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Research Article

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Rice bran oil supplementation protects swine weanlings against diarrhea and lipopolysaccharide challenge

Juncheng HUANG[1,2,3], Wenxia QIN[1,2,3], Baoyang XU[1,2,3], Haihui SUN[4], Fanghua JING[4], Yunzheng XU[1,2,3], Jianan ZHAO[1,2,3], Yuwen CHEN[1,2,3], Libao MA[1,2,3]✉, Xianghua YAN[1,2,3]✉

[1]*State Key Laboratory of Agricultural Microbiology, Hubei Hongshan Laboratory, Frontiers Science Center for Animal Breeding and Sustainable Production, College of Animal Sciences and Technology, Huazhong Agricultural University, Wuhan 430070, China*
[2]*The Cooperative Innovation Center for Sustainable Pig Production, Wuhan 430070, China*
[3]*Hubei Provincial Engineering Laboratory for Pig Precision Feeding and Feed Safety, Wuhan 430070, China*
[4]*Yichun Dahaigui Life Science Co., Ltd., Yichun 336000, China*

Abstract: Early weaned piglets suffer from oxidative stress and enteral infection, which usually results in gut microbial dysbiosis, serve diarrhea, and even death. Rice bran oil (RBO), a polyphenol-enriched by-product of rice processing, has been shown to have antioxidant and anti-inflammatory properties both in vivo and in vitro. Here, we ascertained the proper RBO supplementation level, and subsequently determined its effects on lipopolysaccharide (LPS)-induced intestinal dysfunction in weaned piglets. A total of 168 piglets were randomly allocated into four groups of seven replicates (42 piglets each group, (21±1) d of age, body weight (7.60±0.04) kg, and half males and half females) and were given basal diet (Ctrl) or basal diet supplemented with 0.01% (mass fraction) RBO (RBO1), 0.02% RBO (RBO2), or 0.03% RBO (RBO3) for 21 d. Then, seven piglets from the Ctrl and the RBO were treated with LPS (100 μg/kg body weight (BW)) as LPS group and RBO+LPS group, respectively. Meanwhile, seven piglets from the Ctrl were treated with the saline vehicle (Ctrl group). Four hours later, all treated piglets were sacrificed for taking samples of plasma, jejunum tissues, and feces. The results showed that 0.02% was the optimal dose of dietary RBO supplementation based on diarrhea, average daily gain, and average daily feed intake indices in early weaning piglets. Furthermore, RBO protected piglets against LPS-induced jejunal epithelium damage, which was indicated by the increases in villus height, villus height/crypt depth ratio, and Claudin-1 levels, as well as a decreased level of jejunal epithelium apoptosis. RBO also improved the antioxidant ability of LPS-challenged piglets, which was indicated by the elevated concentrations of catalase and superoxide dismutase, and increased total antioxidant capacity, as well as the decreased concentrations of diamine oxidase and malondialdehyde in plasma. Meanwhile, RBO improved the immune function of LPS-challenged weaned piglets, which was indicated by elevated immunoglobulin A (IgA), IgM, β-defensin-1, and lysozyme levels in the plasma. In addition, RBO supplementation improved the LPS challenge-induced dysbiosis of gut microbiota. Particularly, the indices of antioxidant capacity, intestinal damage, and immunity were significantly associated with the RBO-regulated gut microbiota. These findings suggested that 0.02% RBO is a suitable dose to protect against LPS-induced intestinal damage, oxidative stress, and jejunal microbiota dysbiosis in early weaned piglets.

Key words: Rice bran oil; Gut microbiota; Weaned piglets; Oxidative stress; Lipopolysaccharide

1 Introduction

The strategy of early weaning of piglets aims to improve the efficiency of modern swine production systems but usually results in weaning stress (Campbell et al., 2013). Early weaning stress usually induces severe intestinal bacterial infection, intestinal dysfunction, diarrhea, and ultimately high mortality among piglets (Gresse et al., 2017). High dietary zinc oxide and antibiotics have been widely used to improve pathologies in piglets induced by early weaning (Bhandari et al., 2008; Starke et al., 2014). However, considering their side effects on the gut microbiota and the concerns of public health and environment, European nations and China have implemented bans on the use

✉ Libao MA, malibao@mail.hzau.edu.cn
Xianghua YAN, xhyan@mail.hzau.edu.cn
Libao MA, https://orcid.org/0000-0001-6806-6899
Xianghua YAN, https://orcid.org/0000-0003-2238-6218

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of antibiotics for non-therapeutic purposes and have also limited the dose of dietary zinc oxide (Allen et al., 2013; Wang et al., 2017).

Rice bran oil (RBO), a byproduct of the rice milling industry, contains tocotrienols, tocopherols, and many other phytosterols (Kushwaha, 2018). RBO has been reported to exhibit antioxidant and anti-inflammatory properties in vivo and in vitro (Kushwaha, 2018; Punia et al., 2021). Supplementation with RBO could improve oxidative stress in diabetic rats (Posuwan et al., 2013). For early weaned piglets, weaning stress induces an increase in intestinal permeability and inflammation, facilitating reactive oxygen species (ROS) production and release into the intestinal lumen (Gresse et al., 2017; Zeng et al., 2017). These high ROS levels favor the bloom of facultative anaerobes but lessen obligate anaerobes, and consequently induce gut microbiota dysbiosis in early weaned piglets (Gresse et al., 2017; Zeng et al., 2017). Recent evidence suggests that gut microbiota dysbiosis is a crucial factor that accounts for these intestinal disorders in these animals (Dou et al., 2017; Gresse et al., 2017; Ren et al., 2022). We therefore assumed that RBO may serve as a cost-effective dietary supplementation to ameliorate weaning stress by improving gut microbiota in swine weanlings.

To determine the appropriate dose of RBO supplementation, we evaluated the diarrhea scores and growth performance indices of piglets fed with three concentrations of dietary RBO supplementation, namely 0.01%, 0.02%, or 0.03% (mass fraction). Subsequently, we investigated the protective effects of dietary RBO supplementation against lipopolysaccharide (LPS) challenge in early weaned piglets.

