, which reproduced heat stress-induced intestinal injury (Zhao et al., 2021). Through FMT experiments, this study demonstrated the key role of the intestinal microbiota in the protective effects of *E. faecium* and *C. butyricum*.

5. Conclusions

This study demonstrated that two probiotics, *Enterococcus faecium* and *Clostridium butyricum*, can regulate the intestinal microbiota, thereby repairing heat stress-induced colonic barrier damage in female mice. *Enterococcus faecium* and *Clostridium butyricum* alleviated elevated systemic LPS levels, oxidative stress, and inflammation induced by heat

stress. These protective effects mitigated uterine inflammatory injury, restored reproductive hormone balance, and consequently alleviated fetal growth restriction resulting from preconception heat stress. Probiotics were associated with reduced activation of the TLR4-MyD88 inflammatory pathway. These findings suggest that probiotics may have potential protective effects against heat stress-associated reproductive dysfunction.

CRedit authorship contribution statement

Hongda Zhou: Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Mengwei Chen:** Writing – original draft, Validation, Methodology, Formal analysis, Data curation. **Xueqin Wang:** Writing – original draft, Validation, Investigation, Data curation. **Ying Pan:** Validation, Methodology, Investigation, Data curation. **Xinyu Liu:** Investigation, Formal analysis, Data curation. **Jinbo An:** Visualization, Investigation, Formal analysis. **Zhipeng Liu:** Visualization, Formal analysis, Data curation. **Baohua Ma:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Sha Peng:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Ethics statements

All experimental procedures were reviewed and approved in advance by the Animal Ethical and Welfare Committee of Northwest A&F University (Approval No. GB/T45785-2021). The study strictly adhered to the principles of Replacement, Reduction, and Refinement (the 3Rs) to minimize animal use and suffering. Details regarding animal housing, care, and handling were thoroughly documented to ensure minimal impact on animal welfare during the course of the experiment.

Data availability

The 16S rRNA original sequencing data of this study have been deposited in NCBI Sequence Read Archive (<https://www.ncbi.nlm.nih.gov/>) under the accession numbers PRJNA1504453. Other data supporting the findings of this study are available from the corresponding author upon reasonable request.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work, the authors did not use AI tools, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This research was supported by the Grants from the National Natural Science Foundation of China (32573449), the National Key Research and Development Program of China (2022YFD1300203), and Horizontal Research Funding from Ningbo Second Hormone Factory (TG20250168).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.theriogenology.2026.118192>.

[org/10.1016/j.theriogenology.2026.118192](https://doi.org/10.1016/j.theriogenology.2026.118192).

