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Original Research Article

Maternal dietary inulin intake during late gestation and lactation ameliorates intestinal oxidative stress in piglets with the involvements of gut microbiota and bile acids metabolism

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ABSTRACT

Maternal inulin intake has been shown to alleviate oxidative stress in piglets, but the role of bile acids (BAs) in this process remains unknown. This study aimed to investigate the roles of gut microbiota and BAs metabolism in the amelioration of intestinal oxidative stress in piglets through a maternal inulin diet. A total of 40 sows were allocated into two dietary treatments from day 85 of gestation until the end of lactation: CON (control diet) and INU (diet with 2% wheat bran replaced by inulin). An oxidative model was further established on the intestinal porcine epithelial cell-jejunum 2 cell line (IPEC-J2) to examine the effect of bacterial BAs on intestinal oxidative stress. Results showed that the maternal inulin diet promoted the average daily gain of piglets during suckling and reduced diarrhea rate during weaning ($P = 0.026$ and $P = 0.005$, respectively). Piglets from the INU group had lower serum levels of reactive oxygen species ($P = 0.021$), malondialdehyde ($P = 0.045$), along with higher serum levels of glutathione peroxidase ($P = 0.027$), catalase ($P = 0.043$), and total superoxide dismutase ($P = 0.097$). Compared to the CON group, maternal inulin intake increased fecal ursodeoxycholic acid (UDCA) by 10.84%, hyodeoxycholic acid (HDCA) by 250.64% ($P = 0.026$), and lithocholic acid (LCA) by 16.41% ($P = 0.048$) in piglets. Moreover, the fecal abundance of *Ruminococcus* and *Christensenellaceae_R-7_group* increased by 167.08% and 75.47% in INU piglets ($P = 0.046$ and $P = 0.037$, respectively). Furthermore, the in vitro study using IPEC-J2 cells demonstrated that UDCA, LCA, and HDCA attenuated intestinal oxidative stress by mediating kelch-1ike ECH-associated protein 1/nuclear factor E2-related factor 2 signaling. In conclusion, our results suggested that maternal dietary inulin intake during late gestation and lactation alleviates intestinal oxidative stress of piglets by regulating gut microbiota and BA metabolism.

1. Introduction

Oxidative stress has been associated with early-life disease and mortality in piglets (Hao et al., 2021; Li et al., 2023a; Zhou et al., 2023). Typically, oxidative stress originates from an imbalance between the intercellular reactive oxygen species (ROS) production and the antioxidant ability of cells (Al-Saeed et al., 2023; Kazmi et al., 2023; Lin and Beal, 2006). Low or transient level of ROS activates survival signaling pathways, while excessive ROS causes damage to DNA, protein, and lipids, leading to cell apoptosis (Sindi et al., 2023; Ullah et al., 2023; Zhang et al., 2016). Weanling piglets are particularly

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susceptible to oxidative stress due to the inevitable exposure to both foreign solid feed and microbial pathogens during this period, making the intestine vulnerable to ROS attacks (Yin et al., 2014; Zhu et al., 2012). Intestinal oxidative stress can reduce nutrient digestion and absorption, damage the intestinal barrier, and increase epithelial cell permeability, ultimately leading to increases in diarrhea incidence and mortality among piglets (Li et al., 2022; Meng et al., 2022). Therefore, alleviating intestinal oxidative stress during the weaning phase is of great significance for piglet health.

The developments of the immune and antioxidant systems of piglets have been associated with the establishment of early intestinal flora (Gresse et al., 2017; Guevarra et al., 2018), with microbial metabolites acting as mediators (Dang et al., 2023; Fan et al., 2023). Among others, bile acids (BAs) are synthesized by the liver, secreted into the duodenum, and reabsorbed in the terminal ileum, with about 5% escaping reabsorption for further transformation in the hindgut (Campbell et al., 2020; Wahlstrom et al., 2016). The conversion of primary BAs to secondary BAs is determined by intestinal microbial structure, as these transformations rely on microbial enzymes (Jia et al., 2018). Notably, secondary BAs not only impact immune cell formation and function but also enhance the antioxidant capacity of animals (Hang et al., 2019b; Pi et al., 2023; van Best et al., 2020; Yin et al., 2021). Therefore, regulating early oxidative stress in piglets may be possible by modifying their intestinal microbiota and, consequently, the microbial metabolism of BAs.

Maternal fiber intake during lactation can modulate piglets' intestinal microbial compositions, which may consequently mitigate weaning-associated intestinal damage in piglets (Cheng et al., 2018; Leblois et al., 2017; Shang et al., 2019). Apart from this, a maternal fiber diet also has the potential to enhance piglet growth rates during the suckling period due to its positive effects on sow insulin sensitivity and feed intake (Li et al., 2021c; Shang et al., 2021; Tan et al., 2016). Inulin, a linear D-fructose polymer linked to terminal glucose or fructose units by a beta (2–1) glycosidic bond, is a soluble fiber that acts as a prebiotic in the intestine (An et al., 2024; Yin et al., 2024). Previous studies have reported the positive effects of inulin intake on insulin sensitivity, glucose homeostasis, inflammatory responses, and antioxidant abilities of sows (Chambers et al., 2019; Shang et al., 2018; Wen et al., 2023). Additionally, maternal inulin intake influences the structure of intestinal microbes and ameliorates inflammation in their piglets (Chen et al., 2023; Huang et al., 2023b; Li et al., 2019; Zhou et al., 2018). However, the effects of maternal inulin intake on intestinal oxidative stress in piglets during weaning and whether microbial BAs are involved are still unknown.

Therefore, we hypothesized that maternal inulin intake could enhance piglet growth performance by increasing sow feed intake during lactation. Additionally, maternal dietary inulin supplementation may alter the intestinal BA metabolism of offspring by modulating gut microbiota, thereby impacting oxidative stress in the intestines of weanling piglets. To test this hypothesis, we measured the feed intake of the sows and the growth performance of the piglets during lactation. Furthermore, 16S rRNA sequencing and targeted metabolomics were used to analyze the microbial structure and fecal BAs profiles of piglets, respectively. Finally, the intestinal porcine epithelial cell-jejunum 2 (IPEC-J2) was used to explore the impacts of BAs on intestinal oxidative stress in vitro.

2. Methods and methods

2.1. Animal ethics statement

The experimental protocol and animal management were approved by the Institutional Animal Care and Use Committee of China Agricultural University (AW51211202-1-1).

2.2. Animal experiment designs

A total of 40 multiparous sows (Yorkshire × Landrace, day 85 of gestation) were involved in this study. Before the experiment, all sows were weighed, and the MyLab touch Vet ultrasonic device (Esaote, Genoa, Italy) was used to measure the thicknesses of backfat and loin muscle of the sows at 50 mm from the midline of the last rib of the back. Sows were allotted to two groups with 20 sows per group ensuring equal distribution of body weight, backfat thickness, and parity. From day 85 of gestation, sows were housed in individual gestating stalls and were moved to separate farrowing crates on day 107 of gestation. From day 85 of gestation till the end of lactation, sows were fed with a control diet (CON, a corn-soybean meal basal diet), or a treatment diet (INU). In the treatment diet, 2% of wheat bran was replaced by inulin (mainly fructans, 95%, Orafti, BENEO, Germany). The feed formula for sows is presented in Table 1. All diets were designed to meet the nutrient needs of swine (NRC, 2012). The crude protein, calcium, phosphorus, amino acids, neutral detergent fiber (NDF), and acid detergent fiber (ADF) in feed were analyzed according to the methods of GB/T 6432-2018 (China National Standard, 2018b), GB/T 6436-2018 (China National Standard, 2018a), GB/T 6437-2018 (China National Standard, 2018c), GB/T 18246-2019 (China National Standard, 2020), GB/T 20806-2006 (China National Standard, 2006), and NY/T 1459–2022 (China Agricultural Standard, 2022), respectively. The total dietary fiber (TDF) was obtained by summing the soluble fiber + insoluble fiber,

Table 1
Ingredients and nutrient levels of experimental diets for sows (%, as-fed basis).

