

# Dietary Moutan Cortex Radicis Alters Serum Antioxidant Capacity, Intestinal Immunity and Colonic Microbiota in Weaned Piglets

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## Research

**Keywords:** Moutan cortex radicis, Antioxidant capacity, Intestinal microbiota, NF- $\kappa$ B, Weaned piglets

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# Abstract

**Background:** *Moutan cortex radialis* (MCR), as a common traditional Chinese medicine, has been widely used as antipyretic, antiseptic and anti-inflammatory agent in China. However, few studies have evaluated the positive effects of MCR, as a new feed additives, on alleviating weaning stress and improving intestinal health and microbiom in pigs. This study aimed to investigate the effect of dietary MCR supplementation on serum antioxidant capacity, intestinal morphology, anti-inflammatory mechanism, and microbiota in weaned piglets.

**Results:** Supplemental 2000 mg/kg and 4000 mg/kg MCR increased ( $P < 0.05$ ) the final body weight, ADG and ADFI of weaned piglets, and 2000 mg/kg MCR diet significantly decreased ( $P < 0.05$ ) the F/G ratio and increased ( $P < 0.05$ ) serum catalase activity compared with CON group. Also, the villus height and crypt depth in the ileum and the concentrations of total SCFA, acetic acid, butyric acid and valeric acid in the colonic contents were higher ( $P < 0.05$ ) in the 2000 mg/kg and 4000 mg/kg MCR diets than CON group. Dietary MCR supplementation at 4000 mg/kg MCR significantly increased ( $P < 0.05$ ) total antioxidative capability and the crypt depth in the jejunum but decreased ( $P < 0.05$ ) the mRNA expression levels of Interferon  $\gamma$ , tumor necrosis factor- $\alpha$ , interleukin-1 $\beta$ , inhibiting kappa B kinase  $\beta$  (IKK $\beta$ ), inhibiting nuclear factor kappa-B (I $\kappa$ B $\alpha$ ) and nuclear factor kappa-B (NF- $\kappa$ B) in the jejunum and ileum. Supplemental 8000 mg/kg MCR had the higher total antioxidative capability and catalase activity in the serum but decreased ( $P < 0.05$ ) the villus height and crypt depth in the jejunum compared with the CON group. MCR addition reduced ( $P < 0.05$ ) serum malondialdehyde content, and tended to increase the mRNA expression of zonula occludens-1 in the ileum ( $P = 0.066$ ) compared to the CON group. Microbiota sequencing identified the microbial richness indices (Chao1, ACE, and observed species), the relative abundances of *Firmicutes* and *Lactobacillus* were increased ( $P < 0.05$ ), and the relative abundances of *Bacteroides*, *Parabacteroides*, *unidentified\_Lachnospiraceae* and *Enterococcus* were reduced ( $P < 0.05$ ) by MCR supplemented. Microbial metabolic phenotypes analysis also showed that the richness of aerobic bacteria and facultative anaerobic bacteria, oxidative stress tolerance, and biofilm forming were significantly increased ( $P < 0.05$ ), and the richness of anaerobic bacteria and pathogenic potential of gut microbiota were reduced ( $P < 0.05$ ) by MCR treatment.

**Conclusions:** In antibiotic-free diets, MCR supplementation improved growth performance and serum antioxidant capacity, alleviated intestinal inflammatory by inhibiting IKK $\beta$ /I $\kappa$ B $\alpha$ /NF- $\kappa$ B signaling pathway and affecting intestinal microbiota in weaned piglets.

## Background

Oxidative stress often causes mammalian tissues cells damage, especially the intestinal, which significantly affects health status and decrease performance [1, 2]. Various challenges, such as changes in feed nutrition and environment, pathogenic micro-organisms as well as vaccine and drugs use, induces oxidative stress for weaning piglets [3]. Including weaning stress, these combined stresses result in a decrease in food intake, impaired intestinal barrier function, and disordered intestinal flora, which

contribute to damaging immune function and increasing the susceptibility to disease [4, 5]. Antioxidative enzymes forming an antioxidative defense system protect the body against reactive oxygen species (ROS) overproduction. Besides, oxidative stress and inflammation are closely related. Cytokines are activated and secreted when the systemic inflammatory incidences. The activation of nuclear factor kappa-B (NF- $\kappa$ B), a transcription factor, can promote the pro-inflammatory cytokines expression [6]. Antibiotics have been used as growth promoters and immune enhancers at subtherapeutic levels in feed for many years. However, in recent years, the reduction or removal of dietary antibiotics has become a developing tendency in swine production [7]. Therefore, finding effective and safe feed additives to be instead of antibiotics is a strong demand for the swine industry. Traditional Chinese medicine is a natural substance, safe, and reliable with little toxicity [8]. Due to the extensive antibacterial and synergistic effects, traditional Chinese medicine has no drug resistance and overcomes the shortcomings of antibiotics.

*Moutan cortex radices* (MCR) from the tree peony (*Paeonia suffruticosa*), commonly known as Mu Dan Pi, is a traditional Chinese medicine commonly used for anti-inflammatory, analgesic, antispasmodic and anti-oxidation properties [9, 10]. Traditionally, MCR has the clearing heat, promoting blood circulation and removing blood stasis effects on alleviating sickness in humans. Previous researches have demonstrated that MCR has potent free radicals and superoxide anion radicals scavenging capacity, and inhibits ROS production for alleviating oxidative stress [11, 12]. MCR is rich in various chemical components, including paeonol, paeoniflorin, oxypaeoniflorin, galloylpaeoniflorin, gallic acid, and so on [13]. Paeonol is known to be the main active ingredient, which is reported to inhibit blood coagulation and platelet aggregation for enhancing blood circulation [14], and reduce the production of pro-inflammatory cytokines [15]. Moreover, MCR and its bioactive components have also been reported to alleviate obesity, diabetes, and inflammation [16, 17]. Previous studies showed that in neuro-inflammatory therapy, paeonol inhibited I $\kappa$ B $\alpha$  to suppress the translocation of NF- $\kappa$ B and decrease the release of pro-inflammatory products [18]. In addition, gut microbiota are the important contributor to animal health and growth such as nutritional conversion, immunity, and intestinal mucosal barrier function [19]. However, the positive effect of MCR on weaning stress, inflammatory response, and gut microbiota composition of weaned piglets have not been reported. In the present study, the effects of dietary MCR on growth performance, serum antioxidant indexes, intestinal morphology and anti-inflammatory response, and gut microbiota composition in weaned piglets were explored for the first time.

## Materials And Methods

The animal protocols and care standards of this experiment were accepted and approved by the Committee of Animal Care and Use of the Institute of Subtropical Agriculture, Chinese Academy of Science (Changsha, CAS20190409).

## Animals, experimental design and sample collection

Thirty-two Duroc × Large White × Landrace piglets (castrated male), weaned at an age of 21 d, were allocated randomly into four dietary treatments based on an average initial body weight of  $6.37 \pm 0.10$  kg. Each treatment had eight replicates with one piglet each, and each replicate were assigned into an individual pen. Before starting the study, all piglets were adapted for 3-days and fed a basal diet (corn-soybean meal). Four groups included a basal diet (control, CON), the basal diet + 2000 mg/kg *Moutan cortex radialis* (LMC), the basal diet + 4000 mg/kg *Moutan cortex radialis* (MMC) and the basal diet + 8000 mg/kg *Moutan cortex radialis* (HMC). This experiment lasted for 21 d, and all piglets had unlimited access to feed and water. The formulation of basal diet was to meet the NRC (2012) requirements [20] for growing pigs (Table 1) without antibiotics. *Moutan cortex radialis* was provided by the Institute of Botany, Chinese Academy of Sciences (Beijing, China).

Table 1  
Composition and calculated nutrient levels of the basal diet (air-dry basis)

Items	Content
Ingredients (g/kg)	
Corn (4.52% crude protein)	570.0
Soybean meal (43% crude protein)	220.0
Rice bran meal	50.0
Puffing maize powder	50.0
broken	50.0
Fish meal	20.0
Sucrose	10.0
Calcium lactate	3.00
Zinc oxide	2.00
Acidifier	4.00
Limestone	2.20
Monocalcium phosphate	10.0
Antioxidants	1.50
Lysine (98%)	4.00
Methionine	1.00
Threonine	1.00
Vitamin premix <sup>1</sup>	0.30
Mineral premix <sup>1</sup>	1.00
Total	1000
Nutrient content <sup>2</sup>	
Digestible energy (kcal/kg)	3274.5

<sup>1</sup> Provided per kilogram of diet: vitamin A, 80,000 IU; vitamin D<sub>3</sub>, 20,000 IU; vitamin E, 300 mg; vitamin K, 30 mg; vitamin B<sub>1</sub>, 30 mg; vitamin B<sub>2</sub>, 60 mg; vitamin B<sub>6</sub>, 30 mg; biotin, 0.2 mg; folic acid, 10 mg; niacin, 300 mg; pantothenic acid, 300 mg; Cu (CuSO<sub>4</sub>·5H<sub>2</sub>O), 12 mg; Fe (FeSO<sub>4</sub>·7H<sub>2</sub>O), 150 mg; Mn (MnSO<sub>4</sub>·H<sub>2</sub>O), 5 mg; Se (NaSeO<sub>3</sub>), 0.45 mg; Zn (ZnO), 150 mg.

