Animal Nutrition 20 (2025) 303–310

Contents lists available at ScienceDirect

Animal Nutrition

journal homepage: http://www.keaipublishing.com/en/journals/aninu/

Original Research Article

Dihydroquercetin attenuated *Prevotella copri*-caused intestinal injury by modulating gut microbiota and bile acids in weaned piglets

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ARTICLE INFO

Article history:
Received 18 January 2024
Received in revised form
26 October 2024
Accepted 30 October 2024
Available online 7 November 2024

Keywords:
Prevotella copri
Bile acid
Dihydroquercetin
Gut microbiota
Piglet

ABSTRACT

Gut microbiota disruption during the weaning process is a significant factor of intestinal injury. Our previous studies have suggested that *Prevotella* may play a critical role in causing intestinal inflammation. This study aimed to clarify the impact of *Prevotella copri* on intestinal injury and the protecting effect by dihydroquercetin (DHQ) in weaned piglets. A total of 108 healthy Duroc × Landrace × Yorkshire weaned piglets, aged 21 d, were randomly allocated into 3 groups with 6 replicates and 6 piglets per replicate. The piglets were the following diets for 28 d: 1) a basal diet, 2) basal diet containing 1.0×10^8 CFU/kg *P. copri*, 3) basal diet supplemented with 1.0×10^8 CFU/kg *P. copri* and 100 mg/kg DHQ. Results showed that *P.* copri decreased significantly the average daily gain (ADG) ($P < 0.001$), which was recovered by supplementation of DHQ with decreased serum levels of malondialdehyde (MDA), interleukin (IL)-2 and IL-8 but increased total superoxide dismutase (T-SOD) activity and IL-10 in weaned piglets ($P < 0.001$). Moreover, DHQ increased the expression of tight junction proteins (claudin-2, occludin and tight junction protein zonula occludens protein-1 (ZO-1) and the mRNA expression of glutathione peroxidase 4 (*GPX-4*) in ileum ($P < 0.001$). Intestinal flora analysis showed that *P. copri* increased the relative abundance of *Prevotella* ($P = 0.026$) and *Eubacterium coprostanoligenes group* ($P < 0.001$), but decreased the relative abundance of Lachnospiraceae *NK4A136 group* ($P < 0.001$), while supplementation of DHQ reduced the relative abundance of *Prevotella* ($P = 0.026$). Metabolomics results indicated that *P. copri* enhanced the content of 12-OH bile acid, but decreased the contents of glycodeoxycholic acid (GDCA) and glycochenodeoxycholic acid (GCDCA) ($P < 0.001$), while DHQ reduced the 12-OH bile acid content ($P < 0.001$) and increased the GDCA content ($P = 0.020$). In summary, *P. copri* caused intestinal injury and reduced growth performance in weaned piglets, and DHQ showed a protective effect by modulating gut microbiota and bile acids metabolism.

1. Introduction

Intestinal damage in piglets can result in digestive system disorders and lead to the decline in growth performance (Tang et al., 2022). Various factors such as premature weaning, transition from liquid to solid feed and exposure to bacteria may induce intestinal damage in piglets (Lallès et al., 2007). Our previous studies have indicated that microbiota disorder play a crucial role in intestinal inflammation and barrier impairment in piglets (Hu et al., 2020; 2022). Specifically, the abundance of the *Prevotella* genus is

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Peer review under the responsibility of Chinese Association of Animal Science and Veterinary Medicine.

Production and Hosting by Elsevier on behalf of KeAi

https://doi.org/10.1016/j.aninu.2024.10.002

positive correlated with multiple inflammatory factors. Moreover, recent studies have suggested that *Prevotella copri* may impact the equilibrium of the gut microbiota (Abdelsalam et al., 2023), and modulate a multifaceted metabolic network that generates diverse metabolites (Huang et al., 2022), which may influence nutrient absorption, immune regulation and the proliferation of other bacterial populations in intestine (Barone et al., 2019).

Gut microbiota has been reported to interact with the regulation of immunity, neuro and hormone of the host (Fung et al., 2017). Microbes convert dietary carbohydrates into metabolites such as short-chain fatty acids, bile acids and amino acids (Marchesi et al., 2016; Funabashi et al., 2020), but can also cause intestinal inflammation by inducing secondary bile acid deficiency (Sinha et al., 2020). The abundance of *Prevotella* is closely correlated with intestinal inflammation in our previous study (Hu et al., 2022), and dihydroquercetin (DHQ), a natural polyphenol, can inhibit the proliferation of *P. copri* (Fig. S1). Therefore, this study was designed to explore the effect of *P. copri* on intestinal injury and protective effect by DHQ, focusing on gut microbiota and bile acids.

2. Materials and methods

2.1. Animal ethics statement

The animal experimental protocol was approved by the Institutional Animal Care and Use Committee of Hunan Agricultural University (approval no: 2021056).

2.2. Materials and agents

Dihydroquercetin (≥98%) was provided by Dongzhizishan (Beijing) Technology Co., Ltd., China. *P. copri* (DSM, 18205) was purchased from American Type Culture Collection, Manassas, VA, USA. Claudin-2, occludin and tight junction protein zonula occludens protein-1 (ZO-1) antibodies were purchased from AiFang Biological, Changsha, Hunan, China, and real-time fluorescence quantitative polymerase chain reaction (PCR) primers (Table S1) were purchased from Sangon Biotech, Shanghai, China.

2.3. Activation and culture of P. copri

A single strain of *P. copri* was purchased from ATTC and subsequently cultivated by utilizing a modified Gifu anaerobic medium (Nissui Pharmaceutical, Japan) supplemented with hemin and vitamin K1 at the temperature of 37 °C, with an anaerobic bag employed to sustain the anaerobic conditions. The quantification of the cultured *P. copri* population after a 24-h incubation period was accomplished through the utilization of a McFarland turbidity tube, yielding a concentration of 1.46×10^9 CFU/mL. Next, the *P. copri* precipitate was collected by centrifugation at 11,600 × *g* and 4 °C for 10 min (Xiangzhi Centrifuge Instrument Co., Ltd., Changsha, Hunan, China), and subsequently freeze-dried to powder.

