Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Probiotic Lactobacillus rhamnosus prevents preeclampsia progression by restoring gut–placenta microbial homeostasis and regulating placental LysoPC metabolism

Qiuyue He

YueqHer@163.com

Department of Clinical Laboratory, the First Affiliated Hospital of Kunming Medical University

Yantuanjin Ma

Department of Clinical Laboratory, the First Affiliated Hospital of Kunming Medical University

Yuhang Zhang

Department of Clinical Laboratory, the First Affiliated Hospital of Kunming Medical University

Xingli Deng

Department of Neurosurgery,The First Affiliated Hospital of Kunming Medical college

Yuan Qian

The Central Laboratory Center for Precision Medicine Clinical Diagnosis and Treatment the Affiliated Hospital of Yunnan University

Research Article

Keywords: Preeclampsia, Lactobacillus rhamnosus, Gut–placenta microbial axis, LysoPC metabolism

Posted Date: July 31st, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10018978/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Abstract

Background

Preeclampsia (PE) is a pregnancy-specific hypertensive disorder that poses a serious threat to maternal and fetal health. PE progression is closely associated with gut microbiota dysbiosis and inflammation; however, effective microbiota-targeted interventions remain limited.

Methods

In this study, a PE rat model was established by administering a low dose of lipopolysaccharide (LPS) in early pregnancy to explore the potential protective effects and underlying mechanisms of *Lactobacillus rhamnosus* (LR) supplementation during gestation. We measured blood pressure, urinary protein levels, and placental indices, performed 16S rRNA sequencing to profile changes in gut and placental microbiota, and conducted untargeted metabolomics to identify placental differential metabolites and analyze their correlations with microbial taxa.

Results

The results showed that LPS induced significant hypertension and proteinuria in pregnant rats, reduced α diversity of gut microbiota, and markedly decreased the abundance of butyrate-producing bacteria such as *Roseburia* and *Clostridia_UCG-014*. Concurrently, pro-inflammatory lysophosphatidylcholines (LysoPCs; LysoPC 16:1, 16:2, and 10:0) were significantly elevated in the placenta. LR intervention effectively lowered systolic, diastolic, and mean arterial pressures, attenuated proteinuria, and restored gut microbial balance by increasing the abundance of beneficial taxa and enhancing microbial diversity. Notably, LR supplementation also significantly reduced placental LysoPC accumulation, suggesting its potential as a metabolic intervention target. Furthermore, we identified four key genera (*Roseburia*, *Clostridia_UCG-014*, *Eubacterium_siraeum_group*, and *Lachnospiraceae_UCG-006*) that exhibited similar trends in both placental and cecal samples, supporting the concept of gut–placenta axis microbial translocation. Correlation analyses revealed close associations between these core microbial genera and differential placental metabolites, indicating that LR may alleviate PE phenotypes by modulating microbe–metabolite networks.

Conclusions

Our study provides new experimental evidence and theoretical support for probiotic intervention during pregnancy to alleviate preeclampsia, and also identifies potential microbial and metabolic targets for future exploration of PE prevention and treatment strategies.

Introduction

Preeclampsia (PE) remains one of the leading causes of maternal and perinatal morbidity and mortality worldwide[1], affecting 2% to 8% of pregnant women..Characterized by gestational hypertension and systemic small-artery vasospasm, PE is a complex multisystem disorder that can affect nearly all maternal organs,including renal, cardiac, pulmonary, hepatic, and neurological dysfunctio[1, 2].Moreover, preeclampsia has long-term effects on mother and fetus, increasing the risk of future maternal cardiovascular, metabolic, cerebrovascular diseases, and long-term health of the fetus after birth The clinical diagnosis of PE is based on the onset of hypertension after 20 weeks of gestation accompanied by evidence of organ dysfunction, such as proteinuria, thrombocytopenia, or renal impairment [3] .However, the precise etiology and pathogenesis of PE remain incompletely understood. Current evidence suggests that the disease involves a combination of multiple genetic components, immunologic aberrations [4], uteroplacental ischemia, impaired remodeling of uterine spiral arteries, and metabolic disturbance and gut dysbiosis[5]. Currently, there remains no effective means to prevent or treat PE due to the limited knowledge about preeclampsia pathophysiology, till now the delivery is the only current cure, however this was commonly associated with iatrogenic preterm delivery[6] Therefore, it is particularly important to explore other promising biological approaches for the prevention of PE.

The gut microbiota, a complex and dynamic community residing in the digestive tract, plays critical roles in regulating host metabolism, maintaining immune homeostasis, and facilitating nutrient absorption and transformation[7, 8]. Any alterations in the structure and composition of the gut microbiota may be linked to host metabolic disorders and systemic inflammation, and have been implicated in a range of diseases, including obesity[9]、type 2 diabetes [10]、cardiometabolic disorders [11]、non-alcoholic liver disease [12] and inflammatory bowel disease[13]. In recent years, numerous studies have reported alterations in the gut microbiota of women with preeclampsia (PE), linking dysbiosis to the pathogenesis of the disease. The influence of the microbiota on the maternal immune system, together with the interplay between gut microbial imbalance and metabolic disorders, suggests that the microbiome plays a pivotal role in the development of PE. Notably, patients with PE exhibit reduced gut microbial diversity, with a marked enrichment of *Proteobacteria* and *Fusobacteria* in their intestinal microbiota [14]. Gut dysbiosis can compromise the intestinal barrier, allowing harmful bacterial metabolites such as lipopolysaccharide (LPS) to promote local and systemic inflammation, which in turn may trigger or exacerbate the onset and progression of PE. Moreover, in 2015, the existence of a gut–placenta axis was first demonstrated in non-human primates[15], Since then, accumulating evidence has challenged the view of the placenta as a sterile organ, suggesting that gut microbes and their metabolites can act via the gut–placenta axis to influence placental function and, ultimately, pregnancy outcomes[16].

Probiotics were originally defined in 2001 and refined in 2014 as “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host”[17]. Their mechanisms of action are increasingly understood to include pathogen displacement or growth inhibition, enhancement of barrier integrity through modulation of signaling pathways, and immune regulation [18]. *Lactobacillus*

rhamnosus, a widely used probiotic, is bile- and acid-resistant, capable of sustained colonization, and has been shown to reduce necrotizing enterocolitis (NEC) incidence and improve outcomes in preterm infants [19]. Recent findings link placental microbial colonization with preeclampsia pathogenesis, suggesting that modulation of placental microbiota may offer new preventive and therapeutic avenues. Bacterial translocation to the placenta via the gut–placenta axis likely involves ascending vaginal microbes, gut microbial translocation, and oral microbiota, facilitated by increased epithelial permeability during pregnancy and lactation, enabling bacterial dissemination into maternal circulation and placental colonization [20, 21]. *L. rhamnosus* LGR-1 is the first strain demonstrated to restore vaginal microbiota and reduce recurrent urinary tract infections[22]. However, its role in preeclampsia via gut microbiota modulation remains to be elucidated.

Here, we report for the first time the interplay between *Lactobacillus rhamnosus* and the gut placental microbiota in an LPS-induced preeclampsia rat model, and assess its protective potential role. Through 16S rRNA gene sequencing of fecal and placental samples, we identify microbial taxa closely linked to both preeclampsia pathogenesis and probiotic intervention. Our data reveal evidence of microbial translocation between the gut and placenta, and demonstrate that supplementation of *L. rhamnosus* mitigates preeclampsia by restoring gut microbial balance and normalizing placental metabolism. These findings highlight a promising avenue for novel therapeutic strategies against preeclampsia.

Methods and materials

Animals

Six-week-old Sprague-Dawley rats were obtained from Kunming Medical University, Yunnan, China (license number SYXK (Dian) K2020-0006). Animals were housed under standard laboratory conditions at 20–25°C, 50–55% relative humidity, with a 12-hour light/dark cycle. Food and water were provided *ad libitum*. All experimental procedures were approved by the Science and Technology Committee of Kunming City and the Institutional Animal Care and Use Committee of Kunming Medical University, and were conducted in accordance with institutional guidelines. The ethical approval number for this study is KMMU20221594.

Bacterial culture

Lactobacillus rhamnosus ATCC7469 was purchased from the American Cell Culture Collection. *Lactobacillus rhamnosus* was cultured anaerobically in MRS liquid medium at 37°C, 5% H_2, 10% CO_2 and 85% N_2 for 48h. 10 mL of LR suspension was taken in a centrifuge tube and centrifuged at 5000 rmp for 5 min. Bacterial precipitate was collected and washed three times with sterile PBS. Resuspend the bacteria with PBS to a concentration of approximately $5x10^9$CFU/ml and gavage each mouse with 0.2mL of the bacterial solution.