2 Materials and methods

2.1 Animals and experimental treatments

We randomly allocated 168 early weaned piglets (Landrace×Yorkshire, aged (21±1) d, body weight of (7.60±0.04) kg, half males and half females) into four groups with seven replicates (42 piglets in each group, half males and half females), which received basal diet (Ctrl), or basal diet supplemented with 0.01% RBO (RBO1), 0.02% RBO (RBO2), or 0.03% RBO (RBO3) for 21 d. Then, seven piglets from the Ctrl and RBO groups were treated with an intraperitoneal injection of 200 μL LPS solution per piglet (100 μg/kg body weight (BW), *Escherichia coli* 0111:B4, Sigma, USA), as LPS group and RBO+LPS group, respectively. Meanwhile, seven piglets from the Ctrl were treated with the saline vehicle (Ctrl group). Four hours later, all treated piglets were sacrificed after the injection of sodium pentobarbital (50 mg/kg BW) for sampling (Xu et al., 2021b). The composition of the basal experimental diet (Table S1) was in accordance with the recommendations of the National Research Council (NRC, 2012). The ultra-performance liquid chromatography (UPLC) chromatogram of RBO (10.08% tocopherols; and 7.56% tocotrienols; Yichun Dahaigui Life Science Co., Ltd., Yichun, China) was presented in Fig. S1.

2.2 Piglet growth performance and fecal scores

The growth performance indicators of piglets, including average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR), were also determined. The fecal scores of piglets during trial were established using the following scale: 1 for normal, solid feces; 2 for soft, looser than normal feces, slight diarrhea; 3 for moderate diarrheic feces; 4 for liquid, severe diarrheic feces for all pigs daily (Yi et al., 2005).

2.3 Plasma and intestinal sample collection

Blood samples were collected from the anterior cava using an anticoagulant tube, and then centrifuged at 4 ℃, 3000*g* for 10 min to obtain plasma samples. We sampled the jejunal stools and then took three 2-cm segments of jejunal tissues (one was fixed in 4% (mass fraction) paraformaldehyde solution and the others were frozen in dry ice after slight wash with ice-cold phosphate-buffered saline (PBS)). All frozen samples were then stored at −80 ℃ until further use.

2.4 Histomorphology examination of the jejunum tissue

After fixation in 4% paraformaldehyde for 48 h, segments of jejunal tissues were embedded in paraffin and then sectioned at 5 μm for hematoxylin and eosin (H&E) staining. The villus height, crypt depth, and villus height/crypt depth ratio were determined using an Olympus BX51 microscope with Integrated Digital Imaging Analysis System (Olympus Co., Tokyo, Japan).

2.5 Apoptosis assessment of jejunal epithelium

The apoptosis of jejunal epithelium was evaluated using the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay (Gavrieli et al., 1992). The average optical density (AOD) of TUNEL (green) was identified using ImageJ to assess the apoptosis level of the jejunal epithelium (Schneider et al., 2012).

2.6 Determination of plasma antioxidant indices

The antioxidant indices of plasma were determined using commercial kits (Solarbio Science & Technology, Beijing, China) including total antioxidant capacity (T-AOC, BC1315), malondialdehyde (MDA, BC0025), catalase (CAT, BC0205), superoxide dismutase (SOD, BC0175), diamine oxidase (DAO, BC1285), and nitric oxide (NO, BC1475) according to the corresponding manufacturer's instructions.

2.7 Determination of plasma immune indices

The plasma levels of immunoglobulin A (IgA), IgM, IgG, β-defensin-1, and lysozyme were determined using an enzyme-linked immunosorbent assay (ELISA) kit (Jiangsu Meimian Industrial Co., Ltd., China) according to the manufacturer's instructions.

2.8 Tight junction protein determination of the jejunum tissue

The total protein of jejunal tissue was extracted using radio immunoprecipitation assay (RIPA) buffer containing protease inhibitor buffer (1∶100, volume ratio, the same below), and the protein concentration was quantified using a bicinchoninic acid (BCA) kit (Thermo Fisher Scientific, USA). Rabbit polyclonal antibodies Occludin (91131S, 1:1000 dilution, Cell Signaling Technology Beverly, MA, USA) and Claudin-1 (13050-1-AP, 1∶1000 dilution, Proteintech, Wuhan, China) were used as primary antibodies. The western blot results were analyzed using ImageJ software.

2.9 Gut microbiota profiling

The total microbial genomic DNA in jejunal stool was extracted using a commercial kit (TGuide S96 Magnetic Soil/Stool, TIANGEN, Beijing, China). Qualified genomic DNA was used as a template for polymerase chain reaction (PCR) amplification of the V4 hypervariable regions of 16S ribosomal RNA (rRNA) genes with universal primers 515F and 806R2. The purified amplicons were pooled in equimolar quantities and paired-end sequenced (2×250) on a NovaSeq 6000 platform (Illumina, San Diego, USA) at Biomarker Technologies Co., Ltd. (Beijing, China). The generated sequencing raw data were analyzed using Quantitative Insights into Microbial Ecology software package (Version 2021.6) (Bolyen et al., 2019; Xu et al., 2021a). Principal coordinate analysis (PCoA) and permutational multivariate analysis of variance (PERMANOVA, with 9999 Monte Carlo permutations) based on Bray–Curtis distances were performed using the package "vegan" in R software (Version 4.3.1) (Wang et al., 2019). The marker genera were identified by linear discriminant analysis (LDA) effect size (LEfSe) (Segata et al., 2011).

2.10 Analysis of gut microbial function and metabolites

The function of gut microbiota was determined using PICRUSt2 based on the genes of 16S rRNA data and reference genomes (Douglas et al., 2020). Then, gas chromatography was employed to determine certain gut microbial metabolites, namely short-chain fatty acids (SCFAs) of acetate, propionate, and butyrate in colonic stools (Yan et al., 2020).