2.4. Experimental design and diets

A total of 108 healthy Duroc × Landrace × Yorkshire crossbred piglets weaned at 21 d were randomly divided into 3 groups with 6 replicates and 6 piglets per replicate. The control group (CTL) was fed a basal diet based on NRC (2012) (Table 1), and the *P. copri* group (*P. copri*) was fed a diet containing 1.0×10^8 CFU/kg *P. copri*, while the DHQ group (DHQ) was fed a diet supplemented with 1.0×10^8 CFU/kg *P. copri* and 100 mg/kg of DHQ for 28 d. Piglets were raised in a pen with a half-leaking floor and had free access to clean water and feed throughout the entire process.

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

Item	Content
Ingredients	
Wheat for pigs	37.16
Suckling pig corn	15.00
Low protein whey powder	9.00
Expanded soybean	7.00
Hamlet protein HP300	6.50
Flour	5.00
Soybean meal (46%)	5.00
Fermented soybean meal	5.00
Soybean oil	2.35
Biological feed	2.00
Calcium hydrogen phosphate	1.87
Lysine (95%)	0.95
Vitamin premixed core material[1]	0.60
Stone powder	0.50
Trace mineral element premixed core material	0.40
Sodium chloride	0.30
Acyclol (phosphoric acid, lactic acid)	0.30
Methionine (99%)	0.23
Threonine (98%)	0.22
Acridine (benzoic acid, lactic acid)	0.20
Zinc oxide (95%)	0.19
Valine (98%)	0.12
Choline chloride (60%)	0.08
Tryptophan (98%)	0.06
Total	100.00
Energy and nutrient levels	
Digestible energy, MJ/kg	14.58
Crude protein	19.22
Crude fat	5.43
Crude fiber	2.44
Crude ash	5.85
Neutral detergent fiber	10.22
Acid detergent fiber	3.64
Lysine	1.41
Calcium	0.87
Total phosphorus	0.76

[1] The vitamin premixed core material provided per kilogram of feed: vitamin A, 10,500 IU; vitamin D_3, 3300 IU; vitamin E, 22.5 IU; vitamin K_3, 3 mg; vitamin B_1, 3 mg; vitamin B_2, 7.5 mg; vitamin B_6, 4.5 mg; vitamin B_{12}, 0.03 mg; niacin, 30 mg; pantothenate, 15 mg; folic acid, 1.5 mg; biotin, 0.12 mg.

2.5. Chemical analyses

Diets and fecal samples were weighed for determining dry matter (DM) (method 930.15; AOAC, 2006), and data on feed ingredients were obtained from the provided by the China Feed Database (2020). The energy of diets and fecal samples was determined using a Parr 6400 Automatic Oxygen Bomb Calorimeter (Parr Instrument Co., Moline, IL, USA). Crude protein (N × 6.25) was determined by Kjeldahl method (AOAC, 2006; method 976.05). Concentrations of crude fiber (method 978.10), calcium (method 984.01), and total phosphorus (method 965.17) in feed samples were measured according to the Association of Official Analytical Chemists procedures (AOAC, 2006). Lys was analyzed by spectrophotometry (method 975.44; AOAC, 2006). DE were calculated from the diet composition at the estimated requirement with reference to the NRC (2012). Crude fat and crude ash in the diets were analyzed according to method 920.39 and method 942.05 (AOAC, 2006), respectively. Determinations of neutral detergent fiber (NDF) and acid detergent fiber (ADF) were performed using a filter bag and fiber analyzer (Ankom, NY, USA; Van Soest et al., 1991).

2.6. Sample gathered

On d 28 of the experiment, one piglet from each replicate was sacrificed after anesthesia with sodium pentobarbital. Blood

samples (10 mL) were gathered from the jugular vein using a vacuum tube without anticoagulation, and centrifugated at 1500 × *g* for 10 min to obtain serum after stay for 30 min. Cecal contents were collected immediately into sterile test tubes after the cecum was dissected, and quickly frozen in liquid nitrogen before storage at −80 °C. Two specimens of ileum (around 2 cm long) were obtained at 30 cm from the ileocecal valve, washed by 0.9% saline, and preserved in phosphate-buffered paraformaldehyde (4%, pH = 7.6) for hematoxylin and eosin staining (H&E), or snap-frozen in liquid nitrogen before storage at −80 °C.

2.7. Analysis of serum antioxidant and inflammatory markers

Serum levels of cytokines including interleukin (IL)-2, IL-8 and IL-10 were measured by using their respective ELISA kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manual of the kit, while the levels of total superoxidase (T-SOD), total antioxidant capacity (T-AOC) (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) and malondialdehyde (MDA) (Boxbio, Beijing, China) were determined by respective kits according to the manual.

2.8. Real-time fluorescence quantitative PCR

The mRNA expression levels of occludin, claudin-2, *ZO-1*, *ZO-2*, *GPX-1*, *GPX-4*, *SOD-1*, *SOD-2*, *IL-6*, *IL-8*, *IL-10* and *TNF-α* in ileum tissues were detected by real-time fluorescence quantitative PCR. Briefly, total RNA was isolated using the TRIzol Reagent (Sangon Biotech, Shanghai, China) according to the manufacturer's instruction, and the purity of total RNA was identified spectrophotometrically via usage of optical density (OD) 260 and 280 nm measurements (Merinton Instrument, Inc., Ann Arbor, MI, USA). β-Actin was used as an internal reference gene, and Table S1 shows the gene primers. The total reaction system of PCR was 10 μL, including 5 μL 2 × SybrGreen qPCR Master Mix (Thermo Scientific), 0.2 μL every forward and reverse primers (10 μmol/L), 1 μL of cDNA template and 3.6 μL of sterilized water. PCR was performed on LightCycler480 Real-Time fluorescence quantitative PCR system. The cycle parameters were: a total of 45 cycles, each cycle includes 5 s in 95 °C, 30 s in 60 °C.