Animal experiment

Rats were acclimated for at least 7 days prior to experimentation. Female rats were randomly assigned into three groups: control (physiological saline), preeclampsia model (LPS), and *Lactobacillus rhamnosus* treatment (LPS + LR). The LPS + LR group received oral gavage of *Lactobacillus rhamnosus* at 1×10^9 CFU/mL for 21 days, while the control and LPS groups were gavaged with an equivalent volume of saline. Mice were anesthetized by intraperitoneal injection of ketamine (80 mg/kg) and xylazine (5 mg/kg) before gavage. Subsequently, females were mated with males at a 2:1 ratio. The presence of sperm in vaginal smears was used to confirm successful mating, and this day was designated as embryonic day 0 (D0). On embryonic day 5 (D5), LPS (0.5 μg/kg) was administered via tail vein injection to the LPS and LPS + LR groups, while the control group received an equivalent volume of saline. Throughout pregnancy, the LPS + LR group continued to receive daily gavage of *L. rhamnosus* (1×10^9 CFU/mL), whereas the LPS and control groups received saline (Fig. 1a). At the endpoint, mice were euthanized by cervical dislocation following intraperitoneal sodium pentobarbital (100 mg/kg) sedation. Death was confirmed by absence of heartbeat, respiration, and corneal reflex. Fecal, placental, and blood samples were subsequently harvested for analysis.

Blood Pressure and Proteinuria Measurement

Prior to blood pressure assessment, all rats underwent a one-week acclimation training. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured non-invasively using a tail-cuff system (Softron BP-2010, Japan) daily from embryonic day 3 (D3) to day 18 (D18). For each session, blood pressure was recorded three consecutive times, and the average value was calculated for each animal. Concurrently, urine samples were collected using metabolic cages to determine protein concentration, assessing proteinuria in pregnant rats.

Gut microbiota analysis

Total bacterial DNA was extracted from fecal samples using a commercial DNA isolation kit according to the manufacturer's protocol. The V3–V4 hypervariable regions of the bacterial 16S rRNA gene were PCR-amplified using universal primers with genomic DNA as the template. Amplification was performed with Phusion® High-Fidelity PCR Master Mix containing GC buffer. Libraries were subsequently constructed and sequenced on the NovaSeq 6000 platform. Raw sequencing reads were quality-filtered to remove barcode and primer sequences, and paired-end reads were merged using FLASH to generate raw tags[23]. Raw tags were quality-filtered and corrected using fastp and Vsearch to obtain high-quality clean tags. These clean tags were then compared against reference databases to generate effective tags for downstream analyses[24]. Denoising was performed using the DADA2 plugin within QIIME2, and sequences with an abundance of fewer than five were filtered out to generate amplicon sequence variants (ASVs) and the corresponding feature table. Alpha diversity was assessed using ACE, Chao1, Simpson, and Shannon indices. Weighted and unweighted principal coordinates analysis (PCoA) and

unweighted pair-group method with arithmetic mean (UPGMA) clustering were conducted using QIIME. Linear discriminant analysis effect size (LEfSe) was employed to detect significant differences in the relative abundance of microbial taxa between groups. Functional prediction and comparison of Kyoto Encyclopedia of Genes and Genomes (KEGG) metabolic pathways across groups were performed using PICRUSt2.

Metabolomics

Colon and cerebral cortex tissues from mice were ground separately in liquid nitrogen, while serum samples were directly transferred into tubes and mixed with pre-chilled 80% methanol. Samples were incubated on ice for 5 mins, followed by centrifugation at 15,000 × g for 20 mins at 4°C. The resulting supernatants were subjected to LC-MS/MS analysis[25]. Metabolite profiling was performed using an LC-MS/MS Analytics™ AD system (SCIEX) coupled to a QTRAP® 6500 + mass spectrometer (SCIEX, Novogene Ltd., Beijing, China). The raw data files generated by HPLC-MS/MS were processed with SCIEX OS software version 1.4 for peak integration and correction, with peak areas representing the relative abundance of corresponding metabolites. Metabolites were annotated against the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://www.genome.jp/kegg/). Partial least squares discriminant analysis (PLS-DA) was conducted using the metaX software package[26]. Statistical significance was evaluated using Student's *t*-test. Metabolites with VIP > 1, $P < 0.05$, and fold change (FC) ≥ 2 or ≤ 0.5 were considered differentially abundant. Volcano plots of metabolites of interest were generated using $\log_2(FC)$ and $-\log_{10}(P)$ values with the ggplot2 package in R. For hierarchical clustering heatmaps, metabolite intensity data were normalized to z-scores and visualized using the pheatmap package in R.

Statistical analyses

Statistical analyses were performed using IBM SPSS Statistics 20 and visualised by GraphPad Prism 7.0. Each experiment was carried out at least three times and data are expressed as mean ± standard error of the mean (SEM). Differences between groups were performed by two-tailed Student's t-test and one-way analysis of variance (ANOV A) with post hoc Tukey test. Differences were defined as statistically significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$.

Result

Lactobacillus rhamnosu s supplementation during pregnancy alleviates preeclampsia-like symptoms in rats

We established an early-pregnancy PE model in rats by administering a low dose of LPS on gestational day 5 to the LPS and LPS + LR groups (Fig. 1a). Hypertension and proteinuria are key diagnostic criteria for PE; therefore, we assessed these parameters across treatment groups. As expected, LPS-treated pregnant rats showed no difference in blood pressure on day 3, but beginning on day 6, systolic blood

pressure (SBP), diastolic blood pressure (DBP), and mean arterial pressure (MAP) increased progressively and reached statistically significant elevations compared to the control group ($p < 0.05$, Fig. 1b–1d). Moreover, LPS administration led to marked proteinuria on gestational days 15 ($p < 0.01$) and 18 ($p < 0.05$). Then, we evaluated the effect of *Lactobacillus rhamnosus* on LPS-induced PE. Notably, the rats in the LPS + LR group exhibited significantly lower SBP, DBP, and MAP compared to the LPS group from day 6 to day 18 ($p < 0.01$). Moreover, *L. rhamnosus* intervention significantly reduced urinary protein levels in PE rats ($p < 0.05$). Remarkably, urinary protein levels in the LPS + LR group were comparable to those in the control group ($p > 0.05$, Fig. 1e).

In addition, we observed that LPS-induced PE led to smaller size and lighter weight of placentas relative to controls; however, *L. rhamnosus* supplementation reversed these effects, restoring placental size and weight to levels not significantly different from the control group ($p > 0.05$, Fig. 2a, 2b). Despite these improvements, there were no significant changes in fetal weight or the fetal-to-placental weight ratio among the groups (Fig. 2c, 2d). Collectively, these findings suggest that supplementation with *L. rhamnosus* during pregnancy can alleviate PE-like symptoms in rats.

Effects of Lactobacillus rhamnosus on the gut microbiota of rats with preeclampsia

To investigate the impact of *Lactobacillus rhamnosus* intervention on the gut microbiota of LPS-induced preeclamptic pregnant rats, we collected fecal samples to analyze microbial community composition. Figure 3a shows the observed OTUs detected across different samples. Regarding alpha diversity, the number of observed OTUs in LPS-induced PE rats was lower than that in controls, and this reduction was attenuated by *L. rhamnosus* pretreatment ($p < 0.001$, Fig. 3b). Additionally, compared to the control group, the Chao1 ($p < 0.001$) and Shannon ($p < 0.01$) indices were significantly decreased in PE rats. Importantly, *L. rhamnosus* pretreatment significantly increased both the Chao1 (Fig. 3c) and Shannon indices (Fig. 3d) in PE rats compared to the LPS group ($p < 0.001$). However, no significant difference was observed among the three groups in the Simpson index ($p > 0.05$, Fig. 3e).For beta diversity, fecal microbiota from the three groups clustered into distinct groups, which were clearly separated in the PCoA plot, indicating changes in microbial community structure (Fig. 3f). Similarly, non-metric multidimensional scaling (NMDS) analysis revealed significant structural differences among the three groups (Fig. 3g). Weighted UniFrac analysis (Fig. 3h) showed that *L. rhamnosus* reduced the elevated β-diversity induced by LPS in PE rats ($p < 0.001$). We further analyzed the differential microbial composition among the three groups at the phylum (Fig. 3i), family (Fig. 3j), and genus (Fig. 3k) levels. Notably, LPS-induced preeclampsia disrupted the gut microbial composition compared to controls, whereas intervention with *L. rhamnosus* appeared to partially restore the gut microbiota structure toward that of normal pregnancy.

Subsequently, we performed linear discriminant analysis (LDA) to identify taxa that contributed most to the differences in microbial composition among the three groups. Key bacterial groups such as *Clostridia*, *p_Bacteroidota*, *p_Firmicutes*, and *o_Oscillospirales* were identified as major contributors, and LDA scores confirmed significant differences in gut microbiota composition across the groups (Fig. 4a).