<sup>2</sup> Based on the composition of ingredients provided by the NRC (2012).

Items	Content
Crude protein (%)	17.10
Calcium (%)	0.43
Total phosphorus (%)	0.63
Available phosphorus (%)	0.36
Lysine (%)	1.22
Methionine (%)	0.38
Methionine + cysteine (%)	0.66
<sup>1</sup> Provided per kilogram of diet: vitamin A, 80,000 IU; vitamin D <sub>3</sub> , 20,000 IU; vitamin E, 300 mg; vitamin K, 30 mg; vitamin B <sub>1</sub> , 30 mg; vitamin B <sub>2</sub> , 60 mg; vitamin B <sub>6</sub> , 30 mg; biotin, 0.2 mg; folic acid, 10 mg; niacin, 300 mg; pantothenic acid, 300 mg; Cu (CuSO <sub>4</sub> ·5H <sub>2</sub> O), 12 mg; Fe (FeSO <sub>4</sub> ·7H <sub>2</sub> O), 150 mg; Mn (MnSO <sub>4</sub> ·H <sub>2</sub> O), 5 mg; Se (NaSeO <sub>3</sub> ), 0.45 mg; Zn (ZnO), 150 mg.	
<sup>2</sup> Based on the composition of ingredients provided by the NRC (2012).	

The initial and final body weights, and feed intake were weighted and recorded throughout the experimental stage. The average daily weight gain (ADG), average daily feed intake (ADFI) and F/G ratio were determined. On day 22, twenty-four piglets (8 piglets per treatment group) were stunned (250V, 0.5A, for 5 ~ 6 s) and killed. Blood samples were collected from precaval vein and kept into vacuum tubes at room temperature for 2 h. Serum was obtained from the supernatant of blood after centrifugation at 3500 × g for 15 minutes, and then stored at -20 °C for further analysis. Approximately 2 cm in length segments of duodenum and jejunum were stored in 4% phosphate-buffered paraformaldehyde (pH 7.6) for histological analysis. Other middle intestinal samples flushed with 0.9% ice-cold physiological saline were immediately frozen in liquid nitrogen and stored at -80°C for molecular analysis. Colonic contents were collected for short-chain fatty acids (SCFAs) measurement; one sample of colonic content was allocated for microbiota composition determination.

## Detection of Serum Oxidative stress indexes

The serum contents of total antioxidative capability (T-AOC), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) were evaluated by previous evaluation procedures [21], and by spectrophotometric kits (Nanjing Jiangcheng Biotechnology Institute, Nanjing, China).

## Assessment of Intestinal morphology

The method of Histological hematoxylin eosin (HE) staining, as previously described, was used to evaluate intestinal histomorphological changes [22]. In brief, the middle sections of jejunum and ileum were embedded in paraffin after removed from fixation fluid and dehydrated, and made into

approximately 5 µm thickness transverse sections, then stained with HE. Villus height (VH) and crypt depth (CD) were measured by a computer-assisted microscopy (Leica DMI3000B microscopy, Switzerland, Germany). Morphological indices were measured from ten microscopic fields at 100 × magnification. The ratio of villus height to crypt depth (VH/CD) was calculated and analyzed.

## **Analysis of mRNA expression**

The extraction process of total RNA from intestinal tissues was followed by the description of Xiong et al. (2015) [23]. Briefly, samples were homogenized in the Trizol Reagent (Invitrogen, Carlsbad, CA, USA) for total RNA extraction, then further purified with the RNeasy kit (Eppendorf AG, Hamburg, Germany). In the 10 ul reaction systems, 1.0 µg of total RNA was incubated with DNase I for synthesising first-strand cDNA. Then reverse-transcription using Oligo (dT) primers (Takara, Otsu, Japan) was further to synthesise the double-strand cDNA. Real-time PCR was performed with SYBR Green Master Mix reagent (Takara, Otsu, Japan) and objective gene primer pairs using the LightCycler® 480 Real-Time PCR System (Roche, Switzerland, Germany). Primer 6.0 software was used to design primers of the β-actin housekeeping gene and target genes (Table 2). The fold changes in target genes was determined using the  $2^{-\Delta\Delta C_t}$  method.

Table 2  
Primers used for quantitative real-time polymerase chain reaction

Gene	Accession No.	Primer, 5'–3'	Size (bp)	T <sub>A</sub> (°C)
β-actin	XM_021086047.1	F: CTGCGGCATCCACGAAACT R: AGGGCCGTGATCTCCTTCTG	147	61
IFN-γ	NM_213948.1	F: GCCATTCAAAGGAGCATGGA R: TTCACTGATGGCTTTGCGCT	144	58
TNF-α	NM_214022.1	F: CCCCTGTGAGGGCAGGA R: CAGGCCACACATCCCTGAAT	185	60
IL-1β	NM_214055.1	F: CCTGAGATTGATGCCGTCCA R: TCTTCAAGCCGTGTAGCCAT	267	60
IL-6	NM_214399.1	F: CCTGAGATTGATGCCGTCCA R: TCTTCAAGCCGTGTAGCCAT	267	59
Claudin-1	NM_001244539.1	F: AAGGACAAAACCGTGTGGGA R: CTCTCCCCACATTCGAGATGATT	247	60
Occludin	NM_001163647.2	F: ACGAGCTGGAGGAAGACTGGATC R: CCCTTAACCTTGCTTCAGTCTATTG	238	60
ZO-1	XM_021098896.1	F: CCTGCTTCTCCAAAACTCTT R: TTCTATGGAGCTCAACACCC	252	60
IKKβ	NM_001099935.1	F: GTGACATCGCCTCTGCACTT R: GCAGGACGATGTTTTCTGGC	81	59
IκBα	XM_001924394.6	F: CACCCGAGTTAGAAGGGCTC R: GGTATCTGCTGAGGTGTGCTG	155	59
NF-κB	NM_001048232.1	F: AGCCATTGACGTGATCCAGG R: CGAAATCGTGGGGCACTTTG	248	60

T<sub>A</sub>, annealing temperature; IFN-γ, Interferon γ; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; IL-6, interleukin-6; ZO-1 = zonula occludens-1; IKKβ, inhibiting kappa B kinase β; IκBα, inhibiting nuclear factor kappa-B; NF-κB, nuclear factor kappa-B.

## 16S rRNA high-through sequencing for microbiota analysis

Each group selected six samples of colonic contents (n = 6 per group) for microbiota analysis. According to manufacturer's instruction, total microbiota DNA was extracted using PowerFecal™ DNA Isolation kit

(MO BIO Laboratories, Carlsbad, CA, USA). The Novogene Bioinformatics Technology Co., Ltd was invited to complete the 16S rRNA gene sequencing. Under the PCR reaction procedure: 94 °C for 3 min (1 cycle), 94 °C for 45 s/50 °C for 60 s/72 °C for 90 s (35 cycles), and a last step of 72 °C for 10 min, PCR products of pigs' samples were obtained using phusion high-fidelity PCR Mastermix (New England Biolabs (Beijing) LTD., China), then purified by using the QIAquick Gel Extraction Kit (QIAGEN, Dusseldorf, Germany). Sequencing libraries were generated using TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, USA), which subsequently enriched the manufacturer's recommendations and index codes. The evaluation of library quality was done on the Qubit® 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. Finally, an Illumina MiSeq 2 × 250 platform was used to perform the library sequencing for generating 250 bp paired-end reads by the following protocols described by Caporaso, et al (2012) [24]. FLASH, as a very fast and accurate analysis tool, merged paired-end reads from the original DNA fragments, and then identified each sample based on the unique barcodes [25]. To mine deeper data of microbial diversity of the differences between the samples, significance test were conducted with some statistical analysis methods, including T-test, MetaStat, linear discriminant analysis effect size (LEfSe), Anosim and multi-response permutation procedure (MRPP). Evaluation of the correlation between the gut microbiota and other dimensions was frequently performed with the Spearman's rank correlation test.

## **Microbiological function and phenotypic prediction**

Based on metagenomic 16S rRNA data, Tax4Fun, as a software package, was used for predicting functional profiles [26]. Tax4Fun could perform a mapping of 16 rRNA gene sequences reads to SILVA labeled OUT abundances. Normalized Taxonomic abundances are used to linearly combine the precomputed functional profiles of the KEGG organisms for predicting the microbial functional profile. Bugbase is a tool for measuring high-level phenotypes in the colonic microbiota using 16S RNA datasets and mapping file [27]. Besides, the Spearman correlation analysis between colonic microbiota and metabolites was performed in R software (v3.2.1).