Item	Gestation		Lactation	
	CON[1]	INU[1]	CON[1]	INU[1]
Ingredients				
Corn	55.86	55.86	56.25	56.25
Soybean meal	13.00	13.00	26.00	26.00
Fish meal			3.00	3.00
Soybean oil	0.00	0.00	2.00	2.00
Wheat bran	28.00	26.00	9.00	7.00
Inulin		2.00		2.00
Limestone	1.00	1.00	1.50	1.50
Dicalcium phosphate	1.00	1.00	1.10	1.10
NaCl	0.50	0.50	0.50	0.50
L-Lysine HCl	0.04	0.04	0.05	0.05
DL-Methionine	0.04	0.04	0.04	0.04
L-Threonine	0.05	0.05	0.05	0.05
L-Tryptophan	0.01	0.01	0.01	0.01
Premix[2]	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00
Nutrient levels[3]				
ME, Mcal/kg	3.18	3.19	3.35	3.29
CP	15.33	15.18	19.24	19.66
Ca	0.60	0.62	0.92	0.93
P	0.77	0.74	0.75	0.73
Lys	0.71	0.73	1.05	1.05
Met	0.34	0.33	0.34	0.36
Thr	0.55	0.57	0.75	0.76
Trp	0.16	0.17	0.22	0.21
NDF	15.65	16.33	10.70	11.38
ADF	4.09	4.50	3.76	3.87
TDF	18.40	19.34	15.04	15.45

CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; TDF = total dietary fiber; ME = metabolizable energy.
[1] CON = control; INU = diet with 2% wheat bran replaced by inulin.
[2] Premix provided the following per kilogram of diets: VA 11,000 IU; VD_3 1500 IU; VE 15 IU; VK_3 1.6 mg; VB_1 1.5 mg; VB_2 3.0 mg; VB_6 1.5 mg; VB_{12} 0.015 mg; niacin 22.5 mg; D-pantothenic acid 15 mg; folic acid 2.5 mg; biotin 0.2 mg; Fe 85 mg; Cu 7.5 mg; Zn 75 mg; Mn 35 mg; I 0.5 mg; Se 0.3 mg.
[3] All the values in the nutrient composition are measured except for the calculated values of ME (NRC, 2012).

which was analyzed according to the enzymatic-gravimetric method (method 991.43; AOAC, 1999). Each sow was fed 3.5 kg/d during late gestation. After farrowing, the feed intake increases gradually during the first 10 days. From day 10 of lactation until weaning, sows were fed ad libitum. Water was offered freely during the whole experimental period. The backfat thickness, loin muscle thickness, and body weight of the sows were measured on days 1 and 21 of lactation.

Within 24 h after farrowing, the litter size was standardized to 13 piglets by cross-fostering within the same treatment. During lactation, milk is the only source of nutrition for piglets. The body weight of piglets was recorded every seven days, and the litter weight and average daily gain (ADG) of piglets were then calculated. At day 21 of lactation, piglets were weaned and moved to the nursery (temperature 26 to28 °C, humidity 40% to 50%) and were all fed the same nursery diet ad libitum (Table 2). Feed was added three times per day (07:00, 12:00, and 17:00), and made sure that there was enough feed in the trough throughout the day. The diarrhea incidence and diarrhea scores of piglets were recorded for five days after weaning. The diarrhea rate in each litter was calculated as follows:

Diarrhea rate (%) = [(number of diarrhea piglets × number of diarrhea days)/(number of total piglets × number of test days)] × 100.

2.3. Sample collections

The colostrum was collected manually before first suckling and the milk was collected on day 21 of lactation with the injection of 2 mL oxytocin. The colostrum and milk samples were immediately frozen at −80 °C for further analysis.

On the morning of day 21 of lactation, all piglets were weighed, from 8 randomly chosen litters per treatment group, one piglet with a body weight close to the average weight in their litter was selected for blood and fecal sample collection (four males and four females in each group). Blood samples were obtained on day 2 post-weaning via jugular venipuncture. The blood samples were placed in a 10 mL tube and were subsequently centrifuged at 3000 × *g* and 4 °C for 10 min to acquire the serum. Besides, fresh fecal samples from the same piglets were collected at 07:00 on day 21 of lactation by massaging the rectum and were frozen at liquid nitrogen immediately, then stored at liquid nitrogen.

Table 2
Ingredients and nutrient levels of nursery diets for weanling piglets (%, as-fed basis).

Ingredients	Content	Nutrient levels[2]	Content
Corn	61.95	ME, Mcal/kg	3.41
Soybean meal	10.00	CP	19.23
Extruded soybean meal	5.00	Ca	0.88
Fish meal	5.00	P	0.70
Soybean oil	0.40	Lys	1.30
Expanded soybean	6.00	Met	0.45
Whey powder	8.00	Thr	0.82
Limestone	0.80	Trp	0.22
Dicalcium phosphate	1.15		
NaCl	0.20		
L-Lysine HCl	0.61		
Methionine	0.12		
Threonine	0.22		
Tryptophan	0.05		
Premix[1]	0.50		
Total	100.00		

ME = metabolizable energy; CP = crude protein.
[1] The premix provided the following per kilogram of the diet: VA 12,000 IU, VD 32,500 IU, VE 30 IU, VK 33 mg, VB_5 10 mg, VB_{12} 27.6 μg, niacin 30 mg, choline chloride 400 mg, Mn (as MnO) 40 mg, Fe (as $FeSO_4$ H_2O) 90 mg, Zn (as ZnO) 100 mg, Cu (as $CuSO_4$ $5H_2O$) 8.8 mg, I (as KI) 0.35 mg, Se (as Na_2SeO_3) 0.3 mg.
[2] All the values in the nutrient composition are measured except for the calculated values of ME (NRC, 2012).

2.4. Determination of milk nutrient compositions and calculation of milk production

Milk samples were mixed thoroughly before analyzing. The levels of protein, fat, and lactose in the milk were determined by the Bentley FTS/FCM method (Bentley, MN, USA). The estimated milk production of sows during lactation was obtained by calculating milk energy output and milk energy content for the whole lactation period. The formula (Costermans et al., 2020) used was as follows:

Milk energy (kJ/kg) = [90.6 × fat (%) + 55.4 × protein (%) + 232.5] × 4.184;

Milk energy output (kJ/d) = [4.92 × average daily gain/piglet (g/d) - 90] × number of piglets × 4.184;

Estimated milk production (kg/d) = milk energy output/milk energy.

2.5. Fecal DNA extraction and the analysis of 16S rRNA sequencing data

Microbial DNA in feces was extracted by the QIAamp Stool Extraction Kit (Qiagen, Tübingen, Germany). The concentration and purity of DNA were then detected by a NanoDrop 2000 (Thermo Scientific, Wilmington, USA). A universal primer of 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5-GGACTACHV GGGTWTCTAAT-3′) was used to amplify the V3–V4 region of the 16S rRNA. The sequencing service was provided by Majorbio company (Shanghai, China), and data analysis was performed according to the previous study (Lu et al., 2022).

2.6. Determination of inflammatory factors and antioxidant parameters in serum

The ROS, total superoxide dismutase (T-SOD), total antioxidant capacity (T-AOC), malondialdehyde (MDA), catalase (CAT), and glutathione peroxidase (GSH-PX) in the serum were detected by using commercial kits (Jiancheng Co., Ltd., Nanjing, China) (Widowati et al., 2022). The serum levels of insulin-like growth factor-1 (IGF-1), growth hormone (GH), endotoxin, immunoglobulin A (IgA), immunoglobulin M (IgG), immunoglobulin M (IgM) interleukin-6 (IL-6), interleukin-10 (IL-10), and tumor necrosis factor-α (TNF-α) were measured by ELISA kits (Jiancheng Co., Ltd., Nanjing, China).

2.7. Targeted metabolomic of BAs profiles in feces

About 25 mg of feces were ground with liquid nitrogen and added with 380 μL of the prechilled solution containing 80% methanol and 0.1% formic acid. The mixture was ground by a cryogenic grinder for 6 min (−10 °C, 50 Hz), and then was ultrasound at 5 °C and 40 KHz for 30 min. The mixture was incubated at −20 °C for 5 min and centrifuged at 15,000 × *g*, 4 °C for 10 min. Next, 200 μL supernatant was taken for LC-MS/MS (QTRAP 6500+, AB Sciex, USA) analysis and the raw data were processed by using SCIEX OS (AB Sciex, USA). After normalization to total peak intensity, the calibration equations of different types of BAs were drawn with the peak area of standard concentration BAs and internal standard.