The LEfSe cladogram further illustrated the differentially abundant taxa (Fig. 4b).At the phylum level, statistical analysis showed that LPS-induced preeclampsia rats exhibited a significant decrease in the relative abundance of *Firmicutes* ($p < 0.001$) and a marked increase in *Bacteroidota* ($p < 0.001$). Notably, *Lactobacillus rhamnosus* intervention partially reversed these changes, although the differences did not reach statistical significance (Fig. 4c). At the genus level, *L. rhamnosus* treatment significantly increased the abundance of several bacterial genera that were reduced in LPS-induced PE rats, including *g_Turicibacter*, *g_Clostridia_UCG-014*,*g_Roseburia*,*g_Monoglobus*,*g_Eubacterium_sireaum_group*, *g_Eubacterium_nodatum_group*, and *g_Lachnospiraceae_UCG-006* ($p < 0.05$; Fig. 4d).Correlation analysis revealed that hypertension and proteinuria in rats were positively correlated with *g_Phascolarctobacterium*, while negatively correlated with *g_Roseburia*, *g_Clostridia_UCG-014*, and *g_Eubacterium-*

nodatum_group. Placental weight was positively correlated with *g_Roseburia*, *g_Anaerotruncus*, and *g_Eubacterium_sireaum_group*, and negatively correlated with *g_Phascolarctobacterium*. These findings suggest that alterations in the abundance of these bacteria may play an important role in the development and progression of preeclampsia in rats.

Effects of Lactobacillus rhamnosus on the placental microbiota in preeclamptic rats

Previous studies suggest that gut dysbiosis may promote the development of PE through bacterial translocation. To explore whether *Lactobacillus rhamnosus* modulates LPS-induced PE in rats via the gut–placenta axis, we analyzed changes in the placental microbiota. Figure 5a showed the observed OTUs detected in placental samples from different groups. We found that *L. rhamnosus* significantly increased the number of placental OTUs in PE rats ($p < 0.001$, Fig. 5b). Moreover, the LPS + LR group showed significantly higher Shannon (Fig. 5c) and Chao1 indices (Fig. 5d), and a significantly lower Simpson index (Fig. 5e), indicating increased α-diversity.Regarding β-diversity, both PCoA and NMDS analyses revealed clear structural differences in placental microbial communities among the three groups (Fig. 5f, 5g). Further analysis of microbial composition at the phylum (Fig. 5h) and family (Fig. 5i) levels showed that *L. rhamnosus* intervention appeared to restore the placental microbiota structure toward that of normal pregnancy. Interestingly, we observed that the overall structure of the placental microbiota differed from that of the gut microbiota: at the phylum level, *Proteobacteria* dominated in the placenta of normal pregnant rats, rather than *p_Bacteroidota* or *p_Firmicutes*. At the family level, LPS-induced PE rats exhibited a marked increase in *Pseudomonadaceae* and a decrease in *Lactobacillaceae*. Linear discriminant analysis (LDA) identified key taxa such as *Muribaculaceae*, *Pseudomonas*, and *Gammaproteobacteria* that contributed most to the differences among the three groups, with LDA scores confirming significant differences in placental microbiota composition (Fig. 5j). Differential abundance analysis at the genus level (Fig. 5k) revealed that the relative abundance of *Alistipes*, *Bacteroides*, *Christensenellaceae_R-7_group*, *g_Clostridia_UCG-014*, *Eubacterium_Coprostano*

ligenes_group, *Eubacterium_siraeum_group*, *Parabacteroides*, *Prevotellaceae_UCG-001*, *Roseburia*, *Lachnospiraceae_UCG-006*, and *Butyricimonas* was significantly reduced in the placenta of LPS-induced

PE rats ($p < 0.05$), whereas *L. rhamnosus* intervention increased the abundance of these genera. Notably, at the genus level, we identified four bacterial taxa—*Clostridia_UCG-014*, *Roseburia*, *Eubacterium_siraeum_group*, and *Lachnospiraceae_UCG-006*—that were consistently shared and similarly altered in both placental and cecal (fecal) samples. These findings provide evidence supporting a potential connection between gut and placental microbiota via the gut–placenta axis.

Lactobacillus rhamnosus may alleviate PE by reducing placental LysoPC accumulation

Gut microbial metabolites serve as critical mediators linking intestinal microbiota to host physiological responses. To explore this mechanism, we analyzed placental metabolic profiles in PE rats. Partial least squares discriminant analysis (PLS-DA) showed tight clustering within groups and clear separation between groups (Fig. 6a, 6b). The R^2Y value reflects the model's goodness-of-fit, while Q^2Y assesses predictive ability; in our model, R^2Y exceeded Q^2Y, supporting its reliability (Fig. 6c, 6d). Differential metabolites were identified based on VIP > 1.0, fold change (FC) > 1.2 or < 0.833, and $p < 0.05$. VIP reflects each metabolite's contribution to the clustering model, and FC indicates the magnitude of change. Volcano plots visualized the overall distribution of these differential metabolites. Compared to the control group, the LPS group exhibited 17 differential metabolites, with 10 significantly downregulated and 7 significantly upregulated (Fig. 6e). Compared to the LPS group, the LPS + LR group showed 6 differential metabolites: 3 upregulated and 3 downregulated (Fig. 6f). A heatmap (Fig. 6g) further illustrated 33 metabolites that differed significantly among the three groups.

Notably, *L. rhamnosus* intervention significantly increased levels of UDP-D-glucose, cholesterol, and 5-methyl tetrahydrofolate (5-methyl THF) in the placenta of PE rats, while significantly reducing levels of propionylcholine, LysoPC 16:2 (2N isomer), LysoPC 10:0, and epinephrine bitartrate. Other metabolites, including LysoPC 16:1, L-gulono-1,4-lactone, imatinib, D-gluconic acid, D-glucarate, D-galactonic acid, cytidine 5'-monophosphate-N-acetylneuraminic acid, 2,6-dihydroxybenzoic acid, 4-aminoindole, and Nα-acetyl-L-arginine, also showed trends opposite to those in the LPS group after *L. rhamnosus* treatment, though without statistical significance.Importantly, the most striking finding was the marked increase in placental LysoPC levels (including LysoPC 16:1, LysoPC 16:2, and LysoPC 10:0) in LPS-induced PE rats, which was significantly suppressed by *L. rhamnosus* supplementation. The protective effect of *L. rhamnosus* on PE may involve attenuation of placental LysoPC accumulation.

Correlation analysis reveals links between microbiota and metabolites in PE rats following Lactobacillus rhamnosus intervention

By comparing microbial profiles in the gut and placenta, we observed an intriguing pattern: four bacterial genera—*Roseburia*, *Eubacterium_siraeum_group*, *Clostridia_UCG-014*, and *Lachnospiraceae_UCG-006*—showed consistent trends of change in both sites. This finding suggests the possibility of bacterial translocation between the gut and placenta.To further explore this, we performed correlation analysis between these four microbial taxa and placental differential metabolites. A correlation network heatmap illustrated the connections, with solid lines indicating positive correlations, dashed lines indicating negative correlations, and line thickness reflecting the strength of association (Fig. 7a).The correlation

heatmap revealed that the abundance of *Lachnospiraceae_UCG-006* was negatively correlated with levels of D-gluconic acid, D-glucarate, D-galactonic acid, and L-gulono-1,4-lactone. *Roseburia* abundance was positively correlated with 2,6-dihydroxybenzoic acid, cytidine 5'-monophosphate-N-acetylneuraminic acid, and cholesterol, but negatively correlated with D-glucarate. *Clostridia_UCG-014* abundance showed negative correlations with epinephrine bitartrate, imatinib, LysoPC 16:2, and D-glucarate. Similarly, *Eubacterium_siraeum_group* abundance was negatively correlated with epinephrine bitartrate and D-glucarate (Fig. 7b).These correlations suggest that *L. rhamnosus* may modulate specific gut microbial taxa, which in turn are linked to key placental metabolic changes in PE.

We established a rat preeclampsia (PE) model using low-dose LPS during early pregnancy and systematically evaluated the alleviating effects of Lactobacillus rhamnosus (LR) supplementation on PE-like symptoms during pregnancy. We also combined 16S sequencing and metabolomics to explore the comprehensive regulatory effects of LR intervention on gut microbiota, placental microbiota, and placental metabolic profiles. Our results not only confirm the potential value of LR in alleviating PE phenotypes but also provide multi-level evidence supporting the crucial role of the gut-placenta axis in the pathogenesis and prevention of PE.

Firstly, we found that LPS-induced PE rats exhibited typical clinical features such as hypertension and proteinuria, which were highly consistent with the pathological findings of clinical PE. LR intervention significantly reduced systolic blood pressure, diastolic blood pressure, and mean arterial pressure ($P < 0.01$) in rats, while also significantly reducing urine protein levels ($P < 0.05$) and improving placental size and weight. LPS-induced PE rats exhibited significantly reduced α diversity of gut microbiota, including the number of OTUs, Chao1 index, and Shannon index, indicating decreased microbial richness and evenness. LR supplementation significantly restored these diversity indices ($P < 0.001$) and maintained microbial stability. β diversity analysis also confirmed significant differences in microbial community structure among groups, with LR intervention restoring microbial community structure toward that of normal pregnancy. This is consistent with the findings of Ishimwe et al [27] and Yang et al [28], both of which suggest that gut microbiota intervention can alleviate pregnancy-related hypertension and inflammatory damage.