References

- [1] Silva PS, Hooper HB, Manica E, Merighe GKF, Oliveira SA, Traldi AS, Negrão JA. Heat stress affects the expression of key genes in the placenta, placental characteristics, and efficiency of Saanen goats and the survival and growth of their kids. *J Dairy Sci* 2021;104(4):4970–9. <https://doi.org/10.3168/jds.2020-18301>.
- [2] Han J, Yang D, Liu Z, Tian L, Yan J, Li K, Fang Z, Chen Q, Lin B, Zhang W, Xi Z, Liu X. The damage effect of heat stress and psychological stress combined exposure on uterus in female rats. *Life Sci* 2021;286:120053. <https://doi.org/10.1016/j.lfs.2021.120053>.
- [3] Li H, Cong X, Yu W, Jiang Z, Fu K, Cao R, Tian W, Feng Y. Baicalin inhibits oxidative injuries of mouse uterine tissue induced by acute heat stress through activating the Keap1/Nrf2 signaling pathway. *Res Vet Sci* 2022;152:717–25. <https://doi.org/10.1016/j.rvsc.2022.10.005>.
- [4] Limesand SW, Camacho LE, Kelly AC, Antolic AT. Impact of thermal stress on placental function and fetal physiology. *Anim Reprod* 2018;15(Suppl 1):886–98. <https://doi.org/10.21451/1984-3143-AR2018-0056>.
- [5] Zhao W, Luna Ramirez RI, Rhoads RP, Brown LD, Limesand SW. Fetal programming under maternal heat stress: a focus on skeletal muscle growth and nutrition in livestock. *J Dev Orig Health Dis* 2025;16:e28. <https://doi.org/10.1017/S2040174425100111>.
- [6] Wyrwoll CS. Rising stars: the heat is on: how does heat exposure cause pregnancy complications? *J Endocrinol* 2023;259(1):e230030. <https://doi.org/10.1530/JOE-23-0030>.
- [7] Kou R, Guo Y, Qin Z, Xu X, Liu Y, Wei W, Chen Y, Jian Z, Lan B. Systemic dysregulation of the gut microenvironment plays a pivotal role in the onset and progression of inflammatory bowel disease. *Front Immunol* 2025;16:1661386. <https://doi.org/10.3389/fimmu.2025.1661386>.
- [8] Chelakkot C, Ghim J, Ryu SH. Mechanisms regulating intestinal barrier integrity and its pathological implications. *Exp Mol Med* 2018;50(8):1–9. <https://doi.org/10.1038/s12276-018-0126-x>.
- [9] van der Post S, Jabbar KS, Birchenough G, Arike L, Akhtar N, Sjøvall H, Johansson MEV, Hansson GC. Structural weakening of the colonic mucus barrier is an early event in ulcerative colitis pathogenesis. *Gut* 2019;68(12):2142–51. <https://doi.org/10.1136/gutjnl-2018-317571>.
- [10] Ghosh SS, Wang J, Yannie PJ, Ghosh S. Intestinal barrier dysfunction, LPS translocation, and disease development. *J Endocr* 2020;4(2):bvz039. <https://doi.org/10.1210/jendso/bvz039>.
- [11] Cai H, Qin D, Liu Y, Guo X, Liu Y, Ma B, Hua J, Peng S. Remodeling of gut microbiota by probiotics alleviates heat stroke-induced necroptosis in male germ cells. *Mol Nutr Food Res* 2023;67(18):e2300291. <https://doi.org/10.1002/mnfr.202300291>.