## **Short-chain fatty acids (SCFAs) composition of colonic contents**

The composition of SCFAs in the colonic contents was determined according to the method described by Kong et al. (2016) [28]. About 1.0 g of the fresh colonic contents were mixed thoroughly with 5 mL distilled water in a centrifuge tube, incubated and shaken 30 min, and centrifuged at 10000 × g, 10 min at 4 °C. After transferring the supernatant into a new centrifuge tube, the precipitate was repeatedly extracted twice with 2 mL distilled water. Mix all supernatants (0.9 mL) with 25% metaphosphoric acid solution (0.1 mL) for 3 ~ 4 h at room temperature, then centrifuge at 10000 × g for 10 min at 4 °C. After filtered through a 0.45-µm polysulfone filter, the supernatant portion was subjected for analyses on Agilent 6890 gas chromatography (Agilent Technologies, Inc, Palo Alto, CA, USA). The standard solutions of acetic, propionic, butyric, isobutyric, valeric and isopentanoic acids were prepared at concentrations of 5, 10, 15, 20 and 25 mmol/L.

# Statistical analysis

All statistical analysis were performed using IBM SPSS 22.0 software (SPSS Inc, Chicago, IL, USA) except for microbiome analysis. One-way ANOVA and Tukey-Kramer multiple comparison tests were used to compare the differences among experimental treatments. After nonparametric tests, the 16 rRNA sequencing data were analyzed by a Kruskal–Wallis analysis to determine significant differences. The differences were declared significant at  $P < 0.05$  and a trend at  $0.05 < P \leq 0.10$  in all analyses. Results are expressed as means  $\pm$  standard errors (SEM) unless otherwise noted.

## Results

### Growth performance

As shown in Table 3, the initial body weight of pigs had no significant differences among the treatments ( $P > 0.05$ ). Compared to the CON and HMC groups, the final body weight, ADG, and ADFI were significantly increased in LMC and MMC groups ( $P < 0.05$ ). And then, LMC significantly reduced the F/G ratio compared with the CON group ( $P < 0.05$ ).

Table 3  
Effects of dietary *Moutan cortex radidis* on the growth performance in growing pigs.

Item	Diets <sup>1</sup>				P-value
	CON	LMC	MMC	HMC	
Initial body weight, kg	6.10 $\pm$ 0.21	6.07 $\pm$ 0.18	6.10 $\pm$ 0.18	6.11 $\pm$ 0.17	0.999
Final body weight, kg	7.93 $\pm$ 0.37 <sup>a</sup>	11.84 $\pm$ 0.68 <sup>b</sup>	12.51 $\pm$ 0.6 <sup>b</sup>	8.03 $\pm$ 0.65 <sup>a</sup>	< 0.01
ADG, kg/d	0.08 $\pm$ 0.01 <sup>a</sup>	0.24 $\pm$ 0.02 <sup>b</sup>	0.28 $\pm$ 0.04 <sup>b</sup>	0.11 $\pm$ 0.02 <sup>a</sup>	< 0.01
ADFI, kg/d	0.29 $\pm$ 0.04 <sup>a</sup>	0.41 $\pm$ 0.03 <sup>b</sup>	0.42 $\pm$ 0.04 <sup>b</sup>	0.28 $\pm$ 0.04 <sup>a</sup>	< 0.01
F/G ratio	3.29 $\pm$ 0.29 <sup>b</sup>	1.72 $\pm$ 0.07 <sup>a</sup>	2.44 $\pm$ 0.78 <sup>ab</sup>	2.72 $\pm$ 0.47 <sup>ab</sup>	0.039
<sup>1</sup> CON, control group, basal diet without antibiotics; LMC, the control diet + 2000 mg/kg <i>Moutan cortex radidis</i> ; MMC, the control diet + 4000 mg/kg <i>Moutan cortex radidis</i> ; HMC, the control diet + 8000 mg/kg <i>Moutan cortex radidis</i> .					
Data are expressed as means $\pm$ SEM (n = 8). Means within a row with different superscripts are significantly different ( $P < 0.05$ ).					

### Serum antioxidant indexes

As shown in Table 4, pigs fed the MMC and HMC diets had higher T-AOC activity ( $P < 0.05$ ) compared with the CON and LMC groups. The higher CAT activity ( $P < 0.05$ ) was observed in LMC and HMC pigs compared with CON group. Compared to the MMC group, HMC had a higher CAT activity ( $P < 0.05$ ) in the

serum. Pig fed the CON diet had the highest GSH-Px activity ( $P < 0.05$ ) and MDA concentration ( $P < 0.05$ ) in the serum compared with that of the pigs fed the LMC, MMC, and HMC diets. There was no significant effect of dietary MCR on SOD activity ( $P > 0.05$ ).

Table 4  
Effects of dietary *Moutan cortex radidis* on serum antioxidant indexes in growing pigs.

Item <sup>2</sup>	Diets <sup>1</sup>				P-value
	CON	LMC	MMC	HMC	
T-AOC, U/mL	1.96 ± 0.20 <sup>a</sup>	1.85 ± 0.08 <sup>a</sup>	3.28 ± 0.25 <sup>b</sup>	3.72 ± 0.26 <sup>b</sup>	< 0.01
CAT, U/mL	23.06 ± 2.45 <sup>a</sup>	38.94 ± 3.89 <sup>bc</sup>	32.42 ± 2.92 <sup>ab</sup>	54.47 ± 5.06 <sup>c</sup>	< 0.01
SOD, U/mL	40.44 ± 1.88	48.94 ± 1.68	50.50 ± 4.87	52.22 ± 5.25	0.184
GSH-Px, U/mL	1238.78 ± 39.91 <sup>b</sup>	933.63 ± 52.70 <sup>a</sup>	946.55 ± 89.92 <sup>a</sup>	934.80 ± 54.35 <sup>a</sup>	< 0.01
MDA, nmol/mL	10.35 ± 2.22 <sup>b</sup>	5.11 ± 0.70 <sup>a</sup>	2.81 ± 0.55 <sup>a</sup>	2.53 ± 0.29 <sup>a</sup>	< 0.01
<sup>1</sup> CON, control group, basal diet without antibiotics; LMC, the control diet + 2000 mg/kg <i>Moutan cortex radidis</i> ; MMC, the control diet + 4000 mg/kg <i>Moutan cortex radidis</i> ; HMC, the control diet + 8000 mg/kg <i>Moutan cortex radidis</i> .					
<sup>2</sup> T-AOC, total antioxidant capacity; CAT, catalase; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; MDA, malondialdehyde.					
Data are expressed as means ± SEM (n = 8). Means within a row with different superscripts are significantly different ( $P < 0.05$ ).					

## Jejunal and ileal morphology

The effects of MCR on jejunal and ileal morphology in the pigs are shown in Table 5 and Fig. 1. Compared with the CON and HMC groups, MMC significantly increased the CD ( $P < 0.05$ ) in the jejunum. The LMC diet increased the ratio of VH/CD ( $P < 0.05$ ) in the jejunum compared with the MMC diet. Pig fed with the HMC diet had the shortest VH ( $P < 0.05$ ) compared with that of the pigs fed other three diets. Compared to the CON and HMC diets, LMC and MMC diets markedly increased ( $P < 0.05$ ) the VH and CD in the ileum.

Table 5

Effects of dietary *Moutan cortex radidis* on the morphology of the jejunum and ileum of growing pigs.

Item	Diets <sup>1</sup>				P-value
	CON	LMC	MMC	HMC	
<b>Jejunum</b>					
Villus height, $\mu\text{m}$	370.13 $\pm$ 9.21 <sup>b</sup>	382.23 $\pm$ 9.92 <sup>b</sup>	372.27 $\pm$ 8.58 <sup>b</sup>	340.50 $\pm$ 7.89 <sup>a</sup>	< 0.01
Crypt depth, $\mu\text{m}$	200.39 $\pm$ 7.01 <sup>b</sup>	189.56 $\pm$ 6.53 <sup>ab</sup>	225.77 $\pm$ 6.44 <sup>c</sup>	175.80 $\pm$ 5.47 <sup>a</sup>	< 0.01
Villus height/Crypt depth	2.00 $\pm$ 0.09 <sup>ab</sup>	2.26 $\pm$ 0.11 <sup>b</sup>	1.7 $\pm$ 0.08 <sup>a</sup>	2.06 $\pm$ 0.08 <sup>ab</sup>	< 0.01
<b>Ileum</b>					
Villus height, $\mu\text{m}$	312.28 $\pm$ 8.34 <sup>a</sup>	358.50 $\pm$ 5.29 <sup>b</sup>	343.82 $\pm$ 14.67 <sup>b</sup>	323.20 $\pm$ 7.46 <sup>a</sup>	< 0.01
Crypt depth, $\mu\text{m}$	174.49 $\pm$ 6.20 <sup>a</sup>	212.31 $\pm$ 4.63 <sup>b</sup>	206.47 $\pm$ 5.68 <sup>b</sup>	185.63 $\pm$ 5.19 <sup>a</sup>	< 0.01
Villus height/Crypt depth	1.77 $\pm$ 0.05	1.78 $\pm$ 0.04	1.82 $\pm$ 0.04	1.84 $\pm$ 0.05	0.746
<sup>1</sup> CON, control group, basal diet without antibiotics; LMC, the control diet + 2000 mg/kg <i>Moutan cortex radidis</i> ; MMC, the control diet + 4000 mg/kg <i>Moutan cortex radidis</i> ; HMC, the control diet + 8000 mg/kg <i>Moutan cortex radidis</i> .					
Data are expressed as means $\pm$ SEM (n = 8). Means within a row with different superscripts are significantly different ( $P < 0.05$ ).					