Interestingly, genus-level analysis revealed that the abundance of butyrate-producing bacteria such as Roseburia, Clostridia_UCG-014, Lachnospiraceae_UCG-006, and Eubacterium_siraeum_group was significantly reduced in the LPS group, while LR intervention significantly increased the abundance of these bacteria ($P < 0.05$). Roseburia is an anaerobic, Gram-positive, slightly curved rod-shaped flagellated bacterium that has been shown to prevent intestinal inflammation and maintain energy homeostasis by producing metabolic products.It's the findings that Roseburia is beneficial in various diseases, such as inflammatory bowel disease, type 2 diabetes, antiphospholipid syndrome, and atherosclerosis. [29]Zhao [30] et al. reported that Clostridium difficile alleviated food allergy symptoms in mice by regulating their intestinal immune function, improving the composition of their intestinal microbiota, and strengthening

their intestinal barrier. The increase in these microbial communities may promote SCFA synthesis. A study indicates that metabolic changes associated with energy homeostasis protection during pregnancy are critical for the developing fetus, its future metabolic fate, and maternal health. SCFAs act as signaling molecules, regulating the body's energy balance through G protein-coupled receptors (GPCRs). The GPR41 receptor influences metabolism through the microbiota, while the GPR43 receptor is believed to serve as a molecular link between diet, microbiota, gastrointestinal function, immunity, and inflammatory responses [31]. Additionally, correlation analysis showed that the abundance of Roseburia and Clostridia_UCG-014 was negatively correlated with hypertension and proteinuria, while g_Phascolarctobacterium was positively correlated with hypertension. These results suggest that specific bacterial genera may participate in the development of PE through inflammation and metabolic regulation. Notably, we also analyzed the placental microbiota and found that the placental microbiota of normal pregnant rats was dominated by Proteobacteria, structurally distinct from the intestinal microbiota. However, Roseburia, Clostridia_UCG-014, Eubacterium_siraeum_group, and Lachnospiraceae_UCG-006 exhibit similar trends in both the placenta and cecum, suggesting a potential “gut-placenta axis” connection, i.e., the concept that maternal gut microbiota can migrate to the placenta, thereby enriching experimental evidence for microbial cross-barrier effects.

Metabolomics analysis highlighted that LPS-induced preeclampsia (PE) rat placentas exhibited significantly elevated levels of multiple pro-inflammatory lipids (LysoPC 16:1, LysoPC 16:2, LysoPC 10:0), while LR intervention markedly downregulated their levels, suggesting that LysoPC may be one of the key metabolic markers in PE pathogenesis. LysoPC is a class of biologically active phospholipid molecules that play important roles in metabolism, inflammation, and signal transduction. It can act as an inflammatory mediator, promoting the expression of adhesion molecules and chemokines in endothelial cells, thereby triggering inflammatory responses. It is closely associated with chronic inflammatory diseases such as atherosclerosis, hypertension, and diabetes[32–34]. Recent studies have shown that significantly elevated levels of LysoPC (16:0) and LysoPC (18:0) in umbilical cord blood play a key role in gestational diabetes. It enhances mitochondrial ROS and upregulates HIF-1α expression, impairing mitochondrial homeostasis and leading to trophoblast dysfunction in gestational diabetes[35]. Additionally, correlation analysis revealed that Roseburia abundance was positively correlated with cholesterol and Cytidine 5'-Monophosphate-N-Acetylneuraminic Acid, and negatively correlated with D-Glucarate; Lachnospiraceae_UCG-006 abundance was negatively correlated with D-Gluconic acid, etc.; Clostridia_UCG-014 was negatively correlated with epinephrine bitartrate and lysophosphatidylcholine 16:2. These microbial–metabolite networks suggest that LR modulates specific beneficial bacterial communities, thereby influencing placental lipid and carbohydrate metabolism, improving inflammatory and oxidative stress states, and alleviating PE symptoms.

Summarily, this study revealed the potential mechanism of LR in alleviating preeclampsia (PE) by integrating microbial diversity analysis, abundance of key differential bacterial genera, placental metabolome, and correlation analysis. The results indicate that LR intervention helps upregulate specific SCFA-producing bacteria (such as Roseburia and Clostridia_UCG-014), maintaining microbial ecosystem

stability during pregnancy; simultaneously, it significantly reduces the accumulation of pro-inflammatory lipid LysoPC in the placenta, alleviating inflammation and oxidative stress damage; Furthermore, it improved microbial imbalance in the gut-placental axis, suggesting a potential important microbial migration or metabolic regulatory relationship between maternal gut microbiota and placental function. These findings not only deepen our understanding of the role of gut microbiota in the pathogenesis of PE but also provide new theoretical basis and potential targets for probiotic intervention during pregnancy.

Declarations

Authors' Contributions

Yuan Qian and Xingli Deng conceived and designed the study. Qiuyue and Yantuanjin Ma performed the literature search. Yuhang Zhang conducted the data collection. Qiuyue He prepared the manuscript. All authors read and approved the final version of the manuscript for publication.

Funding

This work was supported by National Natural Science Foundation of China□82360261□;Yunnan Provincial Department of Science and Technology & Kunming Medical University Basic Research Joint Project-Key Project□202401AY070001-036; Health Research Project of Kunming Medical Technology Center Project, 2022-05-02-002; Leader Project of Medical Science and Technology Discipline in Kunming Health Science and Technology Talent Abstract, 2023-SW□Leader)-06.□Yunnan Provincial Department of Science and Technology, Youth Program of Basic Research Projects, 202501AU070081□ Yunnan Provincial Department of Education, Youth Talent Program of Basic Research Projects, 2025J0160.

Data Availability Statement

The raw data presented in this study can be found in the NCBI repository with accession number PRJNA1484749.

Ethics approval and consent to participate

All animal experiments were performed in accordance with the guidelines formulated by Kunming Medical University, ethical approval number KMMU20221594.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflict of interest, financial or otherwise.

References

1. Jeyabalan A. Epidemiology of preeclampsia: impact of obesity [J]. Nutr Rev. 2013;71(1):S18–25.
2. Gestational Hypertension and Preeclampsia. ACOG Practice Bulletin Summary, Number 222 [J]. Obstet Gynecol. 2020;135(6):1492–5.
3. Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy [J]. Obstet Gynecol. 2013;122(5):1122–31.
4. Girardi G. Complement activation, a threat to pregnancy [J]. Semin Immunopathol. 2018;40(1):103–11.
5. Delhaes F, Giza SA, Koreman T, et al. Altered maternal and placental lipid metabolism and fetal fat development in obesity: Current knowledge and advances in non-invasive assessment [J]. Placenta. 2018;69(null):118–24.
6. Hampton T. Do Gut Bacteria Play a Role in Preeclampsia? [J]. Jama-journal Am Med association. 2020;323(21):2120–1.
7. Xiang YT, Mei L. [Research progress in pathogenesis of IBD: the role of gut flora] [J]. Sheng li ke xue jin zhan [Progress in physiology], 2013, 44(4): 247–52.
8. Yang AL, Kashyap PC. A clinical primer of the role of gut microbiome in health and disease [J]. Trop gastroenterology: official J Dig Dis Foundation. 2015;36(1):1–13.
9. Davis HC. Can the gastrointestinal microbiota be modulated by dietary fibre to treat obesity? [J]. Ir J Med Sci. 2018;187(2):393–402.
10. Woldeamlak B, Yirdaw K, Biadgo B. Role of Gut Microbiota in Type 2 Diabetes Mellitus and Its Complications: Novel Insights and Potential Intervention Strategies [J]. Korean J Gastroenterol = Taehan Sohwagi Hakhoe chi. 2019;74(6):314–20.
11. Kunika, Frey N, Rangrez AY. Exploring the Involvement of Gut Microbiota in Cancer Therapy-Induced Cardiotoxicity [J]. Int J Mol Sci. 2023;24(8):null.
12. Sharpton SR, Yong GJM, Terrault NA, et al. Gut Microbial Metabolism and Nonalcoholic Fatty Liver Disease [J]. Hepatol Commun. 2019;3(1):29–43.
13. Kempski J, Huber S. [Role of the gut microbiome in the pathogenesis and treatment of inflammatory bowel diseases] [J]. null. 2022;63(10):1022–7.
14. Zhao Y, Wang B, Zhao X, et al. The effect of gut microbiota dysbiosis on patients with preeclampsia [J]. Front Cell Infect Microbiol. 2022;12(null):1022857.
15. Schlabritz-Loutsevitch N, Li XJ, Rensing C, et al. Microbiota and the gut-placenta axis in non-human primates (PAPIO SPP.) [J]. Placenta. 2015;36(9):A16.
16. Stupak A, Gęca T, Kwaśniewska A, et al. Comparative Analysis of the Placental Microbiome in Pregnancies with Late Fetal Growth Restriction versus Physiological Pregnancies [J]. Int J Mol Sci. 2023;24(8):null.
17. Hill C, Guarner F, Reid G, et al. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term