- [12] Koch F, Thom U, Albrecht E, Weikard R, Nolte W, Kuhla B, Kuehn C. Heat stress directly impairs gut integrity and recruits distinct immune cell populations into the bovine intestine. *Proc Natl Acad Sci U S A* 2019;116(21):10333–8. <https://doi.org/10.1073/pnas.1820130116>.
- [13] Bin Masalam MS, Bahieldin A, Alharbi MG, Al-Masaudi S, Al-Jaouni SK, Harakeh SM, Al-Hindi RR. Isolation, molecular characterization and probiotic potential of lactic acid bacteria in Saudi raw and fermented milk. *Evid Based Complement Alternat Med* 2018;2018:7970463. <https://doi.org/10.1155/2018/7970463>.
- [14] Liu ZL, Chen YJ, Meng QL, Zhang X, Wang XL. Progress in the application of *Enterococcus faecium* in animal husbandry. *Front Cell Infect Microbiol* 2023;13:1168189. <https://doi.org/10.3389/fcimb.2023.1168189>.
- [15] He W, Ni W, Zhao J. *Enterococcus faecium* alleviates gut barrier injury in C57BL/6 mice with dextran sulfate sodium-induced ulcerative colitis. *Gastroenterol Res Pract* 2021;2021:2683465. <https://doi.org/10.1155/2021/2683465>.
- [16] Stoeva MK, Garcia-So J, Justice N, Myers J, Tyagi S, Nemchek M, McMurdie PJ, Kolterman O, Eid J. Butyrate-producing human gut symbiont, *Clostridium butyricum*, and its role in health and disease. *Gut Microbes* 2021;13(1):1–28. <https://doi.org/10.1080/19490976.2021.1907272>.
- [17] Choi Y, Choi SI, Kim N, Nam RH, Jang JY, Na HY, Shin CM, Lee DH, Min H, Kim YR, Seok YJ. Effect of *Clostridium butyricum* on high-fat diet-induced intestinal inflammation and production of short-chain fatty acids. *Dig Dis Sci* 2023;68(6):2427–40. <https://doi.org/10.1007/s10620-023-07835-2>.
- [18] Takahashi M, McCartney E, Knox A, Franceschi M, Oka K, Wada K, Ideno M, Uno K, Kozłowski K, Jankowski J, Gracia MI, Morales J, Kritas SK, Esteve-Garcia E, Kamiya S. Effects of the butyric acid-producing strain *Clostridium butyricum* MIYAIRI 588 on broiler and piglet zootechnical performance and prevention of necrotic enteritis. *Anim Sci J* 2018;89(6):895–905. <https://doi.org/10.1111/asj.13006>.
- [19] Luo X, Kong Q, Wang Y, Duan X, Wang P, Li C, Huan Y. Colonization of *Clostridium butyricum* in rats and its effect on intestinal microbial composition. *Microorganisms* 2021;9(8):1573. <https://doi.org/10.3390/microorganisms9081573>.
- [20] Chen H, Qian Y, Jiang C, Tang L, Yu J, Zhang L, Dai Y, Jiang G. Butyrate ameliorated ferroptosis in ulcerative colitis through modulating Nrf2/GPX4 signal pathway and improving intestinal barrier. *Biochim Biophys Acta Mol Basis Dis* 2024;1870(2):166984. <https://doi.org/10.1016/j.bbdis.2023.166984>.
- [21] Guo X, Xu J, Zhao Y, Wang J, Fu T, Richard ML, Sokol H, Wang M, Li Y, Liu Y, Wang H, Wang C, Wang X, He H, Wang Y, Ma B, Peng S. Melatonin alleviates heat stress-induced spermatogenesis dysfunction in male dairy goats by regulating