2.11 Data processing and statistic analysis

The experimental data were analyzed by one-way analysis of variance (ANOVA) tests, followed by Fisher's least significant difference and the Duncan multiple comparison test with GraphPad 8.0 software. All results were presented as mean±standard error of the mean (SEM) and the significance was set as * $P<0.05$, ** $P<0.01$, and *** $P<0.001$. P values between 0.05 and 0.10 were considered as indicative of a trend.

3 Results

3.1 Effects of dietary RBO supplementation on the diarrhea and growth performance of early weaned piglets

In order to identify the appropriate supplementation level of RBO, the effects of dietary supplementation of 0.01%, 0.02%, and 0.03% RBO on the diarrhea and growth performance of early weaned piglets were determined. As shown in Figs. 1a and 1b, compared

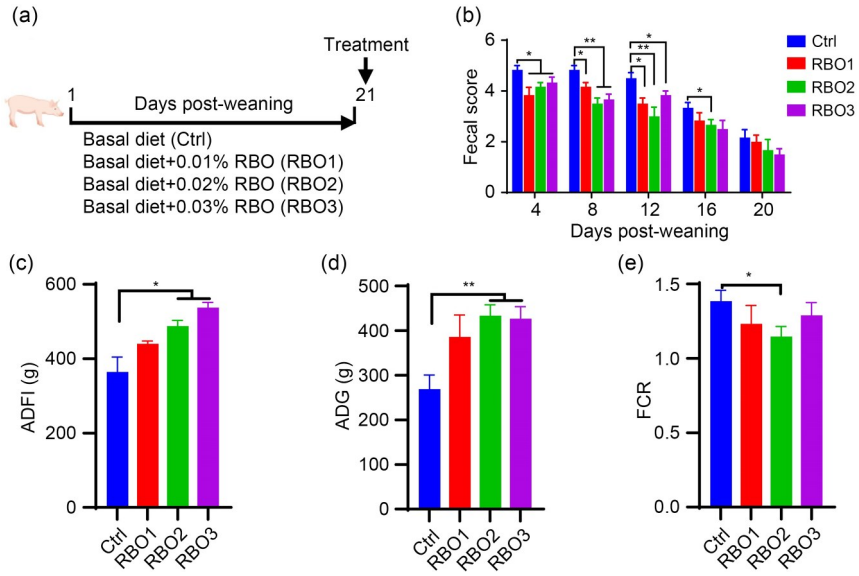

Fig. 1 Effects of dietary rice bran oil (RBO) supplementation on diarrhea and growth performance of early weaned piglets. (a) Experimental design; (b) Fecal scores every four days post-weaning; (c–e) The average daily feed intake (ADFI), average daily gain (ADG), and feed conversion ratio (FCR) from 1 to 21 d post-weaning. Data are expressed as mean± standard error of the mean (SEM), *n*=7. Significance was presented as * *P*<0.05 and ** *P*<0.01.

with the Ctrl group, dietary RBO supplementation at different levels was able to decrease the fecal scores at 4, 8, and 12 d post-weaning. At 16 d post-weaning, only RBO2 decreased the fecal scores compared with the Ctrl group. For growth performance, compared with the Ctrl, RBO2 and RBO3 both increased ADG and ADFI, but only RBO2 improved FCR (Figs. 2c–2e). According to the above indices, 0.02% was the optimal supplementation level of RBO to improve diarrhea and growth performance in early weaned piglets.

3.2 Protective effect of dietary RBO supplementation against LPS-induced jejunal damage in early weaned piglets

In order to determine the effect of dietary RBO supplementation on improving early weaning stress, an LPS challenge was conducted to simulate the enteral infections of post-weaning piglets (Fig. 2a). As presented in Figs. 2b and 2c, LPS crushed jejunal mucosa and reduced the villus height/crypt depth ratio compared to the Ctrl group. LPS also increased the apoptosis of jejunal epithelium (Figs. 2d and 2e) and decreased the Claudin-1 level (Figs. 2f–2h). Compared with the LPS group, RBO improved the jejunal mucosa morphology and villus height/crypt depth ratio (Figs. 2a–2c), as well as the apoptosis of the jejunal epithelium (Figs. 2d and 2e), while it increased the Claudin-1 level (Figs. 2f–2h).

3.3 Effects of dietary RBO supplementation on the antioxidant capacity and immunity function of LPS-challenged early weaned piglets

As shown in Table S2, the LPS challenge increased the plasma levels of DAO and MDA, whereas it decreased the activity of CAT, SOD, and T-AOC. Compared to the LPS group, RBO supplementation significantly increased CAT, SOD, and T-AOC, whereas it decreased the level of DAO and MDA.

As shown in Table S3, for specific immunity indices, LPS challenge decreased IgM, but had no significant effect on IgG or IgA when compared with the Ctrl group. Compared with the LPS, dietary RBO supplementation increased IgA and IgM. For the innate immunity indices, LPS challenge decreased plasma lysozyme, but had no significant effect on β-defensin-1 compared with the Ctrl group. Compared with the LPS, dietary RBO supplementation increased the levels of both β-defensin-1 and lysozyme.