probiotic [J]. Nat Reviews Gastroenterol Hepatol. 2014;11(8):506–14.
18. Petrova MI, Reid G, Ter Haar JA. Lacticaseibacillus rhamnosus GR-1, a.k.a. Lactobacillus rhamnosus GR-1: Past and Future Perspectives [J]. Trends Microbiol. 2021;29(8):747–61.
19. Meyer MP, Alexander T. Reduction in necrotizing enterocolitis and improved outcomes in preterm infants following routine supplementation with Lactobacillus GG in combination with bovine lactoferrin [J]. J neonatal-perinatal Med. 2017;10(3):249–55.
20. Olaniyi KS, Moodley J, Mahabeer Y, et al. Placental Microbial Colonization and Its Association With Pre-eclampsia [J]. Front Cell Infect Microbiol. 2020;10(null):413.
21. Ahmadian E, Rahbar Saadat Y, Hosseiniyan Khatibi SM, et al. Pre-Eclampsia: Microbiota possibly playing a role [J]. Pharmacol Res. 2020;155(null):104692.
22. Bruce AW, Reid G. Intravaginal instillation of lactobacilli for prevention of recurrent urinary tract infections [J]. Can J Microbiol. 1988;34(3):339–43.
23. Magoč T, Salzberg SL. Bioinformatics. 2011;27(21):2957–63. FLASH: fast length adjustment of short reads to improve genome assemblies [J].
24. Haas BJ, Gevers D, Earl AM, et al. Chimeric 16S rRNA sequence formation and detection in Sanger and 454-pyrosequenced PCR amplicons [J]. Genome Res. 2011;21(3):494–504.
25. Want EJ, Masson P, Michopoulos F, et al. Global metabolic profiling of animal and human tissues via UPLC-MS [J]. Nat Protoc. 2013;8(1):17–32.
26. Wen B, Mei Z, Zeng C, et al. metaX: a flexible and comprehensive software for processing metabolomics data [J]. BMC Bioinformatics. 2017;18(1):183.
27. Ishimwe JA, Akinleye A, Johnson AC, et al. Gestational gut microbial remodeling is impaired in a rat model of preeclampsia superimposed on chronic hypertension [J]. Physiol Genom. 2021;53(3):125–36.
28. Yang X, Zhang M, Zhang Y, et al. Ecological change of the gut microbiota during pregnancy and progression to dyslipidemia [J]. NPJ biofilms microbiomes. 2023;9(1):14.
29. Nie K, Ma K, Luo W, et al. Roseburia intestinalis: A Beneficial Gut Organism From the Discoveries in Genus and Species [J]. Front Cell Infect Microbiol. 2021;11(null):757718.
30. Zhao T, Huang HY, Mu RQ et al. Clostridia Enhances Intestinal Immunity to Alleviate Ovalbumin-Induced Allergy [J]. International archives of allergy and immunology, 2025, null(null): 1–20.
31. Ziętek M, Celewicz Z, Szczuko M. Short-Chain Fatty Acids, Maternal Microbiota and Metabolism in Pregnancy [J]. Nutrients. 2021;13(4):null.
32. Cheng H, Yao Q, Song R, et al. Lysophosphatidylcholine activates the Akt pathway to upregulate extracellular matrix protein production in human aortic valve cells [J]. J Surg Res. 2017;213(null):243–50.
33. Qin X, Qiu C, Zhao L. Lysophosphatidylcholine perpetuates macrophage polarization toward classically activated phenotype in inflammation [J]. Cellular immunology, 2014, 289(1–2): 185 – 90.

34. Sheikh AM, Ochi H, Manabe A, et al. Lysophosphatidylcholine posttranscriptionally inhibits interferon-gamma-induced IP-10, Mig and I-Tac expression in endothelial cells [J]. Cardiovascular Res. 2005;65(1):263–71.
35. Hung SC, Chan TF, Chan HC, et al. Lysophosphatidylcholine Impairs the Mitochondria Homeostasis Leading to Trophoblast Dysfunction in Gestational Diabetes Mellitus [J]. Antioxid (Basel Switzerland). 2024;13(8):null.

Figures

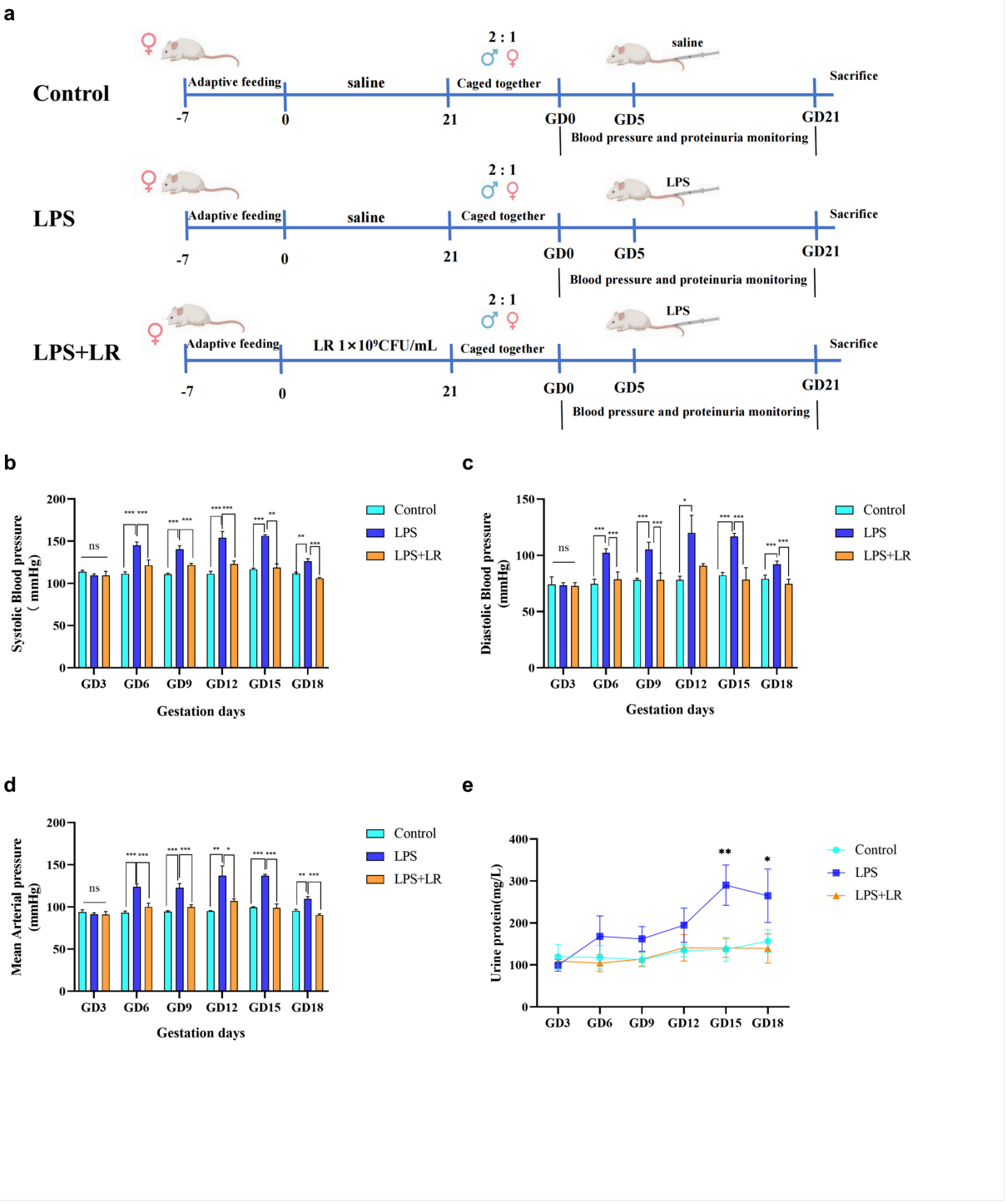


Figure 1

Effects of prenatal supplementation with *Lactobacillus rhamnosus* (LR) on blood pressure, proteinuria and placental parameters in LPS-induced preeclampsia model rats.

(a) Schematic diagram of the experimental procedure, including LPS administration on gestational day (GD) 5 and the timeline of LR intervention.(b–d) Dynamic changes in systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial pressure (MAP) from GD3 to GD18 in different groups.

(e) Time-course of urinary protein levels during gestation. Compared with the control group, LPS treatment significantly increased blood pressure and proteinuria (*p<0.05, ** p<0.01); LR intervention significantly reduced blood pressure and proteinuria, and partially restored placental development (p >0.05 vs. control, not significant). Data are presented as mean ± SD. Statistical significance was determined by one-way ANOVA followed by Tukey's post hoc test.