2.9. Immunofluorescence

Microwave antigen repair was performed on tissue sections using an ethylene diamine tetraacetic acid (EDTA) antigen repair buffer (pH = 8.0). The tissue slices should be lightly dried before being circled with a histochemical pen. After serum blocking, primary claudin-2 antibody incubation was performed before adding the corresponding horseradish peroxidase (HRP) labeled secondary antibody. At the end of the incubation period, 520-TSA was added to continue the incubation, followed by microwave treatment. Occludin and ZO-1 were bound and stained according to the above procedure. Finally, the nuclei were counterstained with DAPI, the autofluorescence was quenched, and the slides were sealed and photographed under microscope (with DAPI staining, nuclei appear blue under ultraviolet illumination, and fluorescein labeling positive expressions red, green, or pink).

2.10. DNA extraction and 16S rDNA sequencing

Amplification of genes V3–V4 area was achieved with primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). For each cecal content, 8-bit barcode sequences were added to the upstream and downstream primers 5′ ends (Allwegene Technology Inc., Beijing, China). PCR was performed on Mastercycler Gradient (Eppendorf, Germany) with a reaction volume of 25 μL, which included 12.5 μL 2×Taq Plus Master Mix, 1 μL forward primer (5 μmol/L), 1 μL reverse primer (5 μmol/L), 2 μL DNA (total template amount was 30 ng), 2 μL bovine serum albumin (BSA) (2 ng/μL) and 5.5 μL ddH_2O. First, 95 °C predenaturation was performed for 5 min, then 28 cycles were run, each lasting 45 s at 95 °C, 50 s at 55 °C, and 45 s at 72 °C, and finally extended 10 min at 72 °C. With the help of the Agencourt AMPure XP purification kit (Beckman Coulter, Inc., USA), PCR products were automatically purified. Finally, the

Table 2
Effects of *P. copri* and dihydroquercetin (DHQ) on growth performance in weaned piglets.[1]

Indexes	CTL	*P. copri*	DHQ	*P*-value
Initial weight, kg	5.90 ± 0.211	6.08 ± 0.452	5.96 ± 0.311	0.651
Final weight, kg	16.74 ± 0.431[a]	15.51 ± 0.291[c]	16.23 ± 0.292[b]	< 0.001
ADG, g	390.01 ± 14.143[a]	335.02 ± 13.783[c]	365.01 ± 14.223[b]	< 0.001
ADFI, g	665.67 ± 24.682[a]	569.33 ± 20.191[c]	619.67 ± 20.061[b]	< 0.001
F:G	1.70 ± 0.011	1.69 ± 0.012	1.69 ± 0.021	0.379

ADG = average daily gain; ADFI = average daily feed intake; F:G = feed-to-gain ratio.
Data were shown as means ± SEM (n = 6). Values with different superscript letters differ significantly (P < 0.05).
[1] CTL = a basal diet; *P. copri* = a basal diet containing 1.0 × 10^8 CFU/kg *P. copri*; DHQ = a basal diet supplemented with 1.0 × 10^8 CFU/kg *P. copri* and 100 mg/kg of dihydroquercetin.

Table 3
Effects of *P. copri* and DHQ on serum inflammatory and antioxidant markers.[1]

Indexes	CTL	*P. copri*	DHQ	*P*-value
IL-2, ng/L	2555.51 ± 55.391[b]	3016.04 ± 195.714[a]	2496.93 ± 102.563[b]	0.038
IL-8, ng/L	7164.64 ± 150.455[b]	8592.87 ± 150.632[a]	7476.03 ± 244.843[b]	0.002
IL-10, ng/L	1156.06 ± 36.121[b]	1106.08 ± 38.011[b]	1572.47 ± 32.464[a]	<0.001
T-AOC, μmol/mL	25.55 ± 3.594	19.77 ± 0.622	27.18 ± 4.912	0.323
MDA, nmol/mL	11.48 ± 1.221[a]	11.47 ± 1.201[a]	7.70 ± 0.594[b]	0.033
T-SOD, U/mL	12.19 ± 0.299[b]	11.14 ± 0.281[b]	14.17 ± 0.771[a]	0.002

IL-2 = interleukin-2; IL-8 = interleukin-8; IL-10 = interleukin-10; T-AOC = total antioxidant capacity; MDA = malondialdehyde; T-SOD = total superoxide dismutase.
Data were shown as means ± SEM (n = 6). Values with different superscript letters differ significantly (P < 0.05).
[1] CTL = a basal diet; *P. copri* = a basal diet containing 1.0 × 10^8 CFU/kg *P. copri*; DHQ = a basal diet supplemented with 1.0 × 10^8 CFU/kg *P. copri* and 100 mg/kg of dihydroquercetin.

sequencing was performed on the Illumina Miseq/Nextseq 2000/Novaseq 6000 (Illumina, Inc.) platform. After running, QIIME (v1.8.0) software was used for data analysis, and R (v3.6.0) software was used for plotting.