## Expression of genes associated with proinflammatory factors, tight junction proteins and NF- $\kappa$ B signaling pathway

As shown in Fig. 2, HMC group significantly decreased the mRNA expression level of interferon  $\gamma$  (IFN- $\gamma$ ,  $P < 0.05$ ) in the jejunum and ileum compared with the CON group. Compared to the CON diet, the MMC diet decreased the mRNA expression level of interleukin-6 (IL-6,  $P < 0.05$ ) in the jejunum and mRNA expression levels of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and IL-1 $\beta$  in the ileum ( $P < 0.05$ ). The LMC diet significantly inhibited the mRNA expression level of TNF- $\alpha$  ( $P < 0.05$ ) in the ileum compared with the CON diet, but had the highest mRNA expression level of TNF- $\alpha$  ( $P < 0.05$ ) in the jejunum of pigs among all treatments. Dietary supplementation of MCR had a significant trend of an inhibited the IL-6 mRNA expression ( $P = 0.054$ ). HMC significantly enhanced ( $P < 0.05$ ) the ZO-1 mRNA expression in the jejunum compared with the LMC diet, and increased ( $P < 0.05$ ) the occludin mRNA expression in the ileum compared to the MMC

diet. And there was a significant trend ( $P = 0.066$ ) toward higher the mRNA expression level of ZO-1 in the ileum pigs fed MCR diets.

The influences of dietary MCR on the IKK $\beta$ /I $\kappa$ B $\alpha$ /NF- $\kappa$ B signaling pathway are shown in Fig. 2C. Compared to the CON and HMC diets, LMC and MMC diets significantly inhibited ( $P < 0.05$ ) the expressions of inhibiting kappa B kinase  $\beta$  (IKK $\beta$ ) and inhibiting nuclear factor kappa-B (I $\kappa$ B $\alpha$ ) in the jejunum. MMC group had a lower ( $P < 0.05$ ) expression level of NF- $\kappa$ B mRNA in the jejunum compared with the HMC group. LMC and MMC had a decreasing tendency for the mRNA expression levels of IKK $\beta$  ( $P = 0.078$ ) and NF- $\kappa$ B ( $P = 0.064$ ) in the ileum compared with CON and HMC groups. Compared to the CON group, MMC also significantly down-regulated the I $\kappa$ B $\alpha$  mRNA expression ( $P < 0.05$ ) in the ileum.

## Concentrations of SCFA in the colonic contents

Analysis of the concentrations of SCFA in the colonic contents revealed differences among all treatments (Fig. 3). The pigs fed the enhanced The concentrations of total SCFA, acetic acid, butyric acid, and valeric acid in the colonic contents were higher in the LMC and MMC groups than in the the CON group ( $P < 0.05$ ). Also, dietary supplementation of MCR showed a tendency to to increase the concentration of isobutyric acid ( $P = 0.062$ ) in the colonic contents of pigs. The concentrations of propionic acid and isopentanoic acid among the four treatments had no significant difference ( $P > 0.05$ ).

## Colonic microbiota diversity and composition

To better understand the differences in richness, the overlaps among treatments were illustrated using a Venn diagram (Fig. 4A). This analysis showed that CON&LMC, CON&MMC and CON&HMC contained 304, 319 and 361 common OTUs, respectively. As shown in Fig. 4B, the microbial richness indices (Chao1, ACE and observed species) were significantly increased ( $P < 0.05$ ) in the gut microbiota of piglets with *Moutan cortex radidis* supplementation, whereas no significant differences were found in the diversity indices (Shannon and Simpson) of gut microbiota. The principal coordinate analysis (PCoA, Fig. 4C) and non-metric multidimensional scaling (NMDS, Fig. 4D) analysis of  $\beta$ -diversity showed a strong difference in the microbiota from the control group to *Moutan cortex radidis*-treated groups. An unweighted Unifrac cluster tree based on the unweighted pair-group method with arithmetic mean (UPGMA) analysis showed the similarity and phylogeny of all observed samples at the phylum level (Fig. 4E), and *Firmicutes*, *Bacteroidetes* and *Proteobacteria* are the dominant bacteria in pigs' colonic microbiota. Further, MetaStat analysis of the microbial community was to explore the significant differences in microbial composition between the MCR-treated group and the control group (Fig. 4F). MCR supplementation significantly elevated the relative abundance of *Tenericutes*, and decreased the relative abundance of *Bacteroidetes* in the colonic microbiota.

As shown in Fig. 5, the phylum level analysis showed that dietary supplementation of MCR significantly increased the relative abundance of *Firmicutes* ( $P < 0.05$ ) and decreased the relative abundance of *Bacteroidetes* ( $P < 0.05$ ). In the genus level, MCR treatment significantly decreased the relative abundances of *Bacteroides*, *Parabacteroides*, *unidentified\_Lachnospiraceae*, and *Enterococcus* in the

colonic microbiota ( $P < 0.05$ ). Compared to the CON group, LMC and MMC groups increased ( $P < 0.05$ ) the relative abundance of *Lactobacillus*.

## Metabolic functions and phenotypes of colonic microbiota

Tax4Fun was performed to determine the effects on metabolic functions of gut microbiota by MCR treatment. Based on KEGG annotation results, the principal components analysis (PCA) showed that the microbial metabolic functions were significantly separated in the CON group and MCR-treated groups (Fig. 6A). As shown in Fig. 6B, KEGG pathways associated with microbial metabolism at level 3, including mismatch repair, pyruvate and purine metabolism, DNA repair and recombination protein, were up-regulated by dietary MCR-treated. Galactose metabolism, oxidative phosphorylation and amino acid-related enzymes were significantly down-regulated. Moreover, based on 16S OTU results to predict bacterial phenotype database, Bugbase can be analyzed the differences among groups simultaneously. Results showed that MCR diet significantly increased ( $P < 0.05$ ) the aerobic bacterial richness, and oxidative stress tolerance and biofilm forming of colonic microbiota compared to the CON group. The richness of Gram-positive bacteria showed a marked increasing trend ( $P = 0.052$ ), while Gram-negative bacteria had a significant decrease trend ( $P = 0.052$ ) by MCR treatments. The pathogenic potential of gut microbiota was reduced ( $P < 0.05$ ) by increasing dietary MCR level. LMC and HMC groups had a lower ( $P < 0.05$ ) richness of anaerobic bacteria and a higher ( $P < 0.05$ ) facultative anaerobic bacteria than the CON group.

Results of Spearman's correlation coefficients between major genera and growth, serum antioxidant parameters makers and colonic SCFA contents were calculated and presented with heatmap (Fig. 7). *Lactobacillus* and *Blautia* had significant positive relations with ADG, serum CAT activity, and the contents of total SCFAs, acetic acid, propionic acid, butyric acid and valeric acid ( $P < 0.05$ ), and was negatively related with the F/G ratio ( $P < 0.05$ ). *Bacteroides* showed significant positive relations with serum GSH-Px activity and MDA content, and was negatively correlated with CAT and the contents of total SCFAs, acetic acid, propionic acid, butyric acid and valeric acid ( $P < 0.05$ ). *Parabacteroides* showed significant positive correlations with serum GSH-Px activity and the F/G ratio ( $P < 0.05$ ), and was negatively correlated with CAT and total SCFAs, propionic acid, butyric acid and valeric acid contents ( $P < 0.05$ ). *Unidentified\_Lachnospiraceae* was positively correlated with ( $P < 0.05$ ) the F/G ratio and GSH-Px activity, and negatively correlated with ( $P < 0.05$ ) serum SOD activity.

## Discussion

In recent years, the misuse of feed antibiotics in the swine industry has seriously threatened human health and food safety, and China has banned the application of antibiotics in feeds in 2020. Therefore, exploring an alternative to antibiotics is necessary for the sustainable development of the livestock industry. Many previous studies have found the positive results of MCR in various animal models of disease [29–31]. In the present study, addition of MCR to the diet without antibiotic firstly showed the effect of promoting growth performance in weaned piglets. The improvement may be due to protecting

piglets from oxidative stress and intestinal inflammation response caused by weaning stress, which was evidenced by the enhanced antioxidant capacity, inhibition of NF- $\kappa$ B signaling pathway and regulation of intestinal flora structure and metabolites in piglets.