3.4 Effect of dietary RBO supplementation on LPS-induced jejunal microbiota dysbiosis in early weaned piglets

The Venn diagram of operational taxonomic unit (OTU) distribution showed that the RBO+LPS group shared 124 OTUs and the LPS group shared 58 OTUs with the Ctrl (Fig. 3a). The PCoA of β-diversity indicated that the LPS group formed a markedly distinct

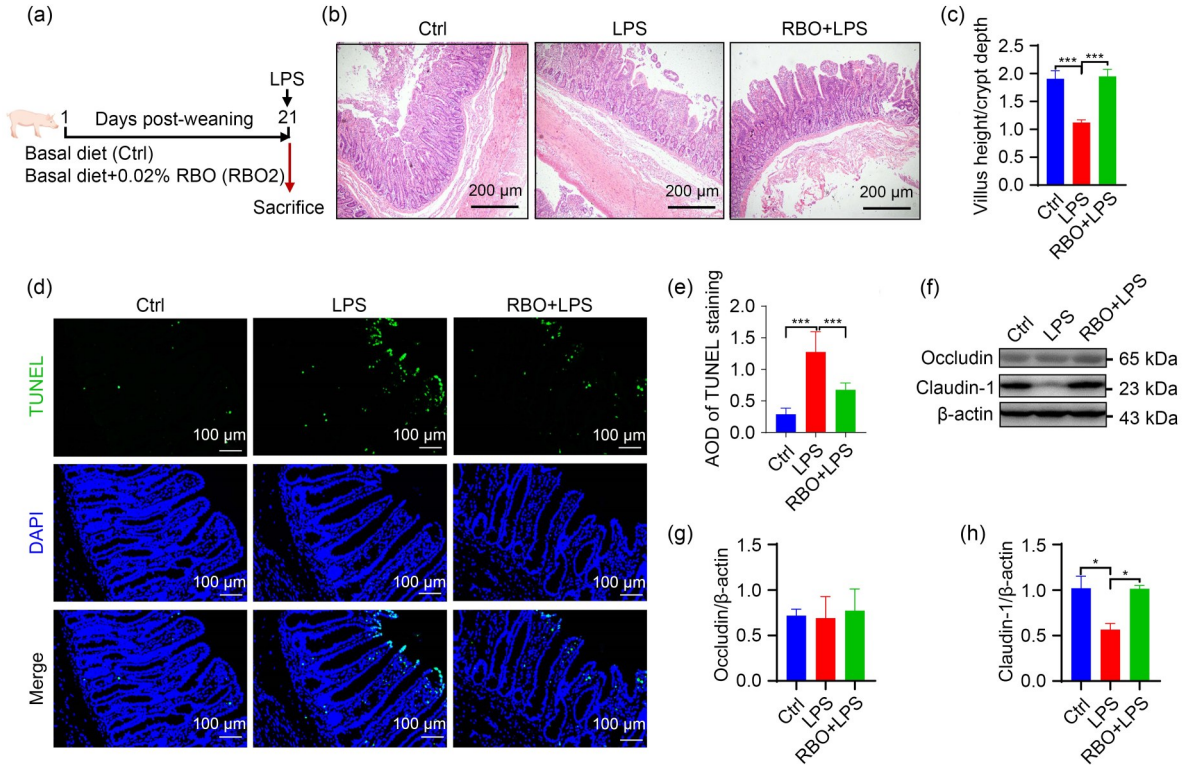

Fig. 2 Effect of dietary rice bran oil (RBO) supplementation on jejunal damage induced by lipopolysaccharide (LPS) challenge. (a) Experimental design; (b) Representative hematoxylin and eosin (H&E)-stained jejunal sections; (c) Villus height/crypt depth ratio; (d) Representative terminal deoxynucleotidyl transferase (TdT) dUTP nick end labeling (TUNEL)-stained jejunal sections; (e) The average optical density (AOD) of TUNEL staining; (f–h) Determination of the barrier proteins Occludin and Claudin-1 by western blot analysis. Data are expressed as mean±standard error of the mean (SEM), n=7. Significance was presented as * P<0.05 and *** P<0.001.

cluster from those of the Ctrl and RBO+LPS groups (Fig. 3b). For a single sample, alpha diversity analysis showed that LPS decreased the indicators of observed species, Chao1, and abundance-based coverage estimator (ACE), while it had no significant effect on Shannon, Simpson, or coverage compared with the Ctrl group (Fig. 3c). Compared with the LPS group, dietary RBO supplementation increased Chao1 (Fig. 3c).

3.5 Effects of dietary RBO supplementation on the relative abundance of jejunal microbiota in early weaned piglets

As shown in Figs. 4a and 4b, LPS shifted the relative abundance of bacteria at both the phylum level (Fig. 4a) and family level (Fig. 4b). The LEfSe results showed that f_Ruminococcaceae, f_Erysipelotrichaceae, c_Erysipelotrichi, and o_Erysipelotrichales were enriched in the Ctrl group, whereas g_*Staphylococcus*, f_Staphylococcaceae, f_Brevibacteriaceae, g_*Ochrobactrum*, o_Actinomycetales, and f_Bacillaceae were enriched in the LPS group (Fig. 4c). In terms of RBO+LPS vs. LPS treatment, the LEfSe results showed that f_Clostridiaceae and s_*Collinsella aerofaciens* were enriched in the RBO+LPS, while f_Staphylococcaceae, f_Brevibacteriaceae, g_*Ochrobactrum*, o_Actinomycetales, and o_Bacillales were enriched in the LPS group (Fig. 4d).

3.6 Effects of dietary RBO supplementation on the function and metabolites of jejunal microbiota in early weaned piglets

The analysis of gut microbial function showed that, compared with the Ctrl, LPS challenge increased the functions of amino acid metabolism, cellular community, digestive system, lipid metabolism, metabolism of terpenoids and polyketides, substance dependence, transport and catabolism, and xenobiotics biodegradation and metabolism, whereas it decreased the function of carbohydrate metabolism (Fig. 5a). Compared with the LPS group, dietary RBO supplementation increased the functions of carbohydrate metabolism, excretory system, and xenobiotics biodegradation