2.11. Bile acid targeted metabolomics

Cecal samples (30 mg) were homogenated in 100 μL distilled water for 60 s, adding 500 μL precooled methanol and 10 mL mixed internal standard (200 ng/mL), and then vortex 60 s before low temperature ultrasound for 30 min, twice. After −20 °C for 1 h precipitation of protein, the supernatants (a relative centrifugal force of 14,000 × *g*) were lyophilized and stored at −80 °C for 20 min. Samples were separated using an Agilent 1290 Infinity LC system (Germany), and placed in an autosampler at 4 °C, column temperature was 45 °C, mobile phase A: 0.1% formic acid aqueous solution, mobile phase B: pure methanol, flow rate was 250 μL/min, and injection volume was 2 μL. One quality control (QC) sample was set up for every certain number of experimental samples in the sample cohort to test and evaluate the stability and repeatability of the system. Sample cohorts set standard mixtures of target substances for correction of chromatographic retention times. Using a 5500 QTRAP mass spectrometer (AB SCIEX, Framingham, MA, USA), negative ions were analyzed. Testing was carried out using multiple reaction monitoring (MRM) mode for ion pairs. A retention time and peak area were extracted using Multiquant 3.0.2 (Applied Biosystems SCIEX, Netherlands). The standard of the target substance was used to correct the retention time for metabolite identification.

2.12. Statistical analysis

The general linear model was applied ($Y_{ij} = \mu + d_i + \varepsilon_{ij}$; Y_{ij}: the observation, μ: the general mean, d_i: the treatment effect, ε_{ij}: the random error). All data were analyzed by one-way ANOVA in SPSS (SPSS 25.0, IBM Corp., Armonk, NY, USA) followed by Duncan's multiple range tests. Each replicate served as the experimental unit f served as the experimental unit for other indicators. A level of $P < 0.05$ was consid-ered statistically significant. Differences were considered statistically significant at $P < 0.05$ and highly significant at $P < 0.01$. The result data were expressed as means ± SEM.

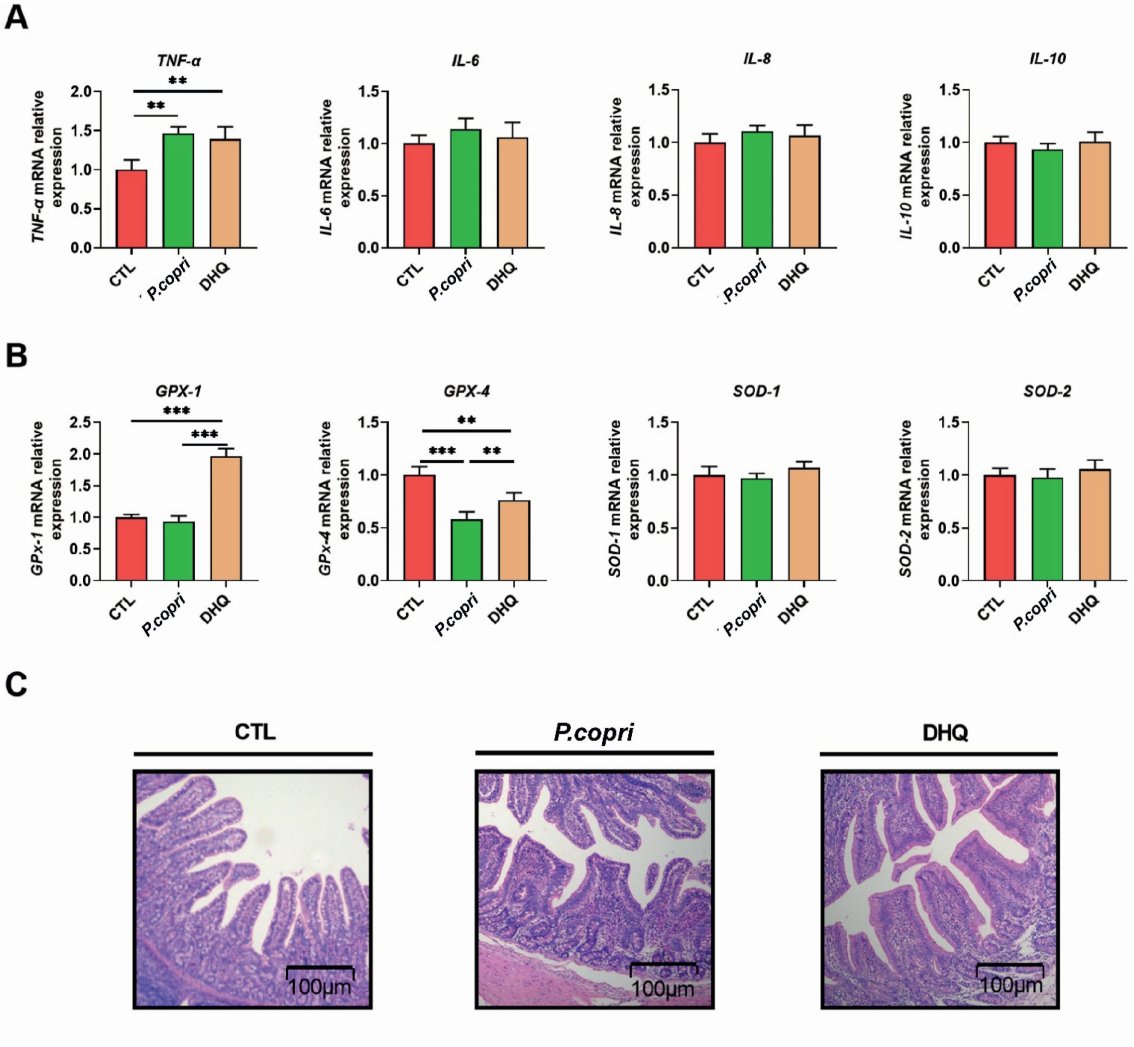

Fig. 1. Modulation of inflammatory and antioxidant genes by *P. copri* and DHQ in ileum tissue. The mRNA expression of inflammatory cytokines (*TNF-α*, *IL-6*, *IL-8* & *IL-10*) (A) and antioxidant markers (*GPX-1*, *GPX-4*, *SOD-1* & *SOD-2*) (B) were quantitated by real-time fluorescence quantitative PCR. The representative sections of ileal tissues stained by hematoxylin and eosin staining (H&E) assay (C). CTL = a basal diet; *P. copri* = a basal diet containing 1.0×10^8 CFU/kg *P. copri*; DHQ = a basal diet supplemented with 1.0×10^8 CFU/kg *P. copri* and 100 mg/kg of dihydroquercetin. Data were shown as means ± SEM ($n = 6$), $^{**}P < 0.01$, $^{***}P < 0.001$.