The depletion of intracellular free-radicals and antioxidants inhibited various antioxidant enzymes activities, which induced oxidative stress [32]. The antioxidant mechanism of polyphenols mainly through increasing antioxidant protective barrier and eliminating intracellular ROS to maintain oxidative balance [33, 34]. Previous studies demonstrated more than 50  $\mu$ g/mL of MCR enhanced the antioxidant defense system by improving the activities of GSH and SOD in glucose-induced oxidative damage [35]. In this study, the activities of T-AOC and CAT were improved, and the GSH-Px activity was decreased in weaned piglets supplemented with MCR. Overall, MCR can play an antioxidant role by increasing antioxidant activity. The mechanism of antioxidative stress and anti-inflammation closely connected to the NF- $\kappa$ B signaling pathway in the body [36, 37]. Dynamic changes of proinflammatory cytokines levels in the intestinal tract tissue act as crucial messengers to stimulate the intestinal inflammatory process. Therefore, during anti-inflammatory therapy, it is necessary to downregulate the production of these pro-inflammatory cytokines [38]. The phosphorylation and degradation of the NF- $\kappa$ B bound protein I $\kappa$ B, activated by the IKK signaling phosphorylation, are directly involved in the activating NF- $\kappa$ B [39]. As demonstrated in the present study MCR has effectively decreased the cytokine productions in jejunum and ileum via inhibiting IKK $\beta$ /I $\kappa$ B $\alpha$ /NF- $\kappa$ B signaling pathway. At the same time, evidences also found that MCR or paeonol could suppress the gene and protein expression of pro-inflammatory cytokines by blocking NF- $\kappa$ B pathway in the LPS-stimulated inflammatory response [30, 40]. Thus it could be suggested that MCR has potential in antioxidant and anti-inflammation therapy in weaned piglets.

Enhanced intestinal morphology and gut barrier are closely associated with nutrients absorption and intestinal integrity [41]. Intestinal morphology significantly changes, including villous atrophy and crypt hyperplasia, which will result in diarrhea and growth retardation in pigs [42]. An increasing villus height/crypt depth ratio is one of the most important indexes of intestinal morphology in evaluating the improvement of intestinal function and enhancement of absorption capacity [43]. A recent study found that dietary supplemented with MCR at 2000 mg/kg improved the ratio of villus height to crypt depth in the jejunum, and increased the villus height and crypt depth in the ileum of weaned piglets. 4000 mg/kg MCR increased the villus height and crypt depth in the ileum, whereas 8000 mg/kg MCR decreased the villus height and crypt depth in the jejunum and ileum compared with 2000 mg/kg and 4000 mg/kg MCR groups. Therefore, we speculated that a high dosage (8000 mg/kg) of MCR does not promote the improvement of intestinal villi and intestinal digestive ability. Tight junctions protein, as the mechanical barrier, constitutes intestinal barrier function and prevents pathogenic antigen invasion [44]. Occludin, claudin-1 and ZO-1 are the main cytoplasmic transmembrane and adaptor protein and jointly constitute the tight intercellular junctions. Improved expression of three crucial proteins can enhance the intestinal barrier function for decreasing permeability of the intestinal wall [45]. Several studies have found that traditional Chinese medicine can alter intestinal permeability dependent on tight junctions protein changes [46, 47]. Our results also demonstrated that ZO-1 and occludin mRNA expression in jejunum and

ileum were increased in piglets fed MCR (8000 mg/kg feed) diet. This suggests that a high dosage of MCR contributed to improving the intestinal barrier integrity in weaned piglets.

The gut microbiome is a complex microbial ecosystem, whose activities and reciprocal relationship has been essential to the host health and disease [48]. The investigation of the gut microbiome has been described as a biomarker for evaluating the effect of specific dietary components on the host. In the current research, MCR shapes intestinal microbiota in weaned piglets, including increases in the microbial richness, the abundances of the phyla *Firmicutes* and the genera *Lactobacillus*, and a decrease in the abundances of the phyla *Bacteroidetes*, and the genera *Bacteroides*, *Parabacteroides*, *unidentified\_Lachnospiraceae* and *Enterococcus*. Piglets fed MCR diets had a higher observed Chao1, ACE and species number for gut microbiota, which indicates that MCR supplementation contributes to improving microbial diversity. *Firmicutes* and *Bacteroidetes*, as two main communities, are associated with the energy metabolism homeostasis [49]. Many previous studies reported that increased *Firmicutes* and reduced *Bacteroidetes* are most common in the obesity phenotype, which led to effectively absorb the calories from food [50]. The abundance of *Lactobacillus* in the intestine is closely related to activating the production of secretory IgA for improving intestinal mucosal immunity, which acts an important role in maintaining intestinal barrier function [51]. *Bacteroides* and *Parabacteroides*, occurring in the early stages of life, have been reported to produce gamma amino butyric acid, associated with growth [52]. The abundance of *Enterococcus* correlated positively with metabolites associated with inducing oxidative stress [53]. Moreover, changed microbial composition has been linked to the production and composition of SCFA in the colon. In the present study, we found that colonic contents of SCFA, including acetic acid, propionic acid, butyric acid, and valeric acid, were increased significantly in piglets fed the MCR diet at 2000 and 4000 mg/kg. SCFA, as an important metabolite of gut microbiota, could favor the energy homeostasis, and relieve inflammations and metabolic syndrome in the colon [54]. Corrêa-Oliveira has demonstrated that the addition of SCFA increased villi height and crypt depth, enhanced the intestinal barrier, and had anti-inflammatory properties in mice [55]. In summary, MCR addition regulates piglets' intestinal microbiota and microbial metabolites for improving intestinal health. And it would be interesting to further investigate whether MCR has a marked influence on lipid metabolism through regulating intestinal microbiota in weaned piglets.

Based on microbial function prediction, results demonstrated that MCR addition increased the pyruvate metabolism, DNA repair and purine metabolism, and decreased oxidative phosphorylation and amino acid-related enzymes. MCR may inhibit the amino acid metabolism and promote the nucleotide metabolism and multi-drug resistance in gut microbial communities. Moreover, the changes of microbial metabolic phenotypes in weaned piglets treated with different doses of MCR, were first revealed. Dietary supplementation of MCR has a strong antimicrobial property against Gram-negative and anaerobic bacteria, but promotes the proliferation of Gram-positive and aerobic bacteria. MCR supplementation also increased biofilm forming and oxidative stress tolerance, while the promoting effect was negatively correlated with the added dose. Biofilm formation and oxidative stress tolerance of microbial communities were found to go together with drug resistance, inflammation, and pathogenesis [56]. Higher MCR levels significantly reduced the pathogenic potential of microbial communities. However, these

metabolic phenotypes changes need to further explore the mechanism. Further, association analysis of growth performance, serum antioxidants, colonic SCFA contents and microbiota first revealed that MCR supplementation has widely influenced the growth and health in piglets.

## Conclusions

In conclusion, dietary supplemented with *Moutan cortex radidis* was able to significantly alleviate weaning stress in piglets, as demonstrated by improving antioxidant capacity and regulating gut microbial communities. *Moutan cortex radidis* increased serum antioxidant capacity, improved intestinal barrier function and inhibited the NF- $\kappa$ B signaling pathway. Additionally, besides improving the richness indices, MCR significantly increased the microbial metabolic phenotypes and functions, and metabolites, which is benefit weaned piglets with better intestinal status and growth potential. The present study contributes to provide theoretical support in applying *Moutan cortex radidis* at 4000 mg/kg for antioxidation and regulating intestinal health in livestock production.

## Abbreviations

ADG

average daily weight gain; ADFI:average daily feed intake; CAT:catalase; CD:crypt depth; GSH-Px:glutathione peroxidase; HE:hematoxylin eosin; IFN- $\gamma$ :Interferon  $\gamma$ ; IL-1 $\beta$ :interleukin-1 $\beta$ ; IL-6:interleukin-6; IKK $\beta$ :inhibiting kappa B kinase $\beta$ :I $\kappa$ B $\alpha$ , inhibiting nuclear factor kappa-B; LEfSe:linear discriminant analysis effect size; MCR:*Moutan cortex radidis*; MDA:malondialdehyde; NF- $\kappa$ B:nuclear factor kappa-B; MRPP:multi-response permutation procedure; ROS:reactive oxygen species; SCFAs:short-chain fatty acids; SOD:superoxide dismutase; T-AOC:total antioxidative capability; TNF- $\alpha$ :tumor necrosis factor- $\alpha$ ; VH:villus height; ZO-1:zonula occludens-1.

## Declarations

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Not applicable.

### Author's contributions

The contributions of the authors were as follows: M. B. conducted the animal work, sample analysis and manuscript writing; H. L., J. D., Y. Y., and Q. S. designed the research and reviewed the manuscript; K. X, X. X. and R. H analyzed the data and helped to revise the manuscript. S. Liu and Q. S. provided experimental materials and analyzed study data. S. W. and J. Z. helped to conduct animal trial and sample analysis. All authors read and approved the final manuscript.

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### **Availability of data and materials**

All data generated or analyzed during this study are available from the corresponding author upon reasonable request.

### **Ethics approval**

The animal protocols and care standards of this experiment were accepted and approved by the Committee of Animal Care and Use of the Institute of Subtropical Agriculture, Chinese Academy of Science (Changsha, CAS20190409).

### **Consent for publication**

Not applicable.

### **Competing interests**

The authors declare that they have no competing interests.