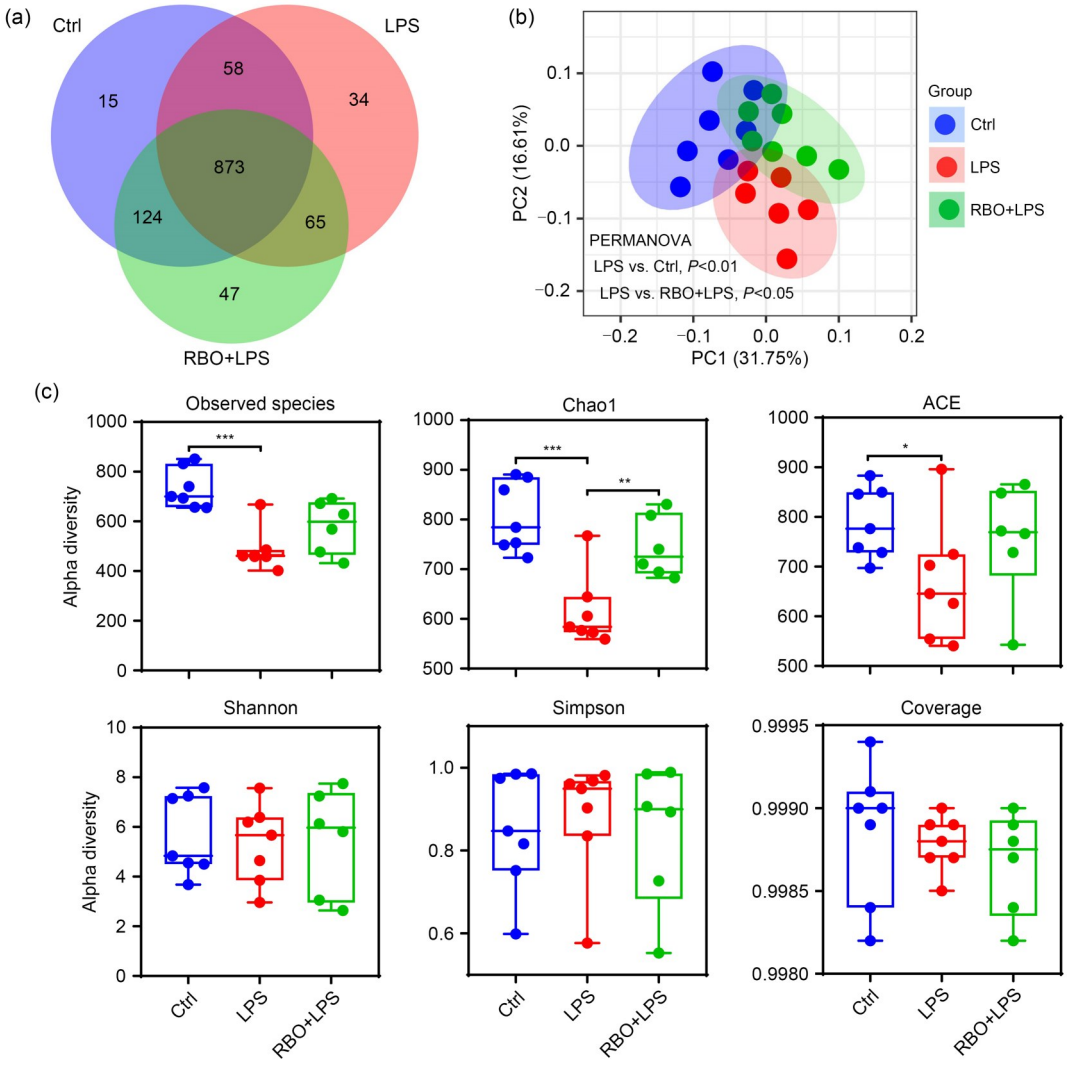

Fig. 3 Effect of dietary rice bran oil (RBO) supplementation on jejunal microbiota dysbiosis induced by lipopolysaccharide (LPS) challenge. (a) Venn diagram of operational taxonomic unit (OTU) distribution among groups; (b) The structure shifts (beta diversity) presented by principal coordinate analysis (PCoA) plot; (c) Alpha diversity indices of jejunal microbiota including observed species, Chao1, abundance-based coverage estimator (ACE), Shannon, Simpson, and coverage (n=7). Significance was presented as * P<0.05, ** P<0.01, and *** P<0.001.

and metabolism, whereas it decreased the functions of folding, sorting and degradation, biosynthesis of other secondary metabolites, energy metabolism, environmental adaptation, and glycan biosynthesis and metabolism (Fig. 5b). Correspondingly, LPS decreased the concentrations of fecal SCFAs including propionate and butyrate, compared with the Ctrl group (Table S4). Compared with the LPS group, dietary RBO supplementation increased the concentrations of fecal SCFAs including acetic acid, propionic acid, and butyric acid (Table S4).

3.7 Correlations among the jejunal microbiota and the indices of immunity, oxidative stress, and jejunum morphology

As shown in Fig. 6, *Collinsella aerofaciens* was positively correlated with Claudin-1 and villus height but negatively correlated with MDA. Clostridiaceae were positively correlated with villus height but negatively correlated with MDA. The alpha diversity index ACE was positively correlated with Claudin-1. The acetate was positively correlated with villus height