3. Results

3.1. Effects of P. copri and DHQ on growth performance in weaned piglets

As shown in Table 2, the final weight, average daily gain (ADG) and average daily feed intake (ADFI) in *P. copri* group were significantly lower than that of control group ($P < 0.001$), but the final weight, ADG and ADFI were increased by supplementation of DHQ ($P < 0.001$), while the feed-to-gain ratio (F:G) kept no significant change.

3.2. Effects of P. copri and DHQ on serum inflammatory and antioxidant markers

The oxidative stress and inflammation of piglets were evaluated by measuring T-AOC, MDA, T-SOD, IL-2, IL-8 and IL-10 in serum. According to Table 3, DHQ decreased significantly the MDA level ($P = 0.033$), and increased the T-SOD activity ($P < 0.001$), while showed limited effect on the level of T-AOC. *P. copri* increased serum levels of IL-2 ($P < 0.001$) and IL-8 ($P < 0.001$), but showed no significant effect on IL-10, as compared to the control group. Supplementation of DHQ decreased the levels of IL-2 and IL-8 ($P < 0.001$), but increased IL-10 level ($P < 0.001$).

3.3. Modulation of inflammatory and antioxidant genes by P. copri and DHQ in ileum tissue

As shown in Fig. 1A, *P. copri* increased the mRNA expression of *TNF-α* in ileum tissue ($P < 0.001$), while *IL-6*, *IL-8* and *IL-10* kept no significant change. Meanwhile, *P. copri* reduced the expression of *GPX-4* (Fig. 1B, $P < 0.001$). Supplementation of DHQ increased the expression of *GPX-1* and *GPX-4* ($P < 0.001$), but showed no significant effect on *SOD-1* and *SOD-2*. These data consist with the H&E assay results of ileum, in which *P. copri* impaired villi and crypt structures, but recovered by DHQ (Fig. 1C).

3.4. Effects of P. copri and DHQ on ileal tight junction protein expression

As shown in Fig. 2 A&B, the fluorescence intensity of tight junction proteins including claudin-2 ($P < 0.001$), ZO-1 ($P < 0.001$) and occludin ($P < 0.001$) in ileum tissue were decreased by *P. copri*, but recovered by dietary supplementation of DHQ ($P < 0.001$). Correspondingly, the mRNA expression of claudin-2 and occludin was reduced by *P. copri* ($P < 0.001$), and increased by DHQ (Fig. 2C, $P < 0.001$).

3.5. Modulation of gut microbiota by P. copri and DHQ

Characterization of cecal microbiota was shown in Fig. 3. *P. copri* showed limited effect on Chao1 index, Shannon index, Observed-species, principal component and microbial phylum (Fig. 3A–C). Analysis on the top ten most abundant microbial families showed that *P. copri* increased the relative abundance of *Eubacterium coprostanoligenes* group and Prevotellaceae (Fig. 3D, $P = 0.038$), but decreased the abundance of Lachnospiraceae and Clostridiaceae (Fig. 3E, $P = 0.040$). However, DHQ reduced significantly the relative abundance of Prevotellaceae and *E. coprostanoligenes* group ($P = 0.019$). Further analysis on the top 20 microbial genera revealed that a total of 9 genera showed significant difference (Fig. 3G), in which *P. copri* enriched *Prevotella* ($P = 0.026$) and *E. coprostanoligenes group* ($P < 0.001$), but reduced the relative abundance of Lachnospiraceae *NK4A136 group* ($P = 0.003$) and *Alloprevotella* ($P = 0.024$), while DHQ decreased the relative abundance of *Prevotella* ($P = 0.026$) (Fig. 3H).

3.6. Effects of P. copri and DHQ on bile acids

Metabolomics analysis was next performed to understand the effects of *P. copri* and DHQ on bile acids. As shown in Fig. 4A–E, *P. copri* and DHQ showed limited effect on total bile acids, primary bile acids, secondary bile acids, free bile acids, conjugated bile acids,