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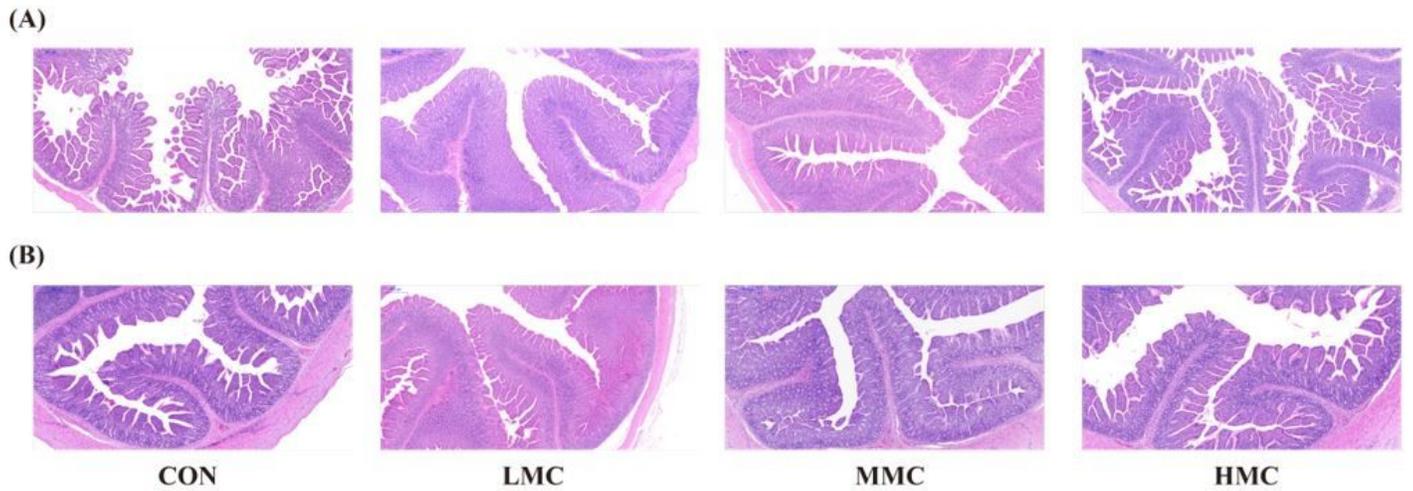
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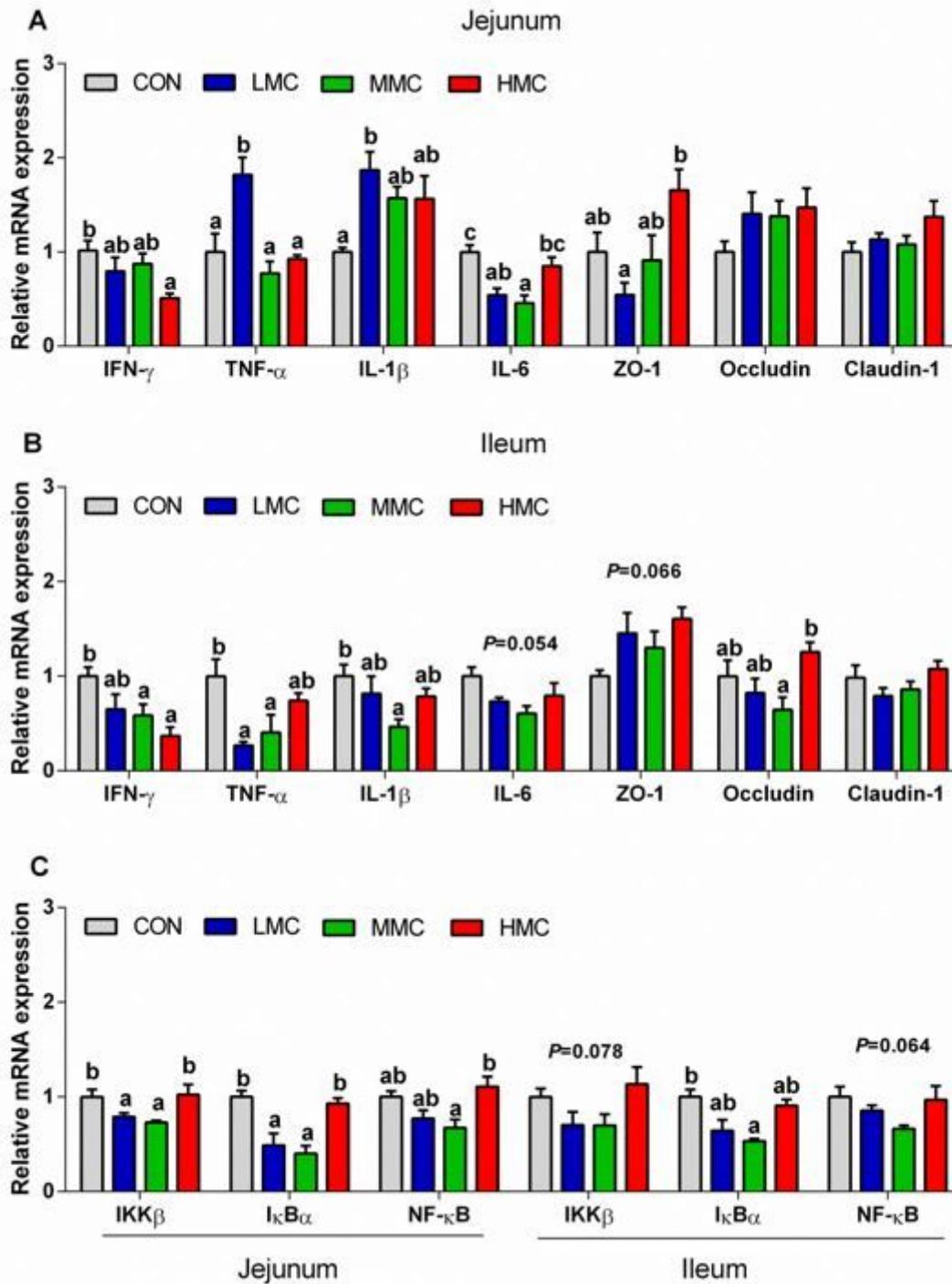
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## Figures



**Figure 1**

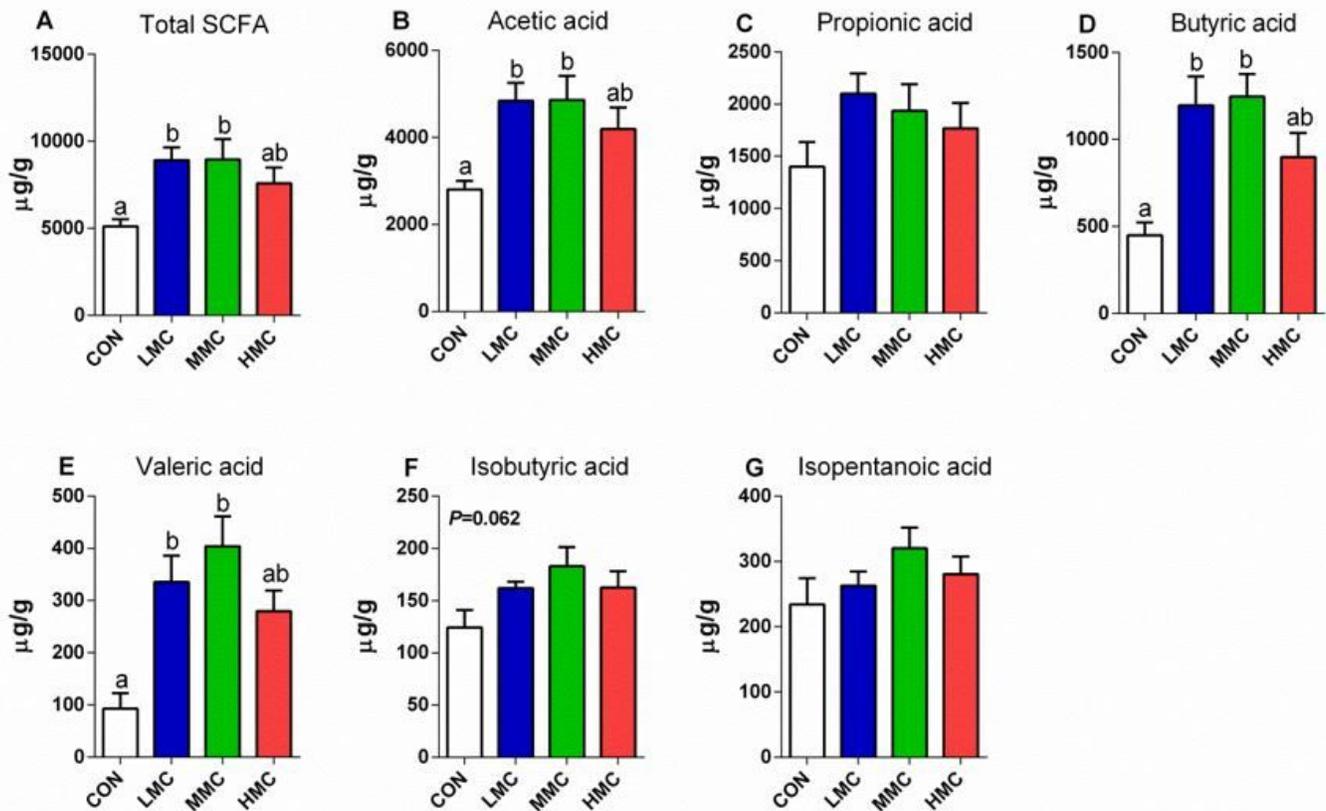
The intestinal morphology was histologically analyzed by hematoxylin and eosin (HE, 500  $\mu\text{m}$ ). (A) Jejunum, (B) Ileum. CON, control group, basal diet without antibiotics; LMC, the control diet + 2000 mg/kg Moutan cortex radice; MMC, the control diet + 4000 mg/kg Moutan cortex radice; HMC, the control diet + 8000 mg/kg Moutan cortex radice.