- arachidonic acid metabolism mediated by remodeling the gut microbiota. *Microbiome* 2024;12(1):233. <https://doi.org/10.1186/s40168-024-01942-6>.
- [22] Bai H, Kawahara M, Kusama K, Sakurai T, Pfarrer C, Takahashi M. Heat stress induces oxidative stress and activates the KEAP1-NFE2L2-ARE pathway in reproduction-related cells. *Anim Sci J* 2025;96(1):e70023. <https://doi.org/10.1111/asj.70023>.
- [23] Yuan L, Dietrich AK, Nardulli AM. 17 β -Estradiol alters oxidative stress response protein expression and oxidative damage in the uterus. *Mol Cell Endocrinol* 2014; 382(1):218–26. <https://doi.org/10.1016/j.mce.2013.09.023>.
- [24] Akbarian A, Michiels J, Degroote J, Majdeddin M, Golian A, De Smet S. Association between heat stress and oxidative stress in poultry: mitochondrial dysfunction and dietary interventions with phytochemicals. *J Anim Sci Biotechnol* 2016;7:37. <https://doi.org/10.1186/s40104-016-0097-5>.
- [25] Newsholme P, Cruzat VF, Keane KN, Carlessi R, de Bittencourt Jr PI. Molecular mechanisms of ROS production and oxidative stress in diabetes. *Biochem J* 2016; 473(24):4527–50. <https://doi.org/10.1042/BCJ20160503C>.
- [26] Mujahid A, Akiba Y, Warden CH, Toyomizu M. Sequential changes in superoxide production, anion carriers and substrate oxidation in skeletal muscle mitochondria of heat-stressed chickens. *FEBS Lett* 2007;581(18):3461–7. <https://doi.org/10.1016/j.febslet.2007.06.051>.
- [27] Cadenas S. Mitochondrial uncoupling, ROS generation and cardioprotection. *Biochim Biophys Acta Bioenerg* 2018;1859(9):940–50. <https://doi.org/10.1016/j.bbambio.2018.05.019>.
- [28] Murata H, Kunii H, Kusama K, Sakurai T, Bai H, Kawahara M, Takahashi M. Heat stress induces oxidative stress and activates the KEAP1-NFE2L2-ARE pathway in bovine endometrial epithelial cells. *Biol Reprod* 2021;105(5):1114–25. <https://doi.org/10.1093/biolre/iaab143>.
- [29] Feng C, Guo L, Gao X, Ji X, Liu Y, Liu J, Liu S, Liu Y, Ma L, He X. Circadian rhythm disruption induces PCOS-like phenotypes by interfering with ovarian steroid hormone synthesis pathways. *J Steroid Biochem Mol Biol* 2026;259:106980. <https://doi.org/10.1016/j.jsbmb.2026.106980>.
- [30] Burton GJ, Jauniaux E. The human placenta: new perspectives on its formation and function during early pregnancy. *Proc Biol Sci* 2023;290:20230191. <https://doi.org/10.1098/rspb.2023.0191>.
- [31] Vento-Tormo R, Efremova M, Botting RA, Turco MY, Vento-Tormo M, Meyer KB, Park JE, Stephenson E, Polański K, Gonçalves A, Gardner L, Holmqvist S, Henriksson J, Zou A, Sharkey AM, Millar B, Innes B, Wood L, Wilbrey-Clark A, Payne RP, Teichmann SA. Single-cell reconstruction of the early maternal-fetal interface in humans. *Nature* 2018;563(7731):347–53. <https://doi.org/10.1038/s41586-018-0698-6>.
- [32] Lager S, Powell TL. Regulation of nutrient transport across the placenta. *J Preg* 2012;2012:179827. <https://doi.org/10.1155/2012/179827>.
- [33] Kamphof HD, Posthuma S, Gordijn SJ, Ganzevoort W. Fetal growth restriction: mechanisms, epidemiology, and management. *Matern Fetal Med* 2022;4(3): 186–96. <https://doi.org/10.1097/FM9.0000000000000161>.
- [34] Wu JJ, Zheng X, Wu C, Ma W, Wang Y, Wang J, Wei Y, Zeng X, Zhang S, Guan W, Chen F. Melatonin alleviates high temperature exposure induced fetal growth restriction via the gut-placenta-fetus axis in pregnant mice. *J Adv Res* 2025;68: 131–46. <https://doi.org/10.1016/j.jare.2024.02.014>.
- [35] Cowell W, Ard N, Herrera T, Medley EA, Trasande L. Ambient temperature, heat stress and fetal growth: a review of placenta-mediated mechanisms. *Mol Cell Endocrinol* 2023;576:112000. <https://doi.org/10.1016/j.mce.2023.112000>.
- [36] Hu C, Patil Y, Gong D, Yu T, Li J, Wu L, Liu X, Yu Z, Ma X, Yong Y, Chen J, Gooneratne R, Ju X. Heat stress-induced dysbiosis of porcine colon microbiota plays a role in intestinal damage: a fecal microbiota profile. *Front Vet Sci* 2022;9: 686902. <https://doi.org/10.3389/fvets.2022.686902>.
- [37] Wu Z, Mehrabi Nasab E, Arora P, Athari SS. Study effect of probiotics and prebiotics on treatment of OVA-LPS-induced of allergic asthma inflammation and pneumonia by regulating the TLR4/NF- κ B signaling pathway. *J Transl Med* 2022; 20(1):130. <https://doi.org/10.1186/s12967-022-03337-3>.