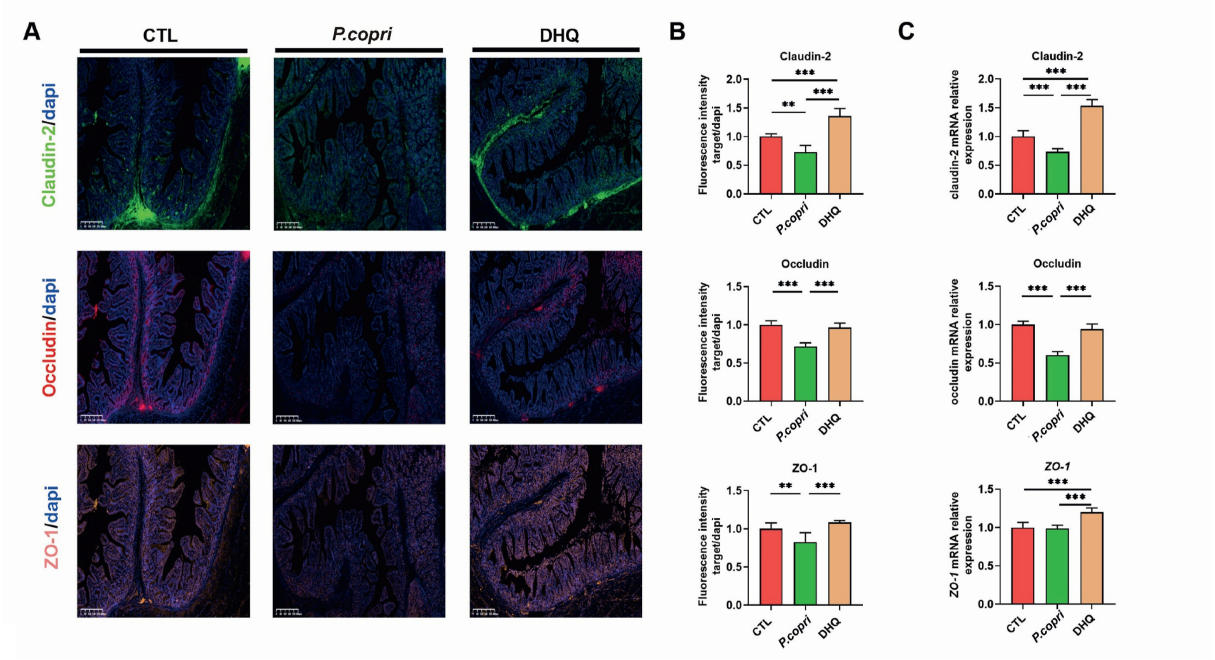

Fig. 2. Effects of *P. copri* and DHQ on ileal tight junction protein expression. The representative sections of claudin-2, occludin and ZO-1 (A), as well as the immunofluorescence intensity (B) were detected by immunofluorescence staining. The mRNA expression of claudin-2, occludin and ZO-1 (C) was quantitated by real-time fluorescence quantitative PCR. dapi = 4′,6-diamidino-2-phenylindole; ZO-1 = zona occludens-1; CTL = a basal diet; *P. copri* = a basal diet containing 1.0×10^8 CFU/kg *P. copri*; DHQ = a basal diet supplemented with 1.0×10^8 CFU/kg *P. copri* and 100 mg/kg of dihydroquercetin. Data were shown as means ± SEM ($n = 6$), $^{**}P < 0.01$, $^{***}P < 0.001$.