**Figure 2**

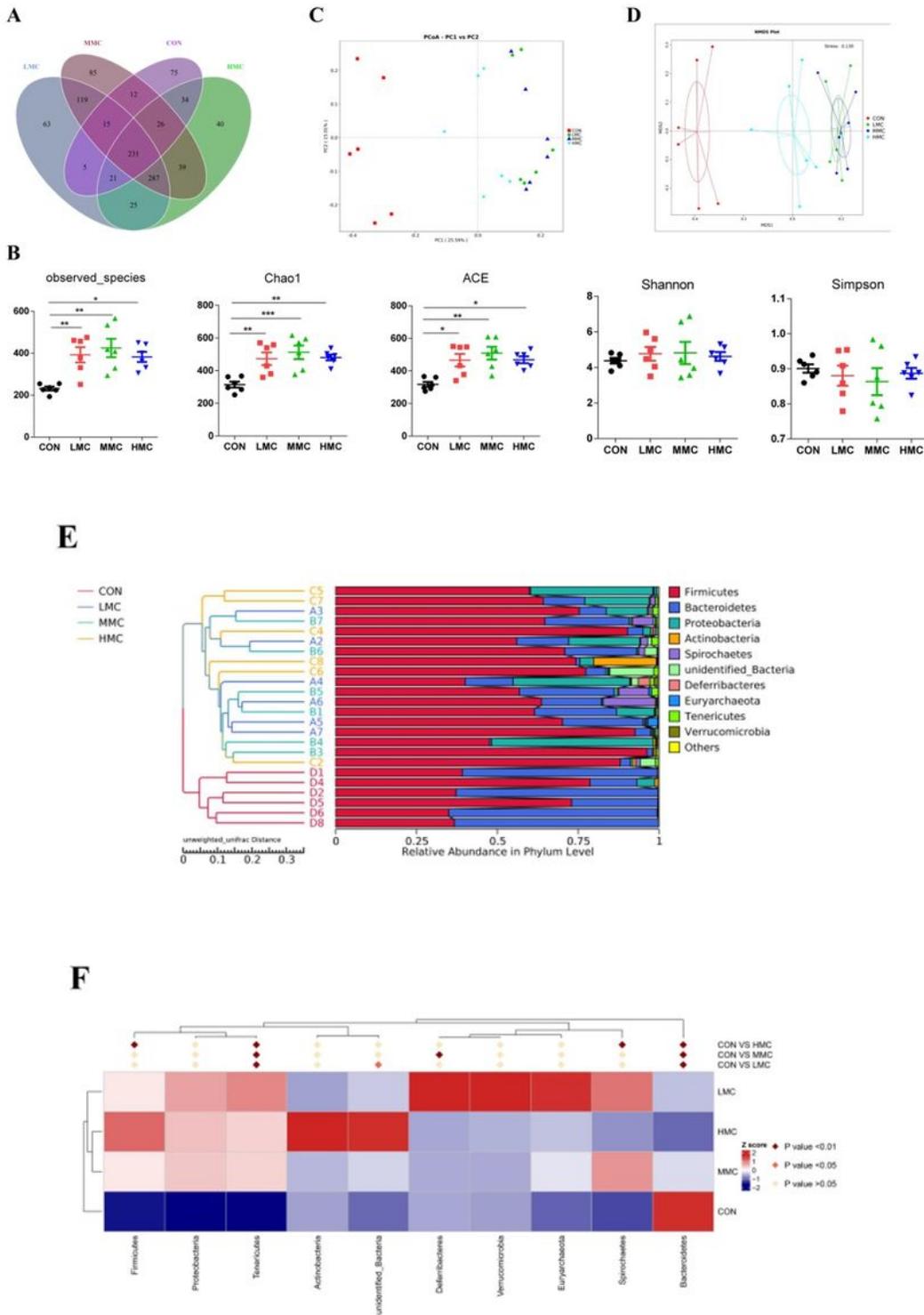
Gene expression levels associated with the proinflammatory factors (IFN- $\gamma$ , TNF- $\alpha$ , IL-1 $\beta$  and IL-6) and tight junction proteins (ZO-1, occludin and claudin-1) of jejunum (A) and ileum (B), and NF- $\kappa$ B signaling pathway in the jejunum and ileum tissues (C) of pigs fed the Moutan cortex radidis diet. CON, control group, basal diet without antibiotics; LMC, the control diet + 2000 mg/kg Moutan cortex radidis; MMC, the control diet + 4000 mg/kg Moutan cortex radidis; HMC, the control diet + 8000 mg/kg Moutan cortex

radicis. Data are expressed as means  $\pm$  SEM (n = 6). Means within a row with different superscripts are significantly different (P < 0.05).



**Figure 3**

Total SCFAs (A), acetic acid (B), propionic acid (C), butyric acid (D), valeric acid (E), isobutyric acid (F), and isopentanoic acid (G) concentrations in colonic contents of pigs fed the Moutan cortex radicis diet for 3 wk. CON, control group, basal diet without antibiotics; LMC, the control diet + 2000 mg/kg Moutan cortex radicis; MMC, the control diet + 4000 mg/kg Moutan cortex radicis; HMC, the control diet + 8000 mg/kg Moutan cortex radicis. Data are expressed as means  $\pm$  SEM (n = 8). Means within a row with different superscripts are significantly different (P < 0.05).

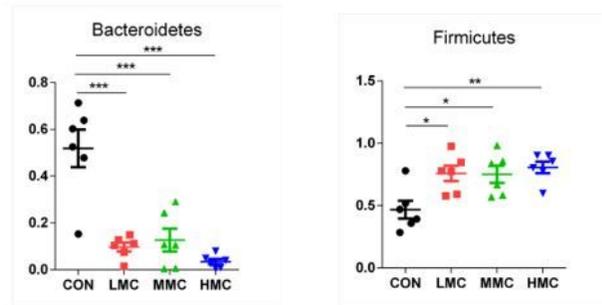
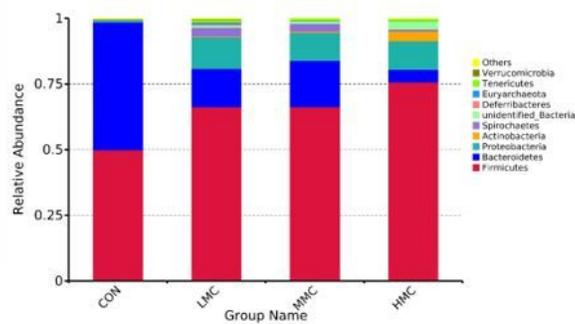


**Figure 4**

Effect of dietary Moutan cortex radices on the colonic microbiota diversity and composition in the pigs. (A) A Venn diagram illustrating the overlaps of OTUs in the gut microbiota; (B) The microbial alpha diversity indexes (Observed-species, Chao1, Shannon, Simpson, ACE) were calculated using the mothur program; (C) Principal coordinate analysis (PCoA); (D) non-metric multidimensional scaling (NMDS) analysis; (E) unweighted unifrac cluster tree based on Unweighted Pair-group Method with Arithmetic

Mean (UPGMA) analysis; (F) The significant different species among groups based on MetaStat analysis. CON, control group, basal diet without antibiotics; LMC, the control diet + 2000 mg/kg Moutan cortex radicis; MMC, the control diet + 4000 mg/kg Moutan cortex radicis; HMC, the control diet + 8000 mg/kg Moutan cortex radicis. Data are expressed as means  $\pm$  SEM (n = 6). \*P < 0.05, \*\*P < 0.01 and \*\*\*P < 0.001.