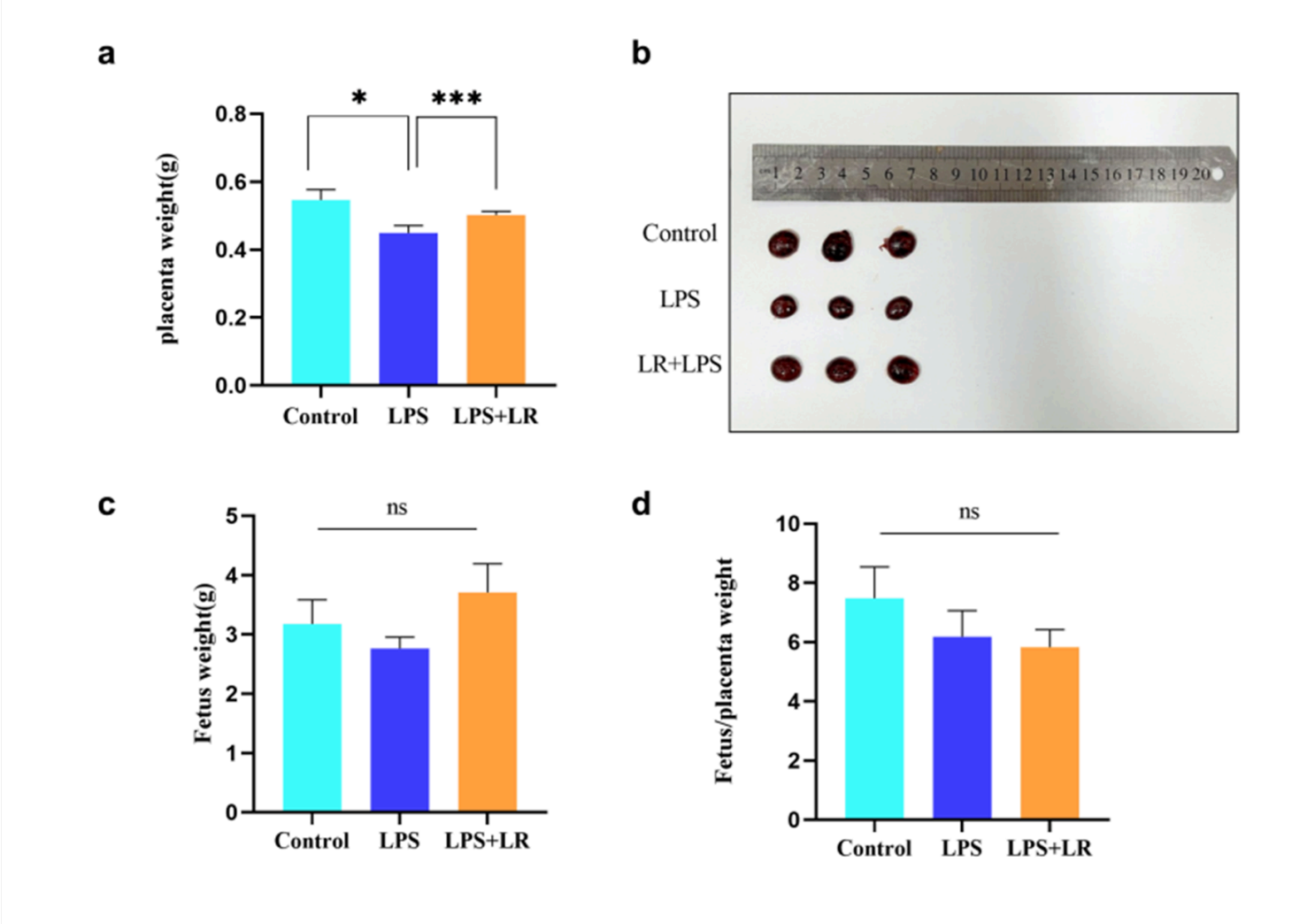


Figure 2

Effects of LR intervention on fetal weight and fetal-to-placental weight ratio in LPS-induced preeclampsia model rats.

(a, b) Mean fetal weight and total fetal weight per litter.

(c, d) Fetal-to-placental weight ratio. LR intervention had no significant effect on fetal weight or fetal-to-placental weight ratio (p >0.05).

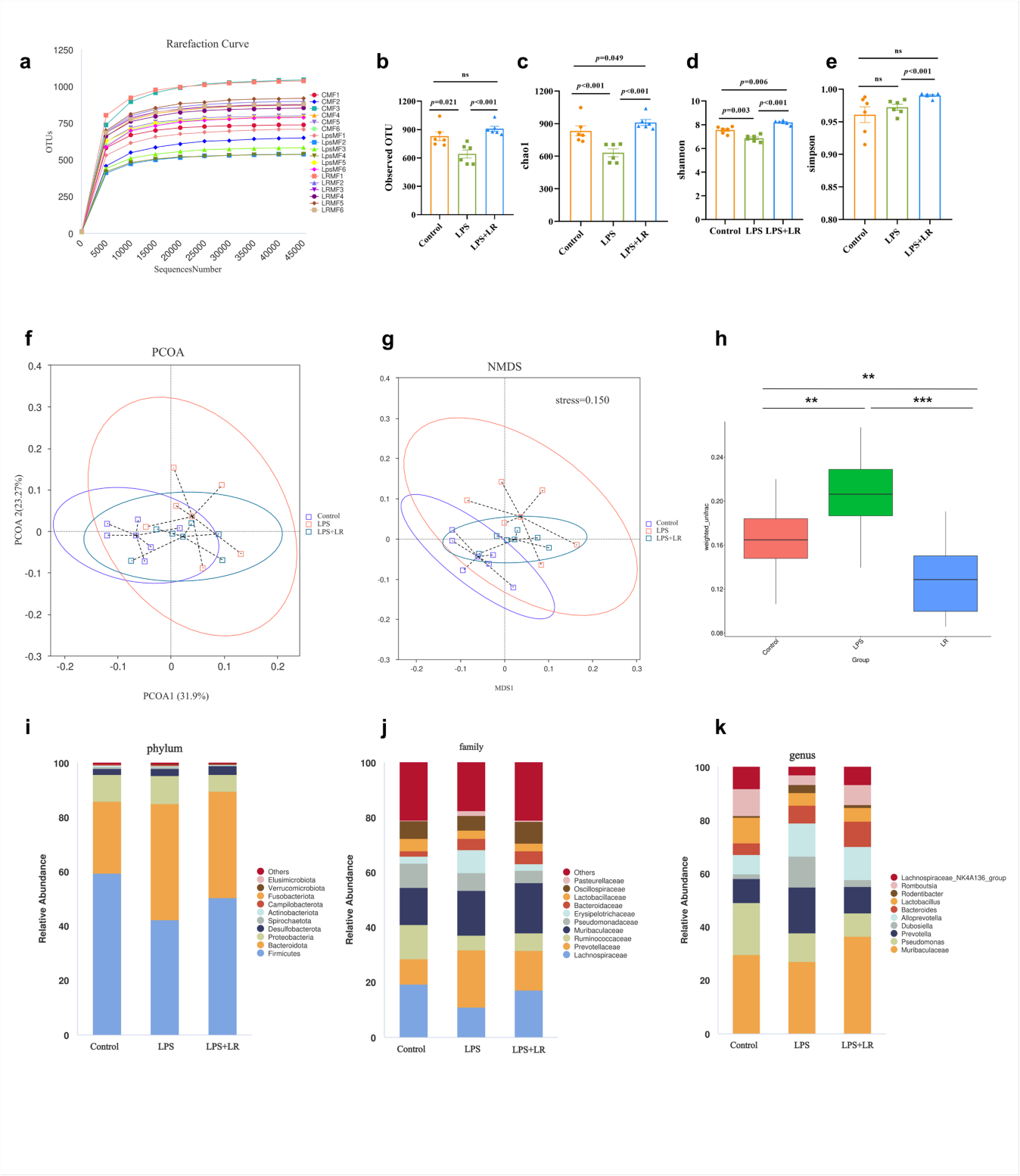


Figure 3

Effects of LR intervention on gut microbiota diversity and community structure in preeclampsia model rats.

(a) Bar plot of OTU abundance distribution. (b–e) α-diversity indices: Observed OTUs, Chao1, Shannon and Simpson indices. (f, g) β-diversity analyses by principal coordinates analysis (PCoA) and non-metric multidimensional scaling (NMDS). (h) Weighted UniFrac analysis comparing β-diversity among groups.

(i–k) Comparison of gut microbiota composition at phylum, family and genus levels. Results showed that LPS significantly reduced gut microbial diversity (P<0.001), whereas LR intervention markedly increased α-diversity and shifted community structure closer to that of normal pregnant rats. These findings suggest that LR may alleviate PE symptoms by restoring gut microbial balance.