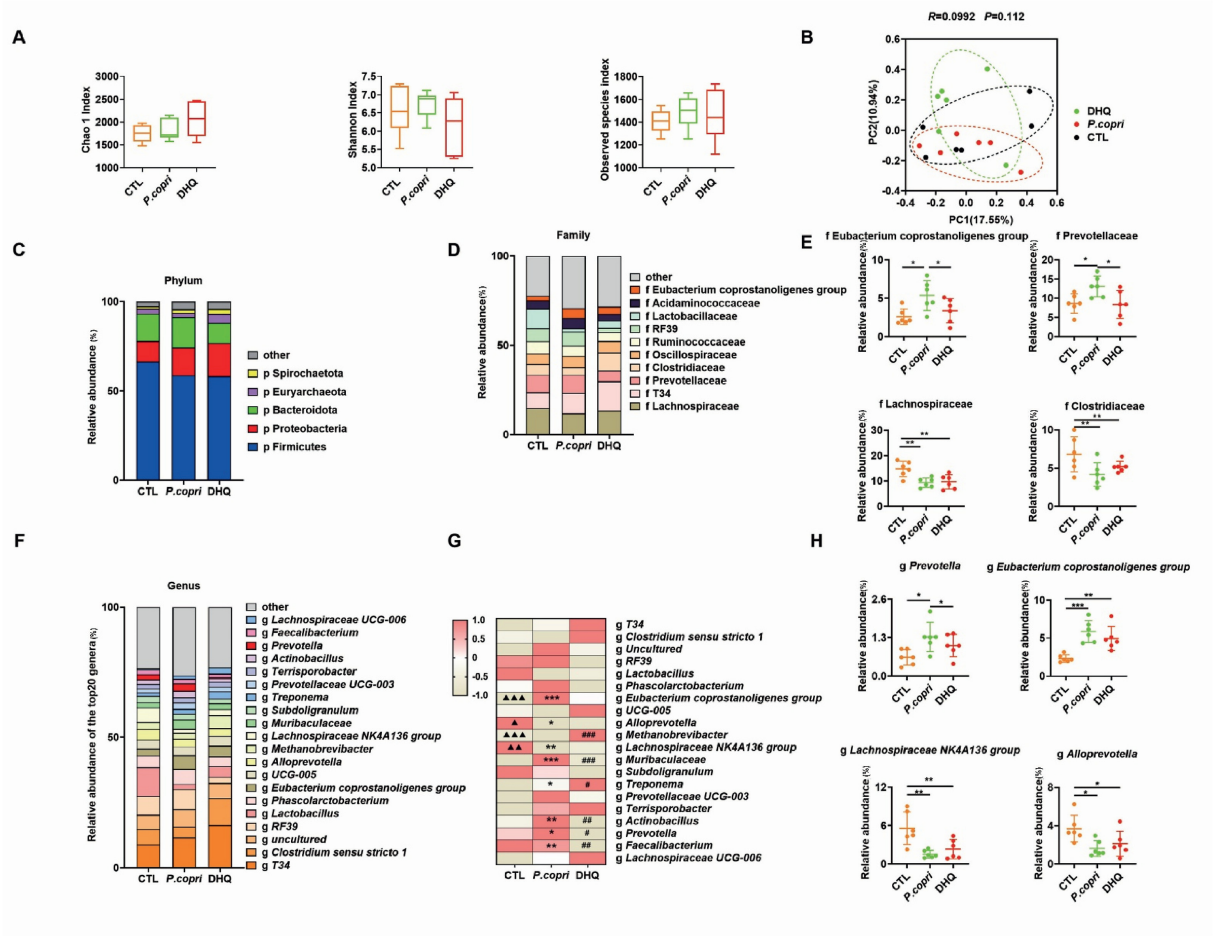

Fig. 3. Modulation of gut microbiota by *P. copri* and DHQ. The Chao1 index, Shannon index and observed species index (A), principal component analysis (B), relative abundance of cecal microbial phyla (C), families (D-E) and genera (F–H) were analyzed by 16S rDNA gene sequencing. CTL = a basal diet; *P. copri* = a basal diet containing 1.0×10^8 CFU/kg *P. copri*; DHQ = a basal diet supplemented with 1.0×10^8 CFU/kg *P. copri* and 100 mg/kg of dihydroquercetin. Data were shown as means ± SEM ($n = 6$), *P. copri* vs CTL, # DHQ vs *P. copri*, ▲ DHQ vs CTL, *, #, ▲ $P < 0.05$, **, ##, ▲▲ $P < 0.01$, ***, ###, ▲▲▲ $P < 0.001$.

as well as the proportion of primary and secondary taurobile acids. However, *P. copri* increased the level of 12-OH bile acid ($P < 0.001$), which was decreased by DHQ (Fig. 4F and G, $P < 0.001$). Moreover, the contents of glycochenodeoxycholic acid (GCDCA) and glycodeoxycholic acid (GDCA) were decreased by *P. copri* ($P < 0.001$), and DHQ increased the GDCA content ($P = 0.020$) (Fig. 4J), although no significant difference was found in primary and secondary glycine bile acids among the 3 groups (Fig. 4H–I).

4. Discussion

Multiple studies have indicated the increase in Prevotellaceae and decrease in *Lactobacillus* in piglets after weaning (Bian et al., 2016; Chen et al., 2017), and *Prevotella* has shown a higher relative abundance in weaned piglets (Guevarra et al., 2018). Our previous studies have also found the enrichment of *Prevotella* in weaned piglets (Hu et al., 2020, 2022). In this study, *P. copri* reduced the final weight, ADG and ADFI of piglets with decreased expression of tight junction proteins including claudin-2, ZO-1 and occludin. Although the concentration of DHQ in feed is far lower than the MIC in vitro, dietary supplementation of DHQ improved the growth performance and tight junction proteins expression.

Dihydroquercetin, a flavonoid known for its antioxidant properties, has been shown to play a significant role in regulating intestinal flora and alleviating intestinal damage (Kanwal et al., 2018; Sunil and Xu, 2019). Recent studies have highlighted the importance of gut microbiota in maintaining intestinal health and its influence on various gastrointestinal disorders (Xu et al., 2021). Dihydroquercetin appears to exert its beneficial effects by modulating the composition of gut microbiota, which in turn can enhance intestinal barrier function and reduce inflammation. In a model of intestinal injury, dihydroquercetin administration was associated with an increase in beneficial microbial populations, such as *Lactobacillus* and *Bifidobacterium*, while decreasing harmful bacteria (Wan et al., 2021). This shift in microbial composition is crucial as it can lead to improved gut health and reduced intestinal permeability, which is often compromised in conditions such as inflammatory bowel disease (IBD) and other gastrointestinal disorders.

Prevotella has been considered as an opportunistic pathogen and often exhibits inflammatory characteristics in the gut microbiota (Larsen, 2017). The abundance of *Prevotella* was found to be increased in a weaning piglet model challenged with *Escherichia coli* (Yu et al., 2021). In this study, *P. copri* caused a notable rise in the abundance of *Prevotella*, which was decreased by DHQ. Metabolomic analysis revealed that *P. copri* increased the content of 12-OH bile acids, but reduced the contents of GCDCA and GDCA, while supplementation of DHQ decreased the content of 12-OH bile acids and GDCA. Bile acids possess ability to modulate gut microbial communities and mediate interactions between microbes and host