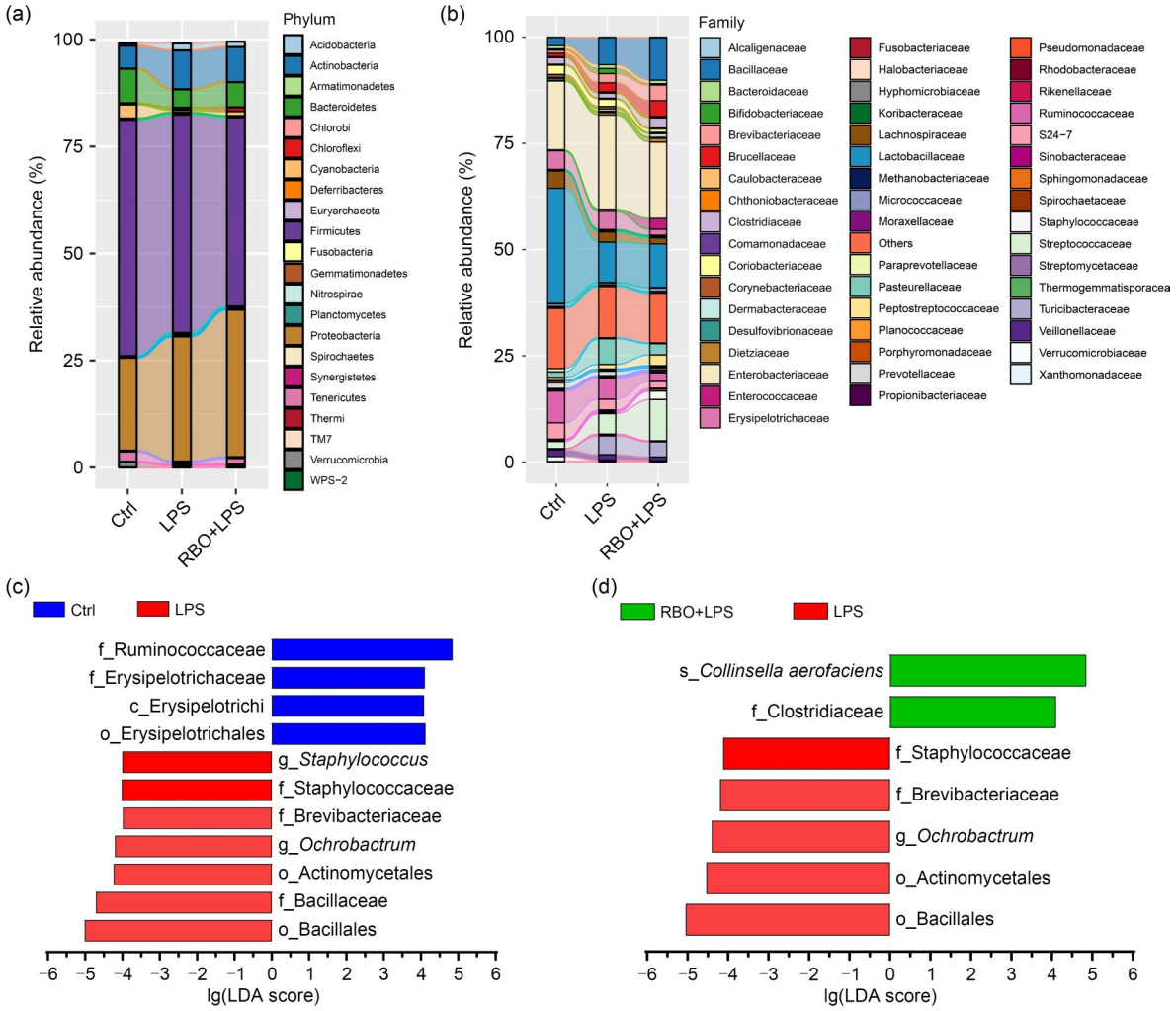

Fig. 4 Effects of dietary rice bran oil (RBO) supplementation on relative abundance of the jejunal microbiota in early weaned piglets. (a, b) The relative abundance of the gut microbiota at phylum level and family level; (c) The marker bacteria identified by linear discriminant analysis (LDA) effect size (LEfSe) between the Ctrl and lipopolysaccharide (LPS) groups; (d) The marker bacteria identified by LEfSe between the RBO+LPS and LPS groups.

and villus height/crypt depth but negatively correlated with DAO. The propionate was positively correlated with T-AOC, villus height, and villus height/crypt depth, but was negatively correlated with DAO. The butyrate was positively correlated with Occludin, Claudin-1, villus height, and villus height/crypt depth, but was negatively correlated with DAO and MDA.

4 Discussion

Early weaned piglets suffer from oxidative stress and enteral infection, which usually leads to gut microbial dysbiosis, serve diarrhea, and even death (Gresse et al., 2017). RBO, a polyphenol-enriched byproduct of the rice milling industry, has been reported to present antioxidant and anti-inflammatory properties both in vivo and in vitro (Kushwaha, 2018; Punia et al., 2021). In this work, we identified the appropriate dose and effect of dietary RBO supplementation on improving weaning stress. Firstly, we determined the effects of dietary 0.01%, 0.02%, and 0.03% RBO supplementation on the diarrhea and growth performance of early weaned piglets and found that 0.02% RBO was a cost-effective supplementation level. Dietary RBO supplementation for 21 d promoted early weaned piglets' growth performance by improving ADG. Compared with these results, supplementation with tocopherols as the main components of RBO could improve the growth performance of both weaned piglets and