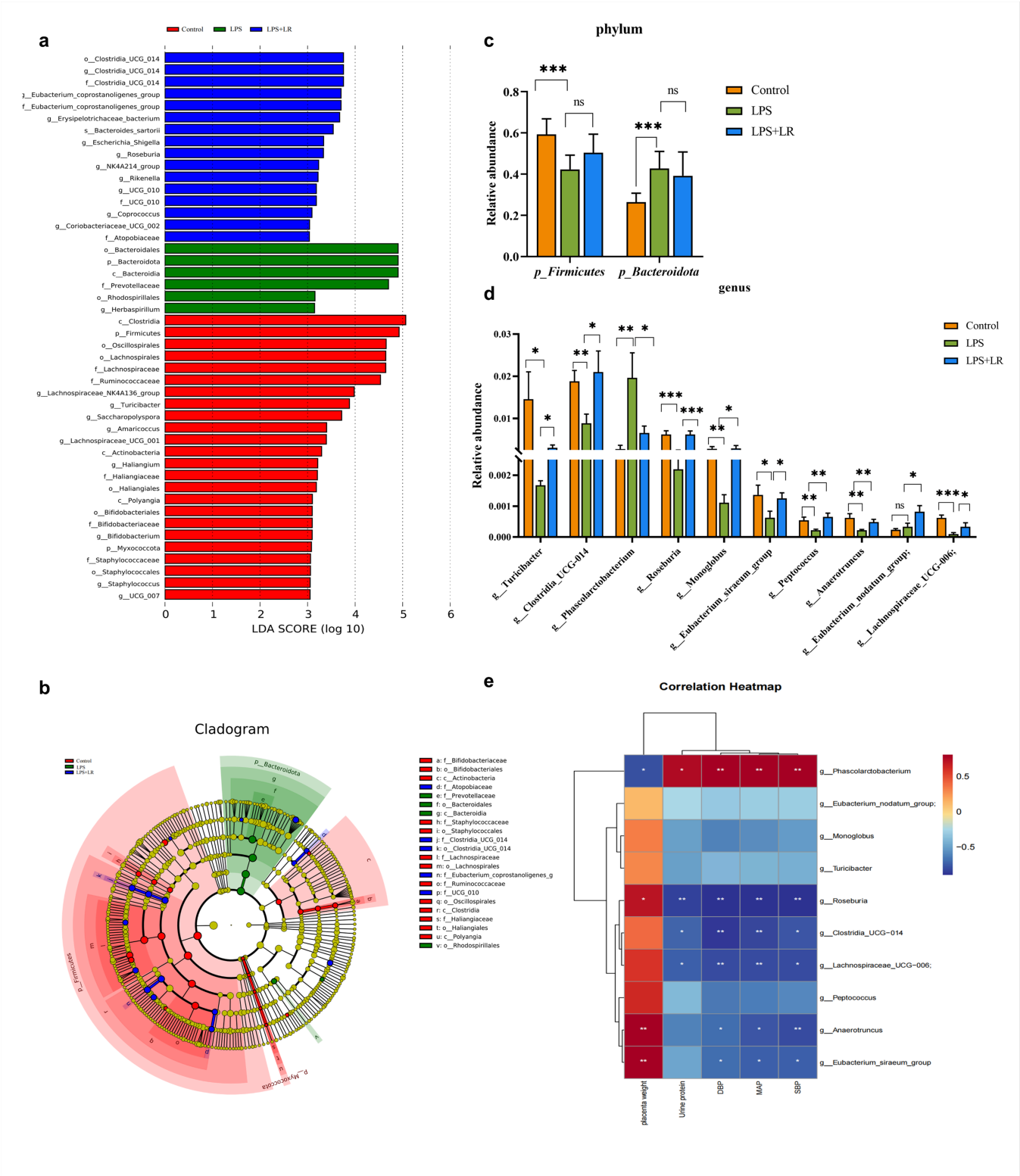


Figure 4

Effects of LR intervention on the abundance and functional characteristics of differential gut microbiota in preeclampsia model rats.

(a) Linear discriminant analysis (LDA) score plot showing key taxa contributing to microbial structural differences among groups.(b) LEfSe cladogram highlighting significantly different taxa.
(c) Comparison of relative abundances of Firmicutes and Bacteroidota at the phylum level: LPS reduced Firmicutes and increased Bacteroidota, while LR intervention partially reversed these changes.n(d) Comparison at the genus level: LR intervention significantly increased the abundance of SCFA-producing genera, including *Turicibacter*, *Clostridia_UCG-014*, *Roseburia*, *Eubacterium_siraeum_group* and *Lachnospiraceae_UCG-006* ($p<0.05$). These results suggest that restoring beneficial or SCFA-producing bacteria may be an important mechanism by which LR alleviatesPE.

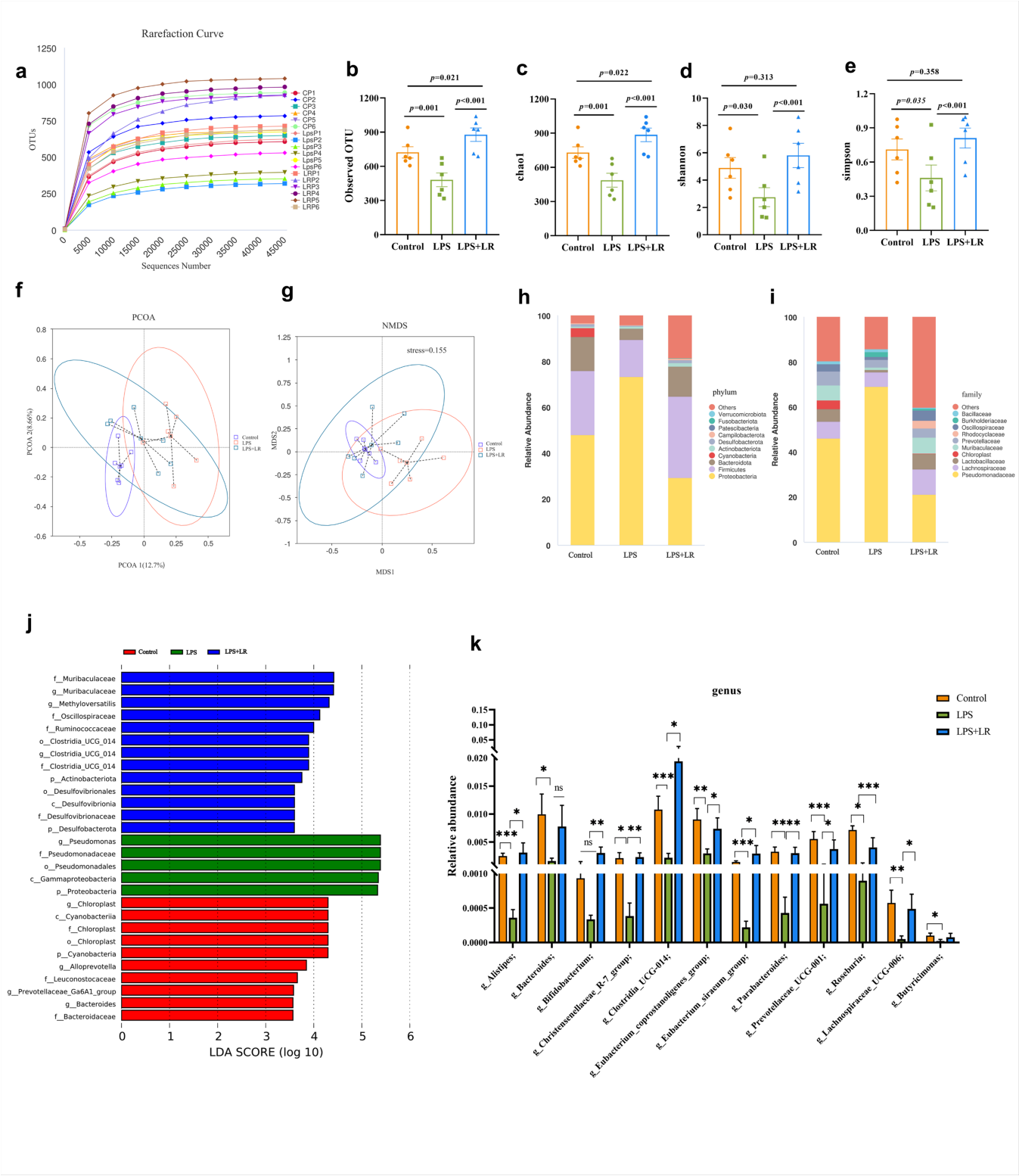


Figure 5

Effects of LR intervention on placental microbiota diversity and community structure in preeclampsia model rats.

(a) OTU abundance distribution.(b–e) α-diversity indices: Observed OTUs, Chao1, Shannon and Simpson indices.(f,g) β-diversity analyses by principal coordinates analysis (PCoA) and non-metric multidimensional scaling (NMDS).(h,i) Comparison of microbial community composition at phylum and

family levels.(j) LDA analysis identifying key differential taxa, such as *Pseudomonas* and *Muribaculaceae*. (k) Comparison of genus-level differential abundances.Results showed that LR intervention significantly increased placental microbiota α-diversity(p<0.001) and shifted the community structure closer to that of normal pregnant rats. These findings suggest that LR may help restore placental microbial balance and contribute to maintaining placental function.