(A) Phylum



(B) Genus

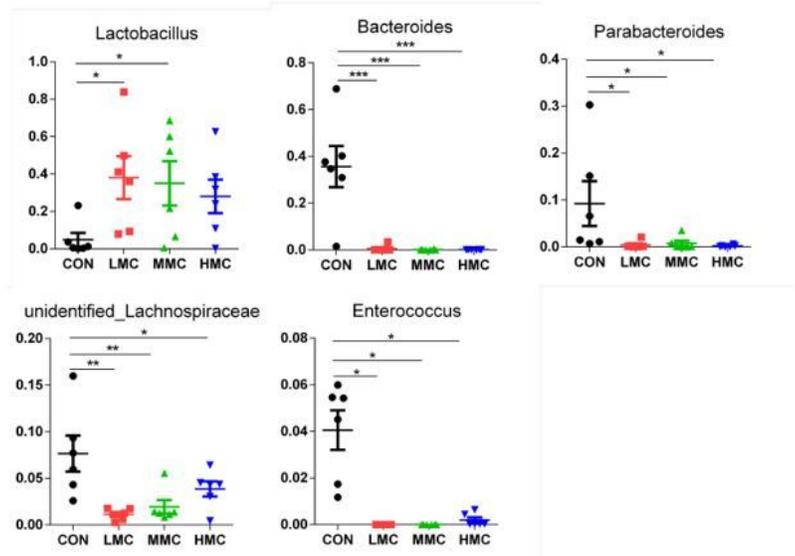
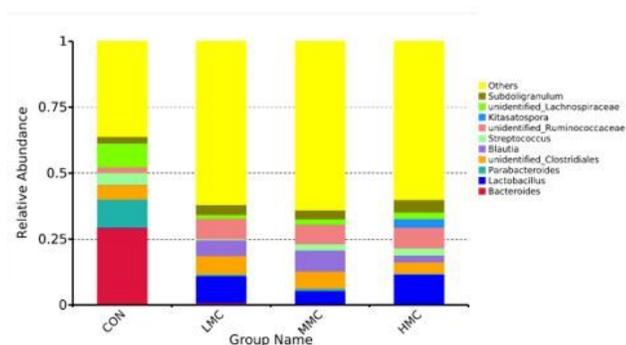
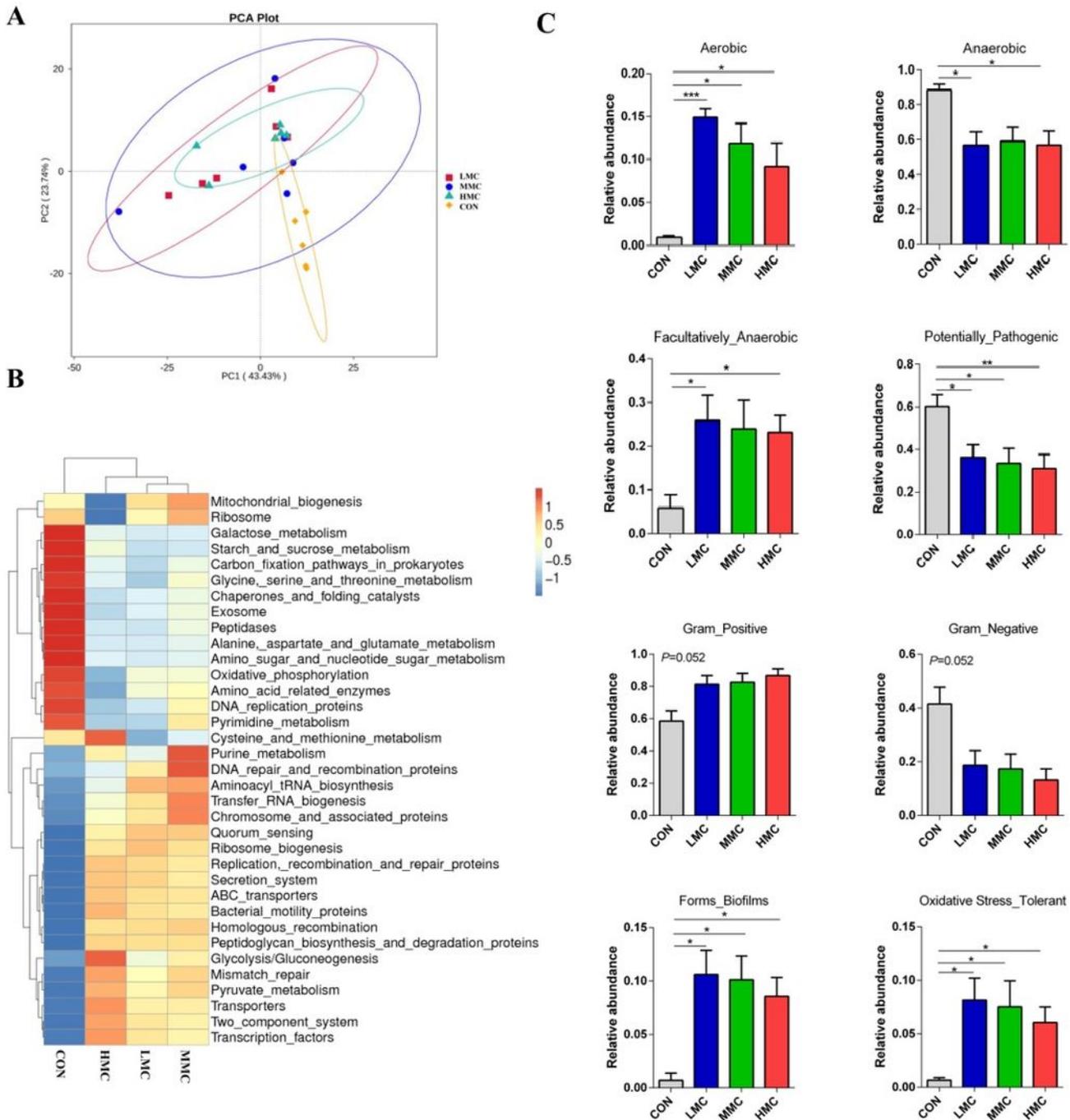


Figure 5

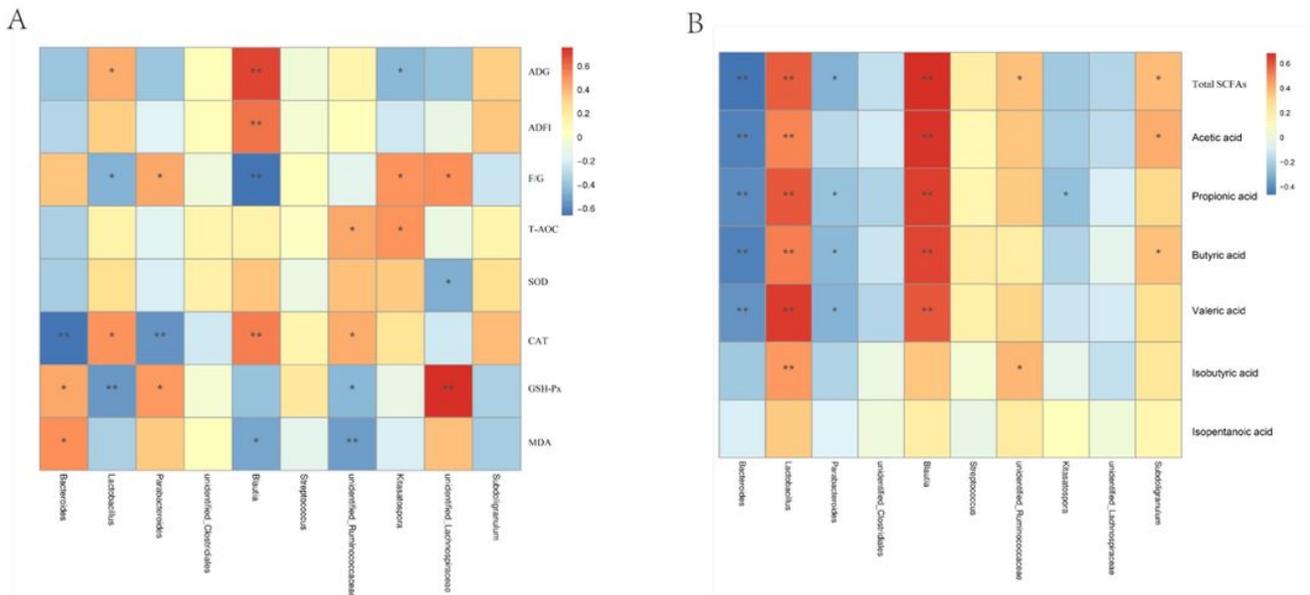
Effects of dietary Moutan cortex radicis on microbial composition at the phylum and genus levels of growing pigs. (A) Relative contribution of the top 10 phylum in each group (left) and the relative abundance of significantly different microorganisms (right); (B) Relative contribution of the top 10 genus in each group (left) and the relative abundance of significantly different microorganisms (right). CON, control group, basal diet without antibiotics; LMC, the control diet + 2000 mg/kg Moutan cortex radicis; MMC, the control diet + 4000 mg/kg Moutan cortex radicis; HMC, the control diet + 8000 mg/kg Moutan cortex radicis. Data are expressed as means  $\pm$  SEM (n = 6). \*P < 0.05, \*\*P < 0.01 and \*\*\*P < 0.001.



**Figure 6**

Dietary supplementation of Moutan cortex radices altered the metabolic functions and phenotypes of colonic microbiota in the pigs. (A) Principal components analysis (PCA) of functional profiles in the gut microbiota; (B) The heatmap tree based on different metabolism related pathway at KEGG level 3; (C) The metabolic phenotypes prediction were compared using BugBase online (<https://bugbase.cs.umn.edu/>). The relative abundances of discrete phenotype was performed using pair-wise Mann-Whitney U tests.

Data are expressed as means  $\pm$  SEM (n = 6). CON, control group, basal diet without antibiotics; LMC, the control diet + 2000 mg/kg Moutan cortex radicis; MMC, the control diet + 4000 mg/kg Moutan cortex radicis; HMC, the control diet + 8000 mg/kg Moutan cortex radicis. \*P < 0.05 and \*\*P < 0.01.



**Figure 7**

Heatmap of the Spearman r correlations analysis. Spearman's correlation coefficients between colonic microbiota and growth and serum antioxidant parameters (A), and colonic SCFA contents (B). \*P < 0.05 and \*\*P < 0.01.