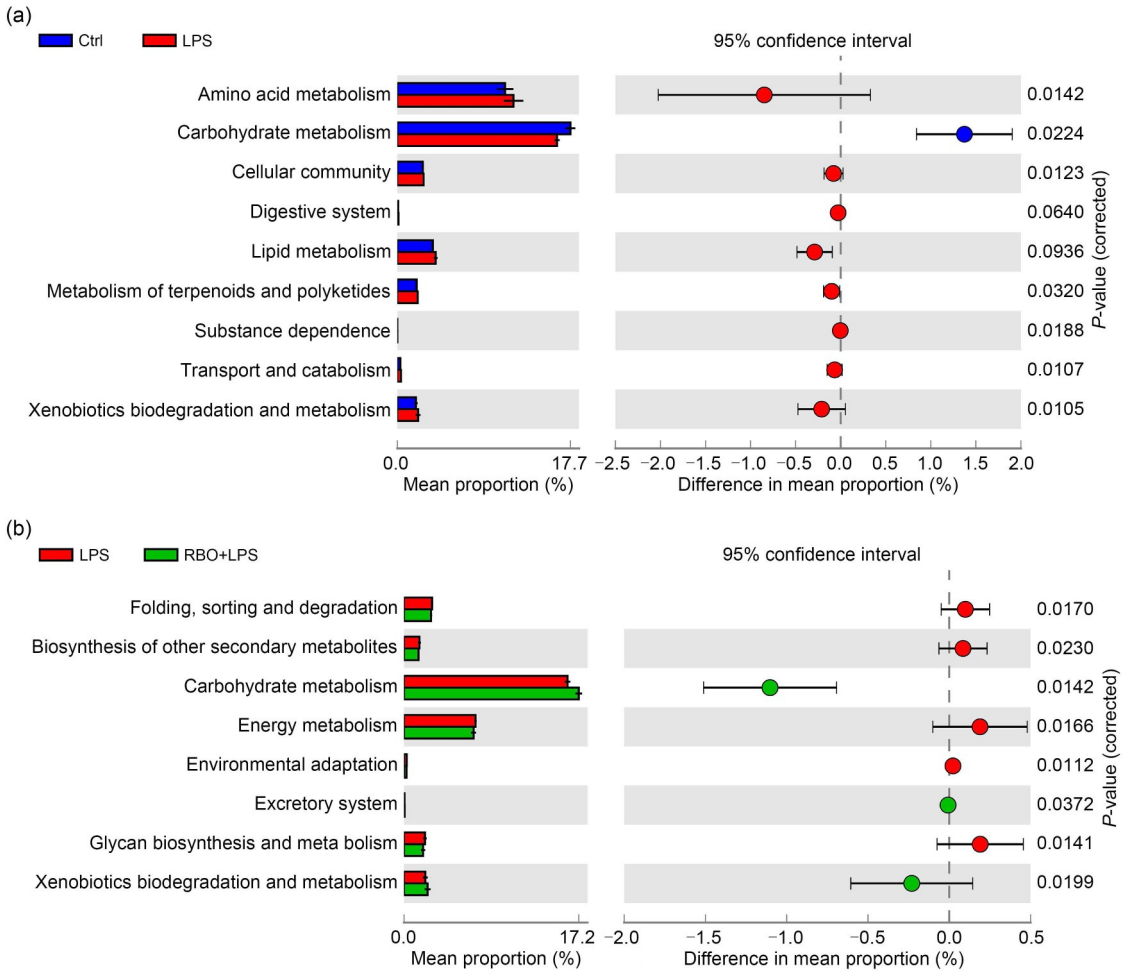

Fig. 5 Effect of dietary rice bran oil (RBO) supplementation on the function of the jejunal microbiota in early weaned piglets. (a) The abundance proportion of different Kyoto Encyclopedia of Genes and Genomes (KEGG) functions between the Ctrl group and the lipopolysaccharide (LPS) group; (b) The abundance proportion of different KEGG functions between the LPS group and the RBO+LPS group. The middle figure shows the difference proportion of functional abundance within the 95% confidence interval, and the value on the far right is the corrected *P* value.

finishing pigs (Boler et al., 2012; Lu et al., 2014). However, another study found that supplementation with 200 IU/kg tocopherols had no significant effects on improving piglets' growth performance (Silva-Guillen et al., 2020). We therefore conducted an LPS challenge to simulate enteral infections that post-weaning piglets generally suffer from (Xu et al., 2021b). Given that piglets' small intestine is the main target of enteral infections and pathogenic bacteria-derived LPS (Xu et al., 2018, 2020; Hu et al., 2022), we analyzed the morphology of jejunum, and the results demonstrated that dietary RBO enhanced piglets' capability to prevent LPS-induced intestinal damage. Specifically, dietary RBO supplementation improved the villus height and villus height/crypt depth ratio in LPS-challenged piglets. Similarly, Chen et al. (2019) reported that dietary tocopherols decreased the crypt depth and increased the villus width by inhibiting the proliferation of intestinal epithelial cells within the jejunum in weaned piglets. We also showed an extraordinary antioxidant activity of RBO regarding the antioxidant capacity indices in the plasma of early weaned piglets. RBO supplementation significantly increased CAT, SOD, and T-AOC, whereas it decreased the levels of DAO and MDA in the plasma of LPS-challenged piglets. These anti-inflammation and anti-oxidative stress functions of RBO had been widely evidenced in human beings and rodent animal studies (Punia et al., 2021). The LPS challenge could induce intestinal barrier function damage and apoptosis of the small intestinal

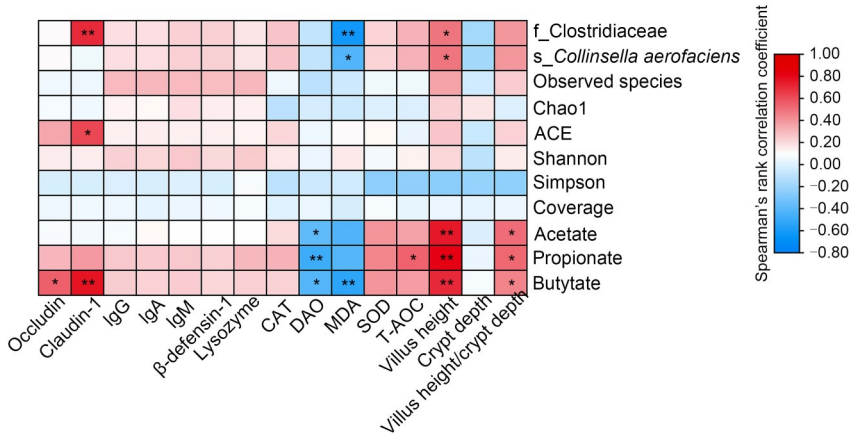

Fig. 6 Correlations among the gut microbiota and the indices of immunity, oxidative stress, and jejunum morphology. Heat maps of the Spearman's rank correlation coefficients and significant tests among the characteristics of jejunal microbiota and the indices of immunity, oxidative stress, and jejunum morphology. A blue color represents a negative correlation, while a red color represents a positive correlation. Significance was presented as * $P<0.05$ and ** $P<0.01$. IgG: immunoglobulin G; CAT: catalase; DAO: diamine oxidase; MDA: malondialdehyde; SOD: superoxide dismutase; T-AOC: total antioxidant capacity (Note: for interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