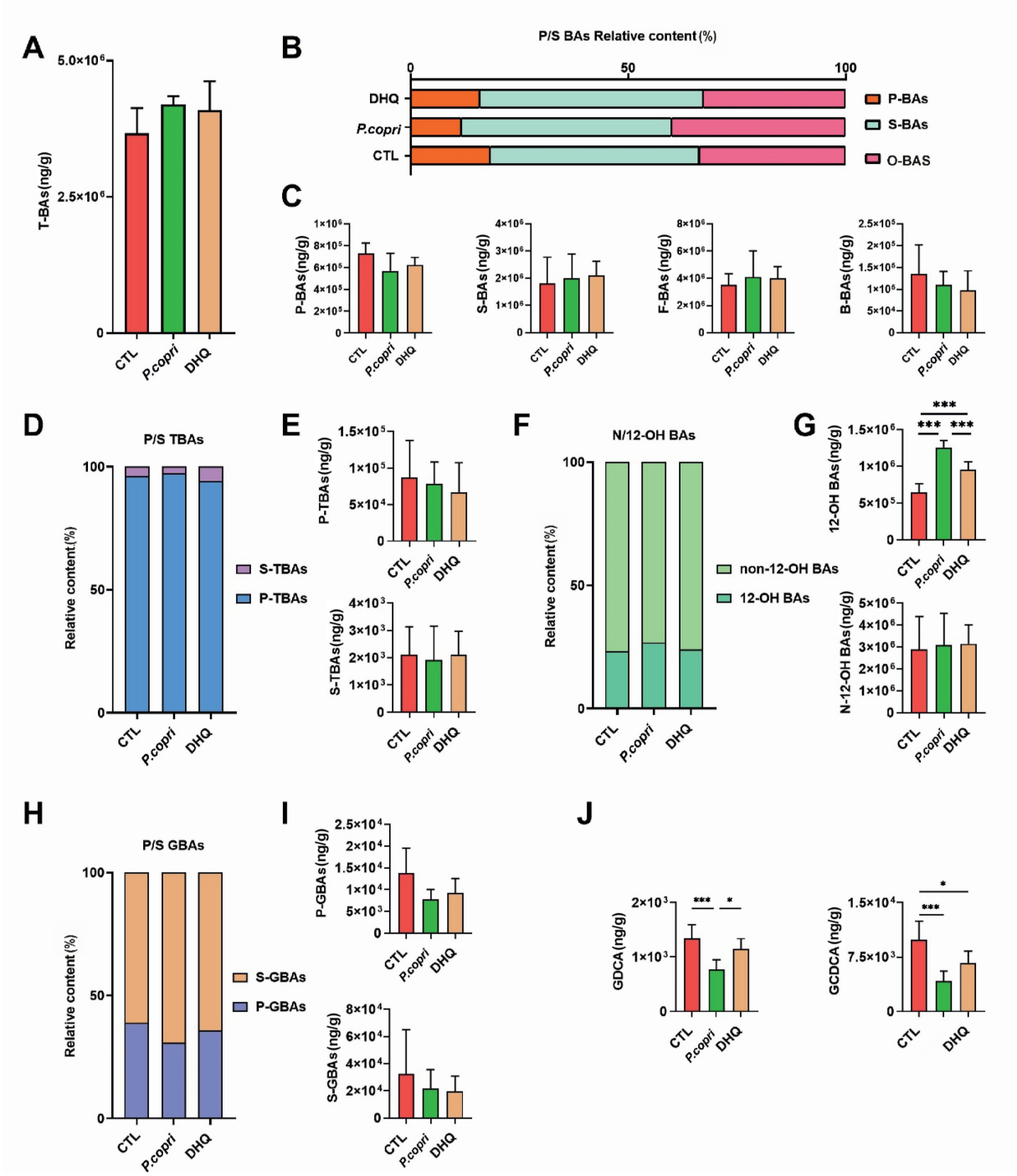

Fig. 4. Effects of *P. copri* and DHQ on bile acids. Total bile acid content (A), the ratio of bile acids (B), contents of primary bile acids, secondary bile acids, free bile acids and bound bile acids (C), the ratio and contents of primary and secondary taurobile bile acids (D-E), the ratio and contents of 12-OH bile acids and non-12-OH bile acids (F-G), the ratio and contents of primary and secondary glycine bile acids (H–I), The contents of GDCA and GCDCA (J) by metabolomics analysis. CTL = a basal diet, *P. copri* = a basal diet containing 1.0×10^8 CFU/kg *P. copri*, DHQ = a basal diet supplemented with 1.0×10^8 CFU/kg *P. copri* and 100 mg/kg of dihydroquercetin. BAs = bile acids, B–BAs = bound bile acids, F–BAs = free bile acids, GCDCA = glycochenodeoxycholic acid, GDCA = glycodeoxycholic acid, N/12-OH BAs = non-12-OH/12-OH bile acids, N-12-OH BAs = non-12-OH bile acids, O–BAs = other bile acids, P–BAs = primary bile acids, P-GBAs = primary glycine bile acids, P-TBAs = primary taurobile acids, P/S BAs = primary/secondary bile acids, P/S GBAs = primary/secondary glycine bile acids, P/S TBAs = primary/secondary taurobile acids, S–BAs = secondary bile acids, S-TBAs = secondary taurobile acids, S-GBAs = secondary glycine bile acids, T-BAs = total bile acids. Data were shown as means ± SEM ($n = 6$), *$P < 0.05$, ***$P < 0.001$.

(Režen et al., 2022; Yu et al., 2023). The liver synthesizes primary bile acids, which are subsequently metabolized by intestinal microbes to generate secondary bile acids. Prior to their entry into the gallbladder, primary bile acids combine with glycine and taurine to create conjugated bile acids (Ay et al., 2022; Guzior and Quinn, 2021), which may influence intestinal inflammation by interacting with bile acid receptors (Wahlström et al., 2016). It has been reported that unconjugated cholic acid (CA) and chenodeoxycholic acid (CDCA) can disrupt the integrity of cell membranes and cause intracellular damage (Urdaneta and Casadesús, 2017), and the

concentration of 12-OH bile acids are positively correlated with inflammatory factors (Yang et al., 2023). Due to *P. copri* increased the content of 12-OH bile acids, and decreased the GCDCA and GDCA contents, it is important to acknowledge the separation of bile acids and the removal of glycine or taurine by bile saline lyase enzymes of gut bacteria (Režen et al., 2022; Ridlon et al., 2016).

5. Conclusion

Taken together, this study demonstrated that *P. copri* can reduce growth performance and cause intestinal injury in weaned piglets, while DHQ, a natural polyphenol, showed a protective effect by regulating the abundance of *Prevotella* and the composition of bile acid pool.

Credit Author Statement

Long Wang: Writing – original draft, Investigation, Formal analysis, Data curation. **Ruizhi Hu:** Formal analysis, Data curation. **Siqi Ma:** Investigation. **Xizi Yang:** Formal analysis, Data curation. **Jiatai Gong:** Formal analysis, Data curation. **Hongkun Xiang:** Investigation. **Mingkun Shi:** Investigation. **Xupeng Yuan:** Formal analysis, Data curation. **Liang Chen:** Writing – review & editing, Resources, Project administration, Funding acquisition. **Hongfu Zhang:** Writing – review & editing, Resources, Funding acquisition. **Bie Tan:** Writing – review & editing, Resources. **Xi He:** Writing – review & editing, Resources, Funding acquisition. **Jianhua He:** Writing – review & editing, Resources, Project administration, Funding acquisition. **Shusong Wu:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

Acknowledgement

This study was partially supported by the funds from the National Natural Science Foundation of China (32102578, U22A20515), and National Key R&D Program of China (2023YFD1302300, 2023YFD1301200).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aninu.2024.10.002.

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