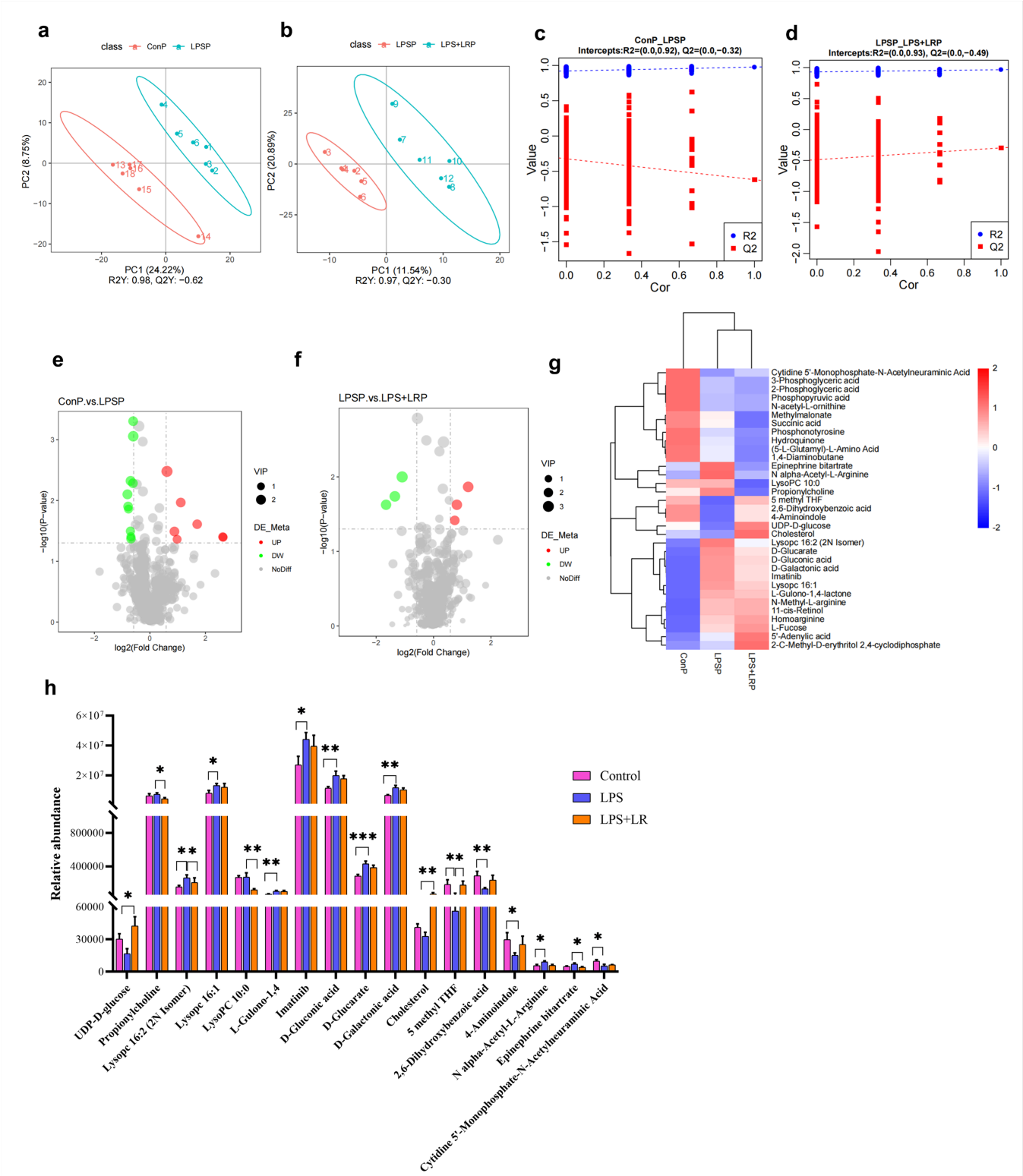


Figure 6

Effects of LR intervention on placental metabolomic profiles in preeclampsia model rats.
(a,b) Partial least squares discriminant analysis (PLS-DA) differentiating LR+LPS, LPS, and control groups.(c,d) R^2Y and Q^2Y values validating model fit and predictive ability. (e,f) Volcano plots of differential metabolites: 17 metabolites altered in LPS vs. control; 6 metabolites altered in LR+LPS vs. LPS. (g) Heatmap clustering of 33 significantly altered metabolites. (h) Differential metabolites identified. LR intervention significantly decreased pro-inflammatory lipids such as lysophosphatidylcholines (LysoPC 16:1, 16:2, 10:0) and upregulated metabolites including UDP-D-glucose, cholesterol, and 5-methyl tetrahydrofolate (5-methyl THF), suggesting metabolic regulation as a key mechanism underlying PE alleviation. (*p<0.05, ** p <0.01, *** p <0.001).

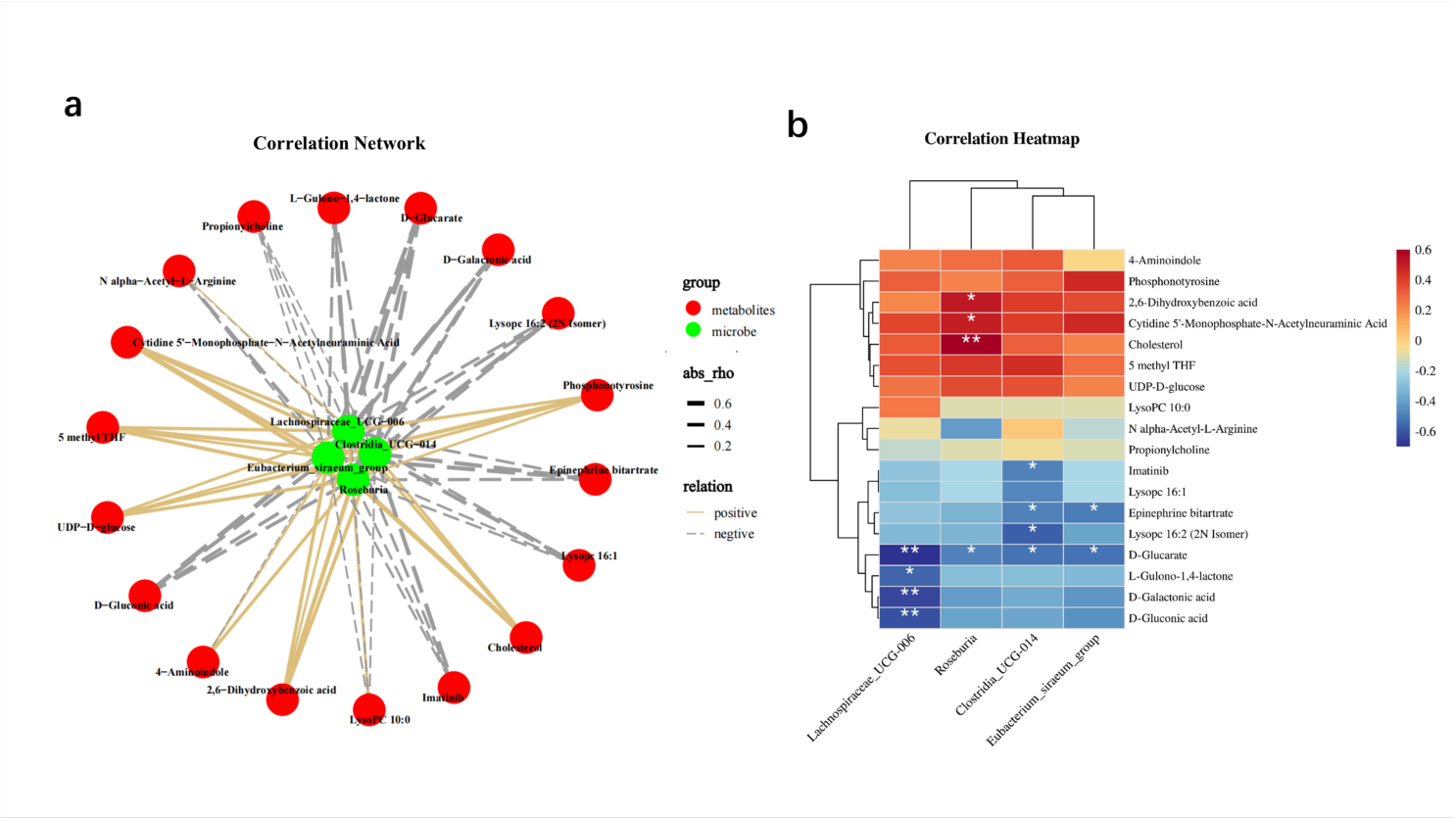


Figure 7

Correlation analysis between key differential genera and placental metabolites under LR intervention.
(a) Correlation network illustrating significant associations between key placental metabolites and four core genera (*Roseburia*, *Clostridia_UCG-014*, *Eubacterium_siraeum_group*, *Lachnospiraceae_UCG-006*) following LR treatment. (b) Heatmap showing positive and negative correlations between selected genera and specific metabolites; for example, *Roseburia* positively correlates with cholesterol and negatively with D-glucarate; *Clostridia_UCG-014*negatively correlates with LysoPC 16:2, among others. These findings suggest that LR may alleviate PE phenotypes by modulating key microbial taxa and their metabolic networks.