2.8. The establishment of the intestinal oxidative stress cell model and the determination of optimal BAs treatment concentration

The density of 5×10^4 cells/mL IPEC-J2 cells was seeded in a plastic culture flask (Corning, USA) by 10 mL DMEM/F12 medium (Invitrogen, USA) and supplemented with 5% fetal bovine serum (Gibco, USA). All cells were incubated under aseptic conditions at 37 °C with 5% CO_2. After forming a confluent monolayer, cells were digested by a pancreatin and then were made into a cell suspension. Next, the resuspended IPEC-J2 cells with a density of 4.0×10^4 cells/mL were seeded in 6-well and 96-well plates at volumes of 2 mL/well and 200 μL/well, respectively. Different concentrations of hydrogen peroxide (H_2O_2) and BAs were added into culture plates after the seeded cells reached 70% to 80% confluency (about 24 h after seeding) according to the experimental design. Specifically, a concentration gradient of H_2O_2 (100, 200, 400, 600, 800, and 1000 μmol/L) was set to identify the optimal concentration of H_2O_2 to induce cellular oxidative stress in IPEC-J2, followed by the cell viability assays. To determine the optimal rescue concentration of BAs in response to IPEC-J2 oxidative stress, three BAs with different concentration gradients, namely ursodeoxycholic acid (UDCA) (0.1, 1, 10, 100, and 1000 μmol/L), lithocholic acid (LCA) (1, 10, 100, and 1000 μmol/L), hyodeoxycholic acid (HDCA) (1, 10, 100, and 1000 μmol/L), were tested under the stimulation of selected concentrations of H_2O_2. The control group was treated with phosphate buffered saline (PBS).

2.9. 3-(4,5-Dimethylthiazol-2-y1)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assays

The MTT kit (MCE, USA) was used to measure the cell viability of IPEC-J2 cells. Briefly, about 200 μL IPEC-J2 cells with a density of 4×10^4 cells/mL were seeded in 96-well plates. After 24 h of cell adhesion and growth, the cells were cultured with different treatment mediums. At the end of the experiment, every well was incubated with 20 μL (5 mg/mL) MTT for 4 h. After that, cells were lyzed with 150 μL dimethyl sulfoxide (DMSO) and detected at 570 nm.

2.10. Real-time quantitative PCR (RT-qPCR)

Total RNA was extracted from IPEC-J2 cells and feces by using a Trizol reagent (Invitrogen, USA). The concentration and purity of RNA were then measured and a total of 1 μg RNA was used to reverse cDNA with a PrimeScript II cDNA Synthesis Kit (TaKaRa, Japan). Next, a SYBR premix kit (TaKaRa, Japan) was applied to the qPCR detection in triplicate. The glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was used as the internal control for mRNA expression in cells, whereas 16S rRNA was used as the internal control for detecting bacterial bile acid-inducible operon J (*BaiJ*), bile salt hydrolase (*Bsh*), and 3α-hydroxysteroid dehydrogenase (*3α-HSDH*) expression. All primer sequences used in this study are presented in Table S1. The fold change of target genes was normalized to *GAPDH* or 16S rRNA in each sample, which was calculated by the $2^{-\triangle\triangle Ct}$ method.

2.11. Statistical analysis

The data are presented as means and standard error of the mean (SEM). The litter represented the experimental unit for growth performance and diarrhea incidence analyses. The piglet was the experimental unit in the analyses of serum and fecal samples, and the sex of the piglet used in these analyses was similar between the two groups. The data of two groups were analyzed by a two-tailed student *t*-test and the data of more than two groups were analyzed by one-way ANOVA with Dunnett's test in SPSS 20.0 software (SPSS Inc., IL, USA). The one-way ANOVA statistical model was as follows:

$$Y_{ij} = \mu + \alpha_i + \varepsilon_{ij.}$$

Among these, Y_{ij} refers to the observation, μ is the general mean, α_i is the treatment effect, ε_{ij} refers to the random error. Moreover, the linear discriminant analysis (LDA) effect size (LEfSe) was applied to analyze microbial differences (LDA >3.0). Correlations between bacteria, serum indexes, and BAs were assessed by using Spearman's correlation based on the data from the CON and INU groups. $P < 0.05$ was considered significant.

3. Results

3.1. Maternal dietary inulin intake promotes the growth of piglets and reduces diarrhea incidence in piglets during weaning

There were no differences in litter weight, average weight, or ADG of piglets between the CON and INU groups during the first two weeks of suckling (Table 3). However, maternal inulin intake increased the litter weight, mean weight, and ADG of piglets from day 14 to 21 of suckling ($P = 0.010$, $P = 0.046$, and $P = 0.042$, Table 3), and promoted the ADG of piglets throughout the entire suckling period ($P = 0.026$, Table 3). The number of weanling piglets and preweaning mortality did not differ between the CON and INU groups ($P = 0.773$ and $P = 0.221$, Table 3). As shown in Table 4, the maternal inulin diet increased the serum GH of piglets at day 21 of suckling ($P = 0.015$). Moreover, the piglets from the INU group presented a lower diarrhea rate and a lower diarrhea index compared with that from the CON group during the 5 days after weaning (Table 4, $P = 0.005$ and $P = 0.07$).

3.2. The effects of maternal inulin intake on body weight, milk composition, milk production, and feed intake of sows during lactation

There were no differences between the CON and INU groups in the body weight loss or backfat loss of sows during lactation

Table 3
Effects of maternal dietary inulin intake on the growth and mortality of piglets during the suckling period[1].

Item	Group		SEM	*P*-value
	CON	INU		
Litter weight, kg				
After cross-fostering	20.19	20.50	0.875	0.833
Day 7	33.96	34.84	2.431	0.270
Day 14	55.60	57.44	3.242	0.225
Day 21	80.83[b]	88.63[a]	4.277	0.010
Mean weight of piglets, kg				
After cross-fostering	1.44	1.46	0.234	0.823
Day 7	2.62	2.68	0.328	0.835
Day 14	4.52	4.73	0.219	0.737
Day 21	6.59[b]	7.28[a]	0.465	0.046
ADG, g/d				
1st week of lactation	166.95	176.83	22.547	0.734
2nd week of lactation	272.00	291.43	34.924	0.183
3rd week of lactation	285.00[b]	359.39[a]	43.505	0.042
Average days 1–21	245.37[b]	275.78[a]	5.368	0.026
Litter size				
After cross-fostering	13.76	13.93	1.032	0.518
At weaning	12.07	12.22	1.157	0.773
Preweaning mortality, %	12.78	12.04	1.416	0.221

ADG = average daily gain.
[a,b] Different letters of peer data shoulder indicate significant differences ($P < 0.05$).
[1] CON = control; INU = diet with 2% wheat bran replaced by inulin. The litter represents the experimental unit, $n = 20$.

Table 4
Effects of maternal inulin intake on serum hormones and diarrhea incidence of piglets during weaning[1].

Item	Group		SEM	*P*-value
	CON	INU		
Serum hormones				
GH, ng/mL	1.68[b]	3.05[a]	0.296	0.015
IGF-1, ng/mL	80.89	93.68	3.643	0.127
Diarrhea incidence				
Diarrhea rate, %	10.50[a]	4.83[b]	0.939	0.005
Diarrhea index	0.53[a]	0.21[b]	0.072	0.007

GH = growth hormone; IGF-1 = insulin-like growth factor-1.
[a,b] Different letters of peer data shoulder indicate significant differences ($P < 0.05$).
[1] CON = control; INU = diet with 2% wheat bran replaced by inulin. The litter represents the experimental unit in diarrhea observation, $n = 20$; the piglet represents the experimental unit for serum hormones detection, $n = 8$.

(Table S2). Interestingly, maternal inulin intake resulted in greater loin muscle loss in sows during lactation (1.06 mm in CON vs. 5.38 mm in INU, $P = 0.010$, Table S2). There were no differences in the nutrient compositions (fat, protein, and lactose) of colostrum and milk between the CON and INU groups (Table S3). However, the estimated milk production was significantly higher in the INU group than in the CON group (14.34 vs. 12.37 kg/d, $P = 0.050$, Table S3). The average daily feed intake (ADFI) of sows in the INU group was higher than that of sows in the CON group during the third week of lactation (7.72 vs. 6.64 kg/d, $P = 0.039$) and the entire lactation period (6.31 vs. 5.70 kg/d, $P = 0.050$) (Table S4).

3.3. Effects of maternal inulin intake on serum immunoglobulins and inflammatory cytokines, endotoxin of weanling piglets

As shown in Table 5, the maternal inulin diet tended to decrease the serum concentration of IL-6 (111.19 pg/mL in INU vs. 142.92 pg/mL in CON, $P = 0.099$) and significantly increased the serum concentration of IL-10 (6.18 pg/mL in INU vs. 6.46 pg/mL in CON, $P = 0.045$). Moreover, piglets from the INU group exhibited higher levels of serum IgA and IgM ($P = 0.028$ and $P = 0.012$). There was no significant difference in serum endotoxin level between the CON and INU groups ($P = 0.167$).

3.4. Effects of maternal inulin intake on serum oxidative/antioxidant indexes of piglets before and after weaning

As shown in Table 6, maternal dietary inulin treatment decreased the serum ROS and MDA ($P = 0.021$ and $P = 0.045$) and increased serum T-SOD, CAT, and GSH-PX activities ($P = 0.097$, $P = 0.043$, and $P = 0.024$) of weanling piglets.

Table 5
Effects of maternal inulin intake on immunoglobulins, inflammatory cytokines, and endotoxin of piglets during weaning[1].

Item	Group		SEM	*P*-value
	CON	INU		
IL-6, pg/mL	142.92	111.19	15.945	0.099
IL-10, pg/mL	4.46[b]	6.18[a]	0.283	0.045
TNF-α, pg/mL	0.33	0.35	0.038	0.743
Endotoxin, EU/mL	3.69	2.39	0.614	0.167
IgG, g/L	27.08	24.42	7.282	0.503
IgA, g/L	7.46[b]	9.97[a]	1.821	0.028
IgM, g/L	7.70[b]	9.68[a]	1.631	0.012

IL-6 = interleukin-6; IL-10 = interleukin-10; TNF-α = tumor necrosis factor-α; IgA = immune globulin A; IgG = immune globulin G; IgM = immune globulin M.
[a,b] Different letters of peer data shoulder indicate significant differences ($P < 0.05$).
[1] CON = control; INU = diet with 2% wheat bran replaced by inulin. The piglet represents the experimental unit, $n = 8$.

Table 6
Effects of maternal inulin intake on serum oxidative and antioxidant indexes of weanling piglets[1].

Item	Group		SEM	*P*-value
	CON	INU		
ROS, U/mL	153.10[a]	132.22[b]	11.733	0.021
MDA, nmol/mL	3.69[a]	2.39[b]	0.390	0.045
T-SOD, U/mL	111.19	142.92	15.945	0.097
CAT, U/mL	4.46[b]	6.18[a]	0.288	0.043
T-AOC, mmol/L	0.33	0.35	0.038	0.742
GSH-PX, U/mL	294.46[b]	317.53[a]	6.272	0.027

ROS = reactive oxygen species; CAT = catalase; T-SOD = total superoxide dismutase; GSH-PX = glutathione peroxidase; MDA = methane dicarboxylic aldehyde; T-AOC = total antioxidant ability.
[a,b] Different letters of peer data shoulder indicate significant differences ($P < 0.05$).
[1] CON = control; INU = diet with 2% wheat bran replaced by inulin. The piglet represents the experimental unit, $n = 8$.

3.5. Effects of maternal inulin intake on the BAs profile of weanling piglets

A total of 41 BAs were quantified in the feces of piglets, with the top five BAs being LCA, HDCA, dehydrolithocholic acid (dehydroLCA), isolithocholic acid (isoLCA), and hyocholic acid (HCA) (Fig. 1A). Maternal inulin intake decreased the concentration of primary BAs, including chenodeoxycholic acid (CDCA), glycochenodeoxycholic acid (GCDCA), HCA, and taurochenodeoxycholic acid (TCDCA) in the feces of piglets ($P = 0.043$, $P = 0.044$, $P = 0.028$, and $P = 0.037$) (Table 7). In terms of secondary BAs, the maternal inulin diet drastically increased the concentration of ω-muricholic acid (ωMCA) and β-muricholic acid (βMCA) ($P = 0.001$ and $P = 0.008$), and significantly increased the levels of α-muricholic acid (αMCA), deoxycholic acid (DCA), HDCA, 3β_ursodeoxycholic acid (3β_UDCA), and LCA ($P = 0.025$, $P = 0.047$, $P = 0.026$, $P = 0.039$, and $P = 0.048$) (Table 7). Levels of LCA derivatives isoLCA and 12-ketolithocholic acid (12_ketoLCA) were also higher in piglet feces from the INU group ($P = 0.052$ and $P = 0.044$) (Table 7). In addition, the deconjugation of BAs was enhanced in the INU group, as indicated by the lower ratios of TCDCA/CDCA, GCDCA/CDCA, tauroohyodeoxycholic acid (THDCA)/HDCA, and taurohyodeoxycholic acid (THCA)/HCA (Table 7). Overall, the production of secondary BAs and total BAs were significantly higher in the INU group compared with the CON group ($P = 0.052$ and $P = 0.037$). However, the ratio of primary BAs/secondary BAs, primary BAs/total BAs, and secondary BAs/total BAs did not differ between the two groups (Table 7). A higher isoLCA/(isoLCA + LCA) was observed in the INU group ($P = 0.075$) (Table 7). Additionally, the relative expression levels of bacterial *Bsh* ($P = 0.088$), *Baij* ($P = 0.020$), and *3α-HSDH* ($P = 0.036$) were elevated in piglets from the INU group (Fig. 1B and C).

3.6. Effects of maternal inulin on microbial composition and diversity of weanling piglets

Piglets from the INU group presented higher microbial richness than those from the CON group, as reflected by the upward trend in the Shannon index ($P = 0.073$, Fig. 2A). Principal-component analysis revealed a distinct clustering in the microbial structure between the CON and INU groups during weaning ($P = 0.049$, Fig. 2B). Firmicutes, Bacteroidetes, and Actinobacteria were the three most dominating phyla, accounting for over 90% of the total bacteria (Fig. 2C). The maternal inulin diet led to a higher relative

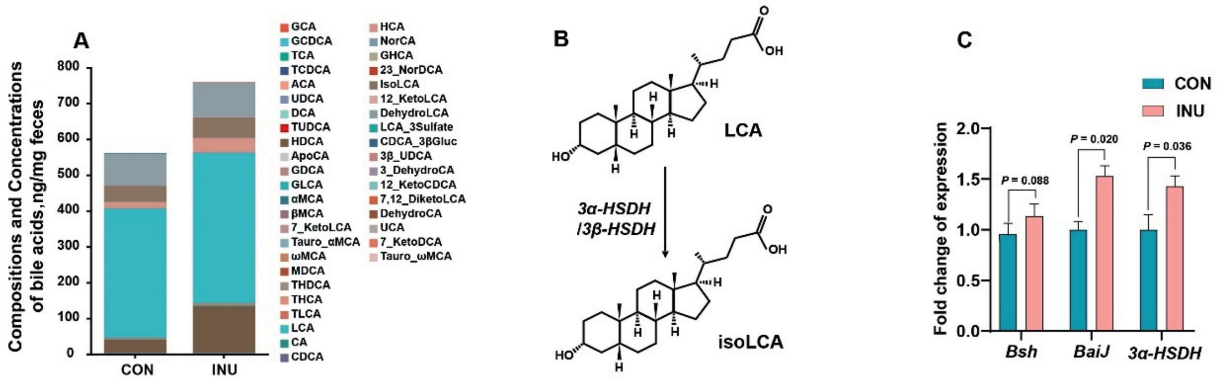

Fig. 1. The bile acids (BAs) profiles and the bacterial gene expression of secondary BAs producing enzymes in the feces of piglets. The piglet represents the experimental unit, $n = 8$. (A) The composition of BAs in piglet feces of two groups and a total of 41 different BAs were identified. (B) The chemical structure of LCA, isoLCA, and their transformation. (C) Relative mRNA expression of bacterial *Bsh*, *BaiJ*, and *3α-HSDH*. CON = control; INU = diet with 2% wheat bran replaced by inulin. The difference between the CON and INU groups was analyzed by a two-tailed student *t*-test. Data are expressed as means ± SEM. GCA = glycocholic acid; GCDCA = glycochenodeoxycholic acid; TCA = taurocholic acid; TCDCA = taurochenodeoxycholic acid; ACA = allocholic acid; UDCA = ursodeoxycholic acid; DCA = chenodeoxycholic acid; TUDCA = tauroursodeoxycholic acid; HDCA = hyodeoxycholic acid; ApoCA = apocholic acid; GDCA = glycodeoxycholic acid; GLCA = glycholithocholic acid; αMCA = α-muricholic acid; βMCA = β-muricholic acid; 7_KetoLCA = 7_keto lithocholic acid; ωMCA = ω-muricholic acid; MDCA = murideoxycholic acid; THDCA = taurohyodeoxycholic acid; THCA = taurohyodeoxycholic acid; TLCA = taurolithocholic acid; LCA = lithocholic acid; CA = cholic acid; CDCA = chenodeoxycholic acid; HCA = hyocholic acid; NorCA = norcholic acid; GHCA = glycohyocholic acid; 23_ NorDCA = 23_ norchenodeoxycholic acid; IsoLCA = isolithocholic acid; 12_KetoLCA = 12_ketolithocholic acid; DehydroLCA = dehydrolithocholic acid; LCA_3sulfate = lithocholic acid_3sulfate; CDCA_3β_gluc = chenodeoxycholic acid_3β_gluc; 3β_UDCA = 3β_ursodeoxycholic acid; 3_DehydroLCA = 3_dehydrolithocholic acid; 12_KetoCDCA = 12_ketochenodeoxycholic acid; 7,12_DiketoLCA = 7,12_diketolithocholic acid; DehydroCA = dehydrocholic acid; UCA = ursocholic acid; 7_KetoDCA = 7_keto chenodeoxycholic acid; Tauro_ωMCA = tauro_ω-muricholic acid; *3α-HSDH* = 3α-hydroxysteroid dehydrogenase; *3β-HSDH* = 3β-hydroxysteroid dehydrogenase; *Bsh* = bile salt hydrolase; *BaiJ* = bile acid-inducible operon J.

abundance of Firmicutes (86.18% in INU vs. 80.43% in CON) and a lower relative abundance of Bacteroidetes (8.25% in INU vs. 10.76% in CON) ($P = 0.045$ and $P = 0.049$). The top seven genera across the two groups were *unclassified_f_Lachnospiraceae*, *Cheristensenellaceae_R-7_group*, *Blautia*, *Subdoligranulum*, *norank_f_Muribaculaceae*, *Eubacterium_hillii_group*, and *Lactobacillus* (Fig. 2D).

The ratio of Firmicutes/Bacteroidetes (F/B) significantly increased in INU piglets compared with the CON piglets (26.40 vs 13.41) (Fig. 2E, $P = 0.037$). As presented in Fig. 2F, maternal inulin intake significantly enhanced the relative abundances of Ruminococcaceae ($P = 0.027$), Clostridiaceae ($P = 0.046$), norank_o__Chloroplast ($P = 0.011$), Enterococcaceae ($P = 0.021$), unclassified_c_Clostridia ($P = 0.021$), and remarkably reduced the relative abundance of Lachnospiraceae at the family level ($P = 0.006$). At the genus level, there were significant promotions in the relative abundances of *Terrisporobacter* ($P = 0.030$), *Ruminococcus* ($P = 0.046$), *Cloacibacillus* ($P = 0.037$), *Enterococcus* ($P = 0.021$), *Eubacterium_ruminantium_group* ($P = 0.026$), *unclassified_c_Clostridia* ($P = 0.021$), and significant reductions in the relative abundances of *Eubacterium_hallii_group* ($P = 0.021$), *norank_f_Lachnospiraceae* ($P = 0.013$) in the INU group.

3.7. The relationship between BAs profile and intestinal microbial structure

As presented in Fig. 3, *Ruminococcus*, *UCG-002*, *norank_f_Ruminococcaceae*, *Christensenellaceae_R-7_group*, and *Clostridium_sensu_stricto_1* were positively correlated with various secondary BAs and the total production of secondary BAs. Among these, *Ruminococcus* was positively correlated with αMCA ($P = 0.007$), βMCA, ωMCA, DCA, UDCA, HDCA, and LCA ($P = 0.037$, $P = 0.030$, $P = 0.044$, $P = 0.045$, $P = 0.037$, and $P = 0.014$) and showed a strong positive relation with both secondary BAs and total BAs (both $P = 0.004$). Moreover, there were negative correlations between the conjugated-BAs (THCA, tauroursodeoxycholic acid [TUDCA], and taurolithocholic acid [TLCA]) and the *Ruminococcus* ($P = 0.157$, $P = 0.057$, and $P = 0.114$) and *Christensenellaceae_R-7_group*. Interestingly, the isoLCA (a derivative of LCA) showed significant positive correlations with *Ruminococcus* ($P = 0.002$), *UCG-002* ($P = 0.043$), and *Christensenellaceae_R-7_group* ($P = 0.037$). Likewise, the 12_ketoLCA (another derivative of LCA) was positively correlated with *Ruminococcus* ($P = 0.030$).

3.8. The correlations between secondary BAs and antioxidants and inflammation of weanling piglets

As shown in Table 8, the serum ROS had strong negative correlations with fecal LCA and HDCA (both $P = 0.005$), while serum CAT was positively correlated with HDCA, UDCA, and LCA ($P = 0.044$, $P = 0.021$, and $P = 0.042$). Regarding inflammatory cytokines, isoLCA showed remarkably negative correlations with IL-6 and TNF-α ($P < 0.001$ and $P = 0.004$) and a greatly positive correlation with IL-10 ($P = 0.002$). Moreover, DCA and LCA were negatively correlated with IL-6 and TNF-α ($P = 0.033$ and $P = 0.040$ for DCA, $P = 0.004$ and $P = 0.044$ for LCA) and UDCA also presented a negative correlation with TNF-α ($P = 0.039$).

3.9. The effects of secondary BAs on the intestinal oxidative stress model

As shown in Table S5, 100, 200, 400, 600, 800, and 1000 μmol/L H_2O_2 significantly reduced cell viability from 100.00% in the control to 98.98%, 75.31%, 13.78%, 6.63%, 6.56%, and 6.36%, respectively ($P < 0.001$). Therefore, the incubation with 200 μmol/L H_2O_2 was used to induce an oxidative stress environment for IPEC-J2 in this study. Moreover, the viability of IPEC-J2 cells was significantly promoted by 21.83% and 15.24% in the H + LCA100 and H + HDCA100 groups, respectively, compared with H_2O_2-treated cells (Tables S6 and S7, both $P < 0.001$). In addition, the cell survival rate of IPEC-J2 increased by 6.53% with 1 μmol/L UDCA treatment compared with the H_2O_2 group ($P < 0.001$, Table S8).

Additionally, HDCA + H, LCA + H, and UDCA + H increased the T-AOC level (164.55, 176.92, and 158.19 mmol/g) compared with the

Table 7
Effects of maternal inulin intake on the BAs profiles of piglets during weaning[1].

Item	Group		SEM	*P*-value
	CON	INU		
Primary BAs, ng/mg				
CA	0.018	0.014	0.0082	0.675
GCA	0.003	0.004	0.0010	0.619
TCA	0.004	0.004	0.0010	0.897
CDCA	0.473[a]	0.242[b]	0.0405	0.043
GCDCA	0.298[a]	0.164[b]	0.0643	0.044
TCDCA	0.310[a]	0.103[b]	0.0594	0.037
CDCA_3β_Glu	1.356	1.327	0.2475	0.962
HCA	11.865[b]	34.463[a]	4.6269	0.028
THCA	0.244	0.120	0.8090	0.074
vGHCA	0.847	0.705	0.2190	0.699
Secondary BAs, ng/mg				
αMCA	0.285[b]	0.886[a]	0.0935	0.025
βMCA	0.177[b]	0.564[a]	0.0477	0.008
ωMCA	0.460[b]	3.204[a]	0.1599	0.001
DCA	0.441[b]	1.153[a]	0.1269	0.047
MDCA	1.210	0.724	0.1553	0.264
3β_UDCA	0.331[a]	0.146[b]	0.0227	0.039
UDCA	3.321	3.686	1.1038	0.827
TUDCA	0.007	0.002	0.0003	0.176
HDCA	34.816[b]	137.833[a]	16.5902	0.026
THDCA	0.250	0.151	0.0485	0.337
LCA	361.837[b]	421.217[a]	67.5408	0.048
GLCA	0.107	0.096	0.0233	0.744
TLCA	0.011	0.006	0.0025	0.222
IsoLCA	45.020	57.203	9.7727	0.052
12_KetoLCA	0.659[b]	0.984[a]	0.1209	0.044
DehydroLCA	89.148	94.845	21.8242	0.885
Total BAs, ng/mg				
Primary BAs	15.420	37.227	12.3605	0.143
Secondary BAs	538.079	722.697	101.3347	0.052
Total of BAs	553.499[b]	759.924[a]	103.5388	0.037
Ratios of different BAs				
Secondary BAs/primary BAs	48.375	34.252	12.3310	0.179
Primary BAs/total BAs	0.027	0.049	0.0061	0.227
Secondary BAs/total BAs	0.972	0.950	0.0065	0.227
TCDCA/CDCA	0.723	0.486	0.0455	0.052
GCDCA/CDCA	0.725	0.667	0.0873	0.677
THDCA/HDCA	0.023[a]	0.001[b]	0.0009	0.012
THCA/HCA	0.037	0.021	0.0050	0.083
IsoLCA/(isoLCA + LCA)	0.107	0.127	0.0232	0.075

BAs = bile acids; CA = cholic acid; GCA = glycocholic acid; TCA = taurocholic acid; CDCA = chenodeoxycholic acid; GCDCA = glycochenodeoxycholic acid; TCDCA = taurochenodeoxycholic acid; CDCA_3β_glu = chenodeoxycholic acid_3β_glu; HCA = hyocholic acid; THCA = taurohyodeoxycholic acid; GHCA = glycohyocholic acid; αMCA = α-muricholic acid; βMCA = β-muricholic acid; ωMCA = ω-muricholic acid; DCA = deoxycholic acid; MDCA = murideoxycholic acid; 3β_UDCA = 3β_ursodeoxycholic acid; UDCA = ursodeoxycholic acid; TUDCA = tauroursodeoxycholic acid; HDCA = hyodeoxycholic acid; THDCA = taurohyodeoxycholic acid; LCA = lithocholic acid; GLCA = glycholithocholic acid; TLCA = taurolithocholic acid; IsoLCA = isolithocholic acid; 12_KetoLCA = 12_keto lithocholic acid; DehydroLCA = dehydrolithocholic acid.
[a,b] Different letters of peer data shoulder indicate significant differences ($P < 0.05$).
[1] CON = control; INU = diet with 2% wheat bran replaced by inulin. The piglet represents the experimental unit, $n = 8$.

H_2O_2 group (147.48 mmol/g) (Fig. 4A and B). Moreover, *SOD1* expression was enhanced in the UDCA + H group (Fig. 4C), and both LCA + H and UDCA + H also increased the relative mRNA expression of *CAT* (Fig. 4D).

3.10. Secondary BAs increase the antioxidant ability of IPEC-J2 by inducing farnesoid X receptor (FXR)/takeda G protein-coupled receptor 5 (TGR5)- kelch-1ike ECH- associated protein 1(Keap1)/ nuclear factor E2-related factor 2 (Nrf2)

HDCA + H increased the relative mRNA expression of *FXR* and *TGR5*, whereas LCA + H and UDCA + H only promoted the *TGR5* mRNA expression (Fig. 5A and B). Moreover, the mRNA expression of *TGR5* was highest in the HDCA + H group. *Keap1*, a negative regulator of *Nrf2*, exhibited a lower level in the LCA + H and UDCA + H groups compared with the H_2O_2 group ($P = 0.041$ and $P = 0.049$, Fig. 5C). Conversely, the expression of *Nrf2* was significantly elevated in the HDCA + H, LCA + H, and UDCA + H groups compared with the H_2O_2 group ($P = 0.004$, $P = 0.037$, and $P = 0.040$, Fig. 5D). Consistently, the heme oxygenase-1 (*HO-1*), an important downstream regulatory gene of *Nrf2*, was highly expressed in the HDCA + H and LCA + H groups compared with the H_2O_2 group (both $P < 0.001$, Fig. 5E).

HDCA + H, LCA + H, and UDCA + H showed lower expression of fas cell surface death receptor (*FAS*) compared with the H_2O_2 group ($P < 0.05$, Fig. 5F). The LCA + H and UDCA + H treatments tended to reduce the B cell leukemia/lymphoma 2-associated X (*BAX*) expression ($P = 0.071$ and $P = 0.096$, respectively, Fig. 5G). Notably, the relative expression of caspase-3 in HDCA + H and LCA + H was lower than that in the H_2O_2 group ($P = 0.043$ and $P = 0.045$, Fig. 5H). Notably, the relative expressions of two tight junction protein genes occludin (Fig. 5I) and zonula occludens-1 (*ZO-1*) (Fig. 5J) were higher in the HDCA + H ($P < 0.001$ and $P = 0.003$) and LCA + H ($P = 0.015$ and $P = 0.035$) groups compared with the H_2O_2 group.

4. Discussion

In this study, we found that maternal inulin intake during late gestation and lactation promoted the growth of suckling piglets, and mitigated intestinal oxidative stress of weanling piglets by regulating microbial structure and BAs metabolism of offspring.

The feed intake of sows during lactation is the determinant factor for the weanling weight of piglets (Eissen et al., 2003). In the present study, a maternal inulin diet was found to increase the feed intake and milk production of sows during lactation, thereby enhancing the growth and weanling weight of piglets. This finding is consistent with the results of the studies in which sows were fed with inulin and soluble fiber diets (Li et al., 2021b; Tan et al., 2015). Insulin resistance develops in the sows during late gestation and lactation physiologically, which results in the decrease of glucose utilization in peripheral tissues. This, in turn, increases the glucose and insulin concentration, suppressing the feeding motivation and reducing the feed intake of sows during lactation (Mosnier et al., 2010a,b). Growing evidence suggests a connection between gut microbiota and insulin resistance (Luo et al., 2020). As a prebiotic, inulin has the potential to increase specific microbiota that are beneficial for alleviating insulin resistance (Li et al., 2021c). Although we did not observe the fecal microbiome of sows in this study, the feces of sows fed inulin have been reported to be rich in *Enterococci* (Passlack et al., 2015). Notably, *Enterococci* can effectively alleviate insulin resistance and enhance the insulin sensitivity of the host (Zhang et al., 2017). These results were also agreed by the studies from Tan et al. and Wu et al., which indicated that the inclusion of soluble fiber in the gestation diet increased the inulin sensitivity of sows, possibly as a result of the contribution of specific bacteria (Tan et al., 2016; Wu et al., 2023). Moreover, a fecal microbial transplantation study conducted on the sow model also confirmed that gut microbiota and insulin sensitivity mediate the increase in feed intake when lactating sows were fed with a fiber diet (Li et al., 2023b). Previous studies have also demonstrated that maternal inulin has long-term effects on the health of offspring piglets, although growth performance during the nursery and finishing periods was not assessed in this study. From late pregnancy through lactation, maternal inulin supplementation increased the expression of genes related to intestinal barrier function and triggered a pro-inflammatory response in the ileum, while promoting an anti-inflammatory response in the colon of piglets at 5 weeks

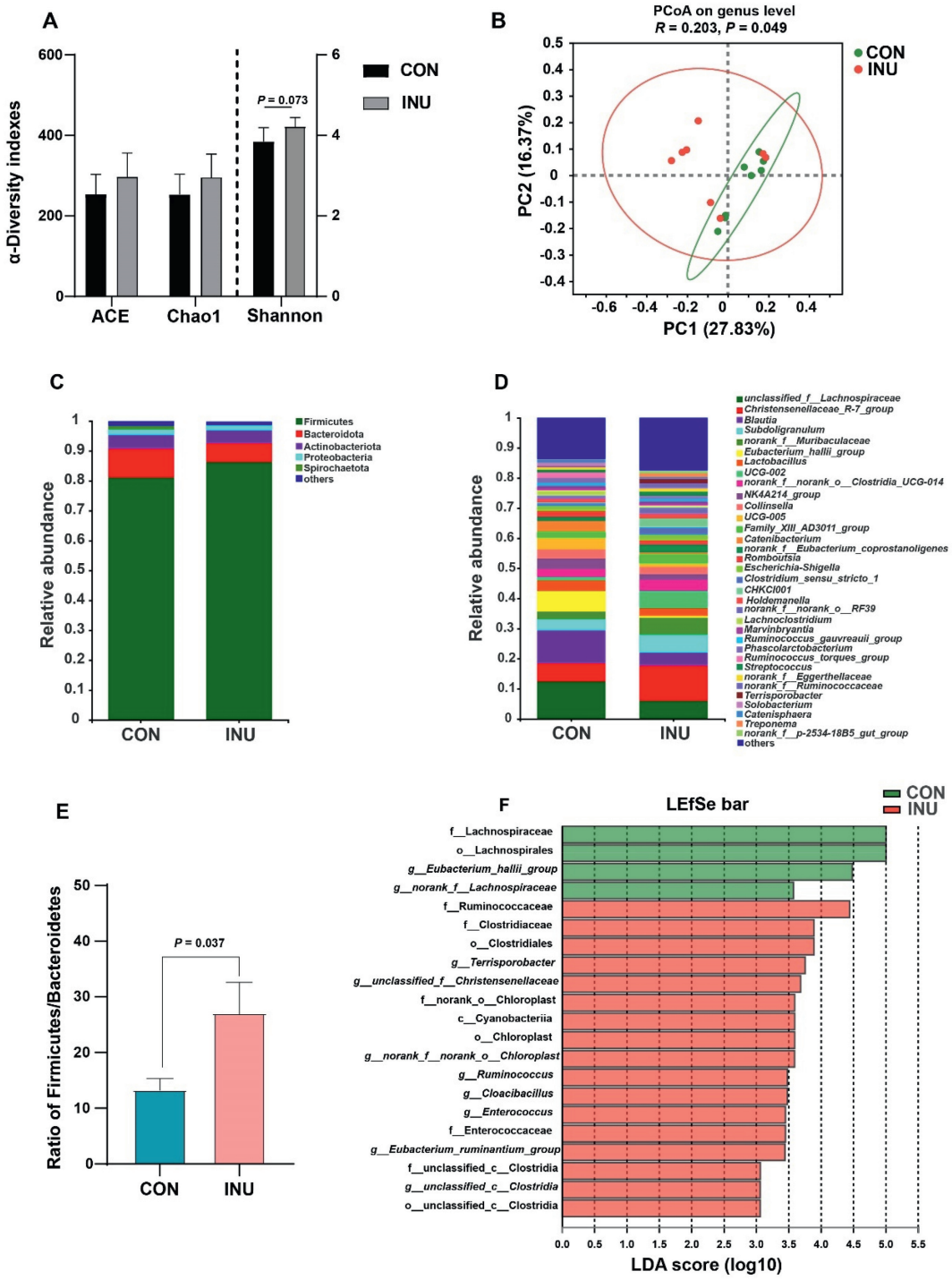

Fig. 2. Maternal inulin intake regulates the microbial structure of piglets. (A) The comparison of fecal microbial α-diversities between the CON and INU groups. (B) Principal-component analysis (PCoA) by unweighted-UniFrac distance. (C) Relative abundances of the bacterial phyla in piglets of the CON and INU groups. (D) Relative abundances of the bacterial genus in piglets of the CON and INU groups. (E) The ratio of Firmicutes to Bacteroidetes in piglets of the CON and INU groups. (F) LEfSe analysis of significantly different bacterial taxonomic units at class, order, family, and genus level, as indicated by the linear discriminant analysis (LDA) effect size. The differences between the CON and INU groups in α-diversity and ratio of Firmicutes/analyzed by a two-tailed student *t*-test and data are expressed as means ± SEM. CON = control; INU = diet with 2% wheat bran replaced by inulin. The piglet represents the experimental unit for, $n = 8$. PC = principal-component.

post-weaning (Sureda et al., 2023). Additionally, Zhou (2018) found that inulin supplementation during gestation significantly increased the liver organ index of offspring at the weaning and fattening stages, while reducing liver lipid deposition in both nursing and fattening piglets. These effects may be attributable to the long-term influence of maternal diet on the offspring's gut microbiome (Fernandes and Lim, 2024).

Primary BAs are metabolized by resident microbes in the gut to form secondary BAs, which in turn regulate the metabolism and immune responses of the host (Wahlstrom et al., 2016). In our

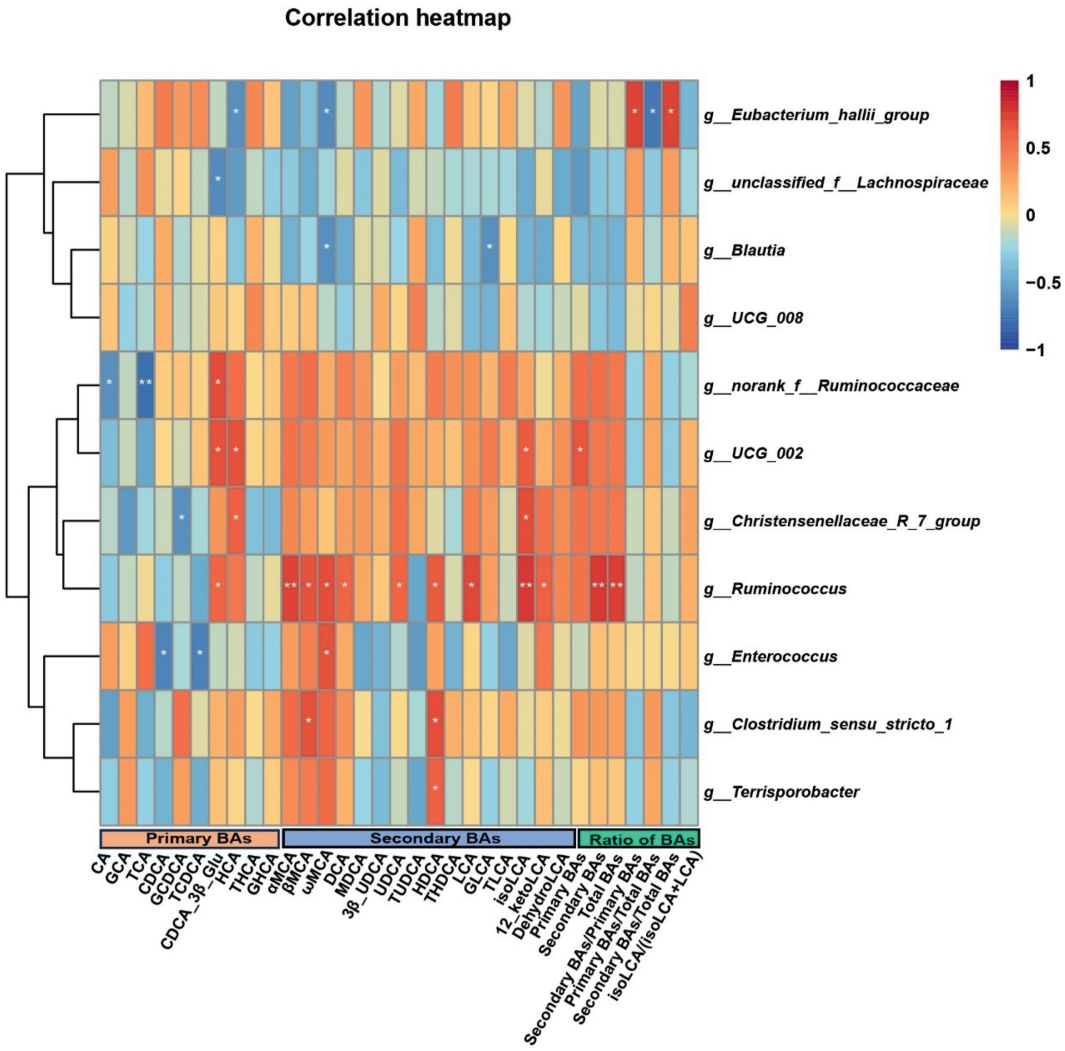

Fig. 3. The correlation analysis between bile acids (BAs) and microbial genera was conducted by a Spearman correlation. Red indicates a positive correlation; Blue indicates a negative correlation. The piglet represents the experimental unit. These data analyses were performed based on all piglets from CON ($n = 8$) and INU ($n = 8$). CON = control; INU = diet with 2% wheat bran replaced by inulin. *, $0.01 < P < 0.05$; **, $P < 0.01$. CA = cholic acid; GCA = glycocholic acid; TCA = taurocholic acid; CDCA = chenodeoxycholic acid; GCDCA = glycochenodeoxycholic acid; TCDCA = taurochenodeoxycholic acid; CDCA_3β_glu = chenodeoxycholic acid_3β_glu; HCA = hyocholic acid; THCA = taurohyodeoxycholic acid; GHCA = glycohyocholic acid; αMCA = α-muricholic acid; βMCA = β-muricholic acid; ωMCA = ω-muricholic acid; DCA = deoxycholic acid; MDCA = murideoxycholic acid; 3β_UDCA = 3β_ursodeoxycholic acid; UDCA = ursodeoxycholic acid; TUDCA = tauroursodeoxycholic acid; HDCA = hyodeoxycholic acid; THDCA = taurohyodeoxycholic acid; LCA = lithocholic acid; GLCA = glycholithocholic acid; TLCA = taurolithocholic acid; isoLCA = isolithocholic acid; 12_KetoLCA = 12_keto lithocholic acid; DehydroLCA = dehydrolithocholic acid.

study, higher levels of DCA, LCA, and HDCA were found in the INU piglets. Moreover, DCA, LCA, and isoLCA showed strongly negative correlations with serum inflammation cytokines. DCA and LCA can increase intestinal epithelial integrity, reduce inflammatory reactions (Guo et al., 2023; van der Lugt et al., 2022), and inhibit IL-1β-induced IL-8 secretion in the intestine (Duboc et al., 2013). An increase in primary BAs and a corresponding decrease in secondary BAs, including DCA and LCA, were found in the patients with intestinal inflammation (Franzosa et al., 2019). Moreover, an enriched isoLCA by maternal insulin intake might help to prevent intestinal inflammatory disorder in this study by inhibiting TH17 cell differentiation and increasing Treg cell differentiation (Hang et al., 2019a; Paik et al., 2022).

Secondary BAs have been pointed out to possess antioxidant abilities (Diaz et al., 2017). We demonstrated that UDCA, LCA, and HDCA relieved intestinal oxidative stress in piglets in this study. Although LCAs may promote oxidative stress in liver and breast cancer studies (Kovacs et al., 2019; Ma et al., 2022), it has been proven to protect against intestinal oxidative injury by improving intestinal calcium absorption under oxidant conditions (Diaz et al., 2017; Marchionatti et al., 2017, 2018). From the perspective of mechanism, BAs receptors (*TGR5* and *FXR*) may be associated with the activation of the antioxidant system. In the presence of *TGR5* shRNA, the expressions of *Nrf2* and *HO-1* decreased, suggesting that *TGR5* exerted antioxidant effects through the Nrf2/HO-1 pathway (Deng et al., 2019). Moreover, FXR/Nrf2 signaling was also involved in the amelioration of metabolic disorders, oxidative stress, inflammation, fibrosis, and myocardial dysfunction (Ma et al., 2022; Wu et al., 2019). Notably, LCA, HDCA, and UDCA increased the *FXR* and *TGR5* expression, accompanied by the higher expressions of *Keap1* and *HO-1* in our study, which suggested that secondary BAs might alleviate intestinal injury under an oxidative condition through FXR/TGR5-Keap1/Nrf2 pathway.

Maternal dietary intervention can effectively modulate the intestinal microbiome of offspring and thereby microbial BAs metabolism (Huang et al., 2023a). The microbial transfer from the sows

Table 8
Correlations between secondary BAs and serum levels of oxidative parameters, and inflammatory cytokines during weaning[1].

Secondary BAs	Correlation index					
	T-SOD	ROS	CAT	IL-6	TNF-α	IL-10
DCA	0.55	−0.45	0.34	−0.63*	−0.57*	0.42
LCA	0.43	−0.74**	0.56*	−0.79**	−0.57*	0.64*
HDCA	0.67*	−0.76**	0.67*	−0.45	−0.28	0.21
UDCA	0.56*	−0.42	0.58*	−0.23	−0.65*	0.34
IsoLCA	0.46	−0.38	0.53	−0.85**	−0.78**	0.79**

BAs = bile acids; DCA = chenodeoxycholic acid; UDCA = ursodeoxycholic acid; HDCA = hyodeoxycholic acid; LCA = lithocholic acid; IsoLCA = isolithocholic acid; ROS = reactive oxygen species; CAT = catalase; T-SOD = total superoxide dismutase; IL-6 = interleukin-6; IL-10 = interleukin-10; TNF-α = tumor necrosis factor-α.
[1] The data were evaluated by Spearman correlation analysis of the Euclidean distance. The piglet represents the experimental unit. These data analyses were performed based on all the piglets from CON ($n = 8$) and INU ($n = 8$). CON = control; INU = diet with 2% wheat bran replaced by inulin.
* Means significant correlation ($P < 0.05$).
** Means strongly significant correlation ($P < 0.01$).

to the piglets already occurs during pregnancy (Leblois et al., 2017), which may explain why the maternal condition influences piglet intestinal microbiota during the perinatal period. Moreover, early mother-child contacts (feces, skin, vagina, saliva, breast milk) strengthen the influence of maternal microbiota on the construction of offspring intestinal microorganisms (Liu et al., 2019; Tian et al., 2023). Therefore, maternal diet during pregnancy and lactation likely shifts the microbial transmission to newborns and primes the neonatal microbial profile with potential outcomes (Selma-Royo et al., 2021). In this study, maternal inulin intake increased the relative abundance of the genus *Ruminococcus* and the family Ruminococcaceae in offspring piglets during weaning. Similarly, Shang et al. revealed that maternal fiber supplements during late gestation and lactation increased the richness of Lachnospiraceae and Ruminococcaceae in weanling piglets (Shang et al., 2019). Ruminococcaceae presented at a significantly lower amount in stool from UC patients, which can explain why there were low inflammation markers in the serum of INU piglets (Sinha et al., 2020). The deconjugation abilities of the intestinal microbiota can be reflected by the ratio of Tauro-BAs/BAs or Glyco-BAs/BA (Li et al., 2021a). Notably, maternal inulin intake increased the ratio of TCDCA/CDCA and THDCA/HDCA of piglets, which was also in line with the bacterial *Bsh* gene expression in our study. According to the correlation analysis, the family Ruminococcaceae and the genus *Ruminococcus* were identified as significant contributors to the production of secondary BAs from primary BAs and the transformation from LCA to isoLCA in our study. Family Ruminococcaceae is one of the few taxa comprising 7α-dehydroxylation bacteria, which generates DCA and LCA by promoting the 7α-dehydroxylation of CA and CDCA, respectively (Sinha et al., 2020). Moreover, *Rumimococcus* is a BAs derivates-producing bacteria in the gut as it produces bacterial *3α-/3β-HSDH* (Cai et al., 2022; Sato et al., 2021), which are the key enzymes in the transformation of LCA to 3-oxoLCA and isoLCA (Paik et al., 2022). Indeed, our study found higher levels of *3α-HSDH* and isoLCA in INU piglets and a strong positive correlation between *Rumimococcus* and the isoLCA. As we discussed earlier, isoLCA was able to suppress TH17 cell differentiation and increase Treg cell differentiation, thereby decreasing inflammatory disorders and intestinal inflammation (Hang et al., 2019a; Paik et al., 2022). In line with our findings, recent studies