epithelial cells (Sun et al., 2021; Zhang et al., 2022). Here, we found that dietary RBO supplementation not only reduced the apoptosis of jejunal epithelium but also ameliorated the intestinal barrier function damage by elevating the Claudin-1 level in LPS-challenged piglets. In vitro evidence suggested that tocopherols were involved in the modulation of oxidative stress, proliferation, inflammation, and apoptosis by interacting with related genes (Mocchegiani et al., 2014). The protective effect of tocotrienol against oxidative insults was more potent than that of tocopherol (Mocchegiani et al., 2014). α-Tocopherol succinate is synthesized by the esterification of α-tocopherol, which could protect mice from radiation-induced gastrointestinal damage by inhibiting apoptosis, promoting the regeneration of crypt cells, and inhibiting the translocation of gut bacteria (Singh et al., 2011). Therefore, tocotrienols and tocopherols, the main biological active components of RBO, may play a vital role in ameliorating apoptosis and barrier function damage of jejunal epithelium in LPS-challenged piglets. LPS challenge is able to decrease the serum concentrations of IgG and IgM in piglets (Prates et al., 2021). Comparably, we found that LPS challenge decreased the plasma concentrations of IgM and lysozyme. Dramatically, dietary RBO supplementation could improve the immunological function against LPS challenge by increasing the plasma concentrations of IgA, IgM, β-defensin-1, and lysozyme in LPS-challenged piglets. Fragou et al. (2004) also found that the tocopherol supplementation could boost the immune system development of piglets in the first two weeks after weaning.

Gut microbiota dysbiosis can facilitate enteral bacterial infections and intestinal barrier function damage during the weaning transition of piglets (Dou et al., 2017; Gresse et al., 2017; Ren et al., 2022). The increased intestinal permeability and inflammation of early weaned piglets facilitate ROS accumulation in the intestinal lumen, which triggers gut microbiota dysbiosis (Gresse et al., 2017; Zeng et al., 2017). Liu et al. (2021) found that tocopherols modulated the gut microbiota during mitigating colitis and protecting the intestinal barrier function in mice. Bao et al. (2021) found phenolic profile of jujube fruit subjected to gut microbiota fermentation and its antioxidant potential against ethyl carbamate-induced oxidative damage in vivo. In this study, we found that LPS challenge resulted in jejunal microbiota dysbiosis by reducing the alpha diversity and shifting the microbial structure, which was improved by dietary RBO supplementation in early weaned piglets. Trillions of microbes that inhabit the mammalian intestine are extremely oxygen-sensitive and therefore occupy separate ecological niches along the intestine lumen (Albenberg et al., 2014; Frese et al., 2015). The accumulation of nitrate and ROS confers aerobic and facultative anaerobic microbes with growth superiority over obligate anaerobic bacteria in the inflamed intestine (Winter et al., 2013; Yardeni et al., 2019). In this study, the intestinal oxidative stress induced by LPS challenge decreased

the alpha diversity and shifted the structure of the jejunal microbiota, which were restored at least partly by dietary RBO supplementation. Taken together, dietary RBO supplementation may prevent the dysbiosis of jejunal microbiota by improving the intestinal inflammation and oxidative stress induced by LPS challenge.

Ruminococcaceae as extremely oxygen-sensitive bacteria are able to degrade indigestible polysaccharides and produce SCFAs (Shang et al., 2016). Erysipelotrichaceae, Erysipelotrichia, and Erysipelotrichales are positively correlated with fat/lipid metabolism (Greiner and Bäckhed, 2011; Kaakoush, 2015). Therefore, shifts in the relative abundance of these bacteria may be due to the oxidation of dietary fat/lipid by ROS in the inflamed jejunal lumen induced by LPS challenge (Gresse et al., 2017). Dietary RBO supplementation increased the functions of carbohydrate metabolism, excretory system, and xenobiotics biodegradation and metabolism, whereas it decreased the functions of folding, sorting and degradation, biosynthesis of other secondary metabolites, energy metabolism, environmental adaptation, and glycan biosynthesis and metabolism. Correspondingly, LPS challenge decreased the concentrations of fecal SCFAs including propionate and butyrate. On the contrary, compared with the LPS group, dietary RBO supplementation increased the concentrations of fecal SCFAs including acetate, propionate, and butyrate. There were significant associations between the characteristics of gut microbiota (taxon, diversity indices, and metabolites) shifted by dietary RBO supplementation and the indices of inflammation, immunity, oxidative stress, and jejunum morphology. Comparably, dietary antioxidants including resveratrol, quercetin, ferulic acid, vanillic acid, and pyrroloquinoline quinone could attenuate intestinal inflammation and oxidative damage in association with the modification of gut microbiota (Huang et al., 2021; Qiu et al., 2021; Xu et al., 2021a; Hu et al., 2022). Overall, we demonstrated that dietary 0.02% RBO supplementation improves antioxidant capacity, intestinal damage, and immunity in association with the gut microbiota in LPS-challenged piglets.

5 Conclusions

Taken together, our findings suggested that dietary supplementation with RBO, the byproduct of rice processing, could improve growth performance and reduce LPS-induced intestinal damage, oxidative stress, and inflammation in early weaned piglets. These beneficial effects of dietary RBO supplementation occurred in association with the simultaneous modification of gut microbiota.

Acknowledgments

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Author contributions

Juncheng HUANG, Wenxia QIN, and Baoyang XU conducted this study, participated in the animal experiments, analyzed the samples and the data, and wrote and revised the manuscript. Haihui SUN, Fanghua JING, Yunzheng XU, Jianan ZHAO, and Yuwen CHEN participated in the animal experiments. Baoyang XU, Libao MA, and Xianghua YAN designed this study and analyzed the data. All authors have read and approved the final manuscript, and therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Juncheng HUANG, Wenxia QIN, Baoyang XU, Haihui SUN, Fanghua JING, Yunzheng XU, Jianan ZHAO, Yuwen CHEN, Libao MA, and Xianghua YAN declare that they have no conflict of interest.

Our animal experiments were conducted in accordance with the Animal Experimental Ethical Inspection of Laboratory Animal Centre, Huazhong Agriculture University, Wuhan, China (No. HZAUSW20210012). All institutional and national guidelines for the care and use of laboratory animals were followed.

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Supplementary information

Tables S1–S4; Fig. S1