- [38] Su Q, Li L, Sun Y, Yang H, Ye Z, Zhao J. Effects of the TLR4/Myd88/NF- κ B signaling pathway on NLRP3 inflammasome in coronary microembolization-induced myocardial injury. *Cell Physiol Biochem* 2018;47(4):1497–508. <https://doi.org/10.1159/000490866>.
- [39] Zhang D, Jing B, Chen ZN, Li X, Shi HM, Zheng YC, Chang SQ, Gao L, Zhao GP. Ferulic acid alleviates sciatica by inhibiting neuroinflammation and promoting nerve repair via the TLR4/NF- κ B pathway. *CNS Neurosci Ther* 2023;29(4): 1000–11. <https://doi.org/10.1111/cns.14060>.
- [40] Wen C, Li S, Wang J, Zhu Y, Zong X, Wang Y, Jin M. Heat stress alters the intestinal microbiota and metabolomic profiles in mice. *Front Microbiol* 2021;12:706772. <https://doi.org/10.3389/fmicb.2021.706772>.
- [41] Song J, Xiao K, Ke YL, Jiao LF, Hu CH, Diao QY, Shi B, Zou XT. Effect of a probiotic mixture on intestinal microflora, morphology, and barrier integrity of broilers subjected to heat stress. *Poult Sci* 2014;93(3):581–8. <https://doi.org/10.3382/ps.2013-03455>.
- [42] Tian X, Yu Z, Feng P, Ye Z, Li R, Liu J, Hu J, Kakade A, Liu P, Li X. Lactobacillus plantarum TW1-1 alleviates diethylhexylphthalate-induced testicular damage in mice by modulating gut microbiota and decreasing inflammation. *Front Cell Infect Microbiol* 2019;9:221. <https://doi.org/10.3389/fcimb.2019.00221>.
- [43] Groschwitz KR, Hogan SP. Intestinal barrier function: molecular regulation and disease pathogenesis. *J Allergy Clin Immunol* 2009;124(1):3–22. <https://doi.org/10.1016/j.jaci.2009.05.038>.
- [44] Johansson ME, Sjövall H, Hansson GC. The gastrointestinal mucus system in health and disease. *Nat Rev Gastroenterol Hepatol* 2013;10(6):352–61. <https://doi.org/10.1038/nrgastro.2013.35>.
- [45] Peterson LW, Artis D. Intestinal epithelial cells: regulators of barrier function and immune homeostasis. *Nat Rev Immunol* 2014;14(3):141–53. <https://doi.org/10.1038/nri3608>.
- [46] Assimakopoulos SF, Triantos C, Maroulis I, Gogos C. The role of the gut barrier function in health and disease. *Gastroenterol Res* 2018;11(4):261–3. <https://doi.org/10.14740/gr1053w>.
- [47] Fasano A. Intestinal permeability and its regulation by zonulin: diagnostic and therapeutic implications. *Clin Gastroenterol Hepatol* 2012;10(10):1096–100. <https://doi.org/10.1016/j.cgh.2012.08.012>.
- [48] Matar A, Damianos JA, Jencks KJ, Camilleri M. Intestinal barrier impairment, preservation, and repair: an update. *Nutrients* 2024;16(20):3494. <https://doi.org/10.3390/nu16203494>.
- [49] Chen Y, Liu N, Chen F, Liu M, Mu Y, Wang C, Xia L, Peng M, Zhou M. Alleviation effects of Lactobacillus plantarum in colitis aggravated by a high-salt diet depend on intestinal barrier protection, NF- κ B pathway regulation, and oxidative stress improvement. *Food Funct* 2025;16(7):2718–36. <https://doi.org/10.1039/d4fo06377e>.
- [50] Sivri D, Şeref B, Şare Bulut M, Gezmen Karadağ M. Evaluation of the effect of probiotic supplementation on intestinal barrier integrity and epithelial damage in colitis disease: a systematic review. *Nutr Rev* 2025;83(7):e1782–97. <https://doi.org/10.1093/nutrit/nuae180>.
- [51] Wu J, Zhou B, Pang X, Song X, Gu Y, Xie R, Liu T, Xu X, Wang B, Cao H. Clostridium butyricum, a butyrate-producing potential probiotic, alleviates experimental colitis through epidermal growth factor receptor activation. *Food Funct* 2022;13(13):7046–61. <https://doi.org/10.1039/d2fo00478j>.
- [52] Li H, Shang Z, Liu X, Qiao Y, Wang K, Qiao J. Clostridium butyricum alleviates enterotoxigenic Escherichia coli K88-induced oxidative damage through regulating the p62-Keap1-Nrf2 signaling pathway and remodeling the cecal microbial community. *Front Immunol* 2021;12:771826. <https://doi.org/10.3389/fimmu.2021.771826>.
- [53] Li Z, Wan M, Wang M, Duan J, Jiang S. Modulation of gut microbiota on intestinal permeability: a novel strategy for treating gastrointestinal related diseases. *Int Immunopharmacol* 2024;137:112416. <https://doi.org/10.1016/j.intimp.2024.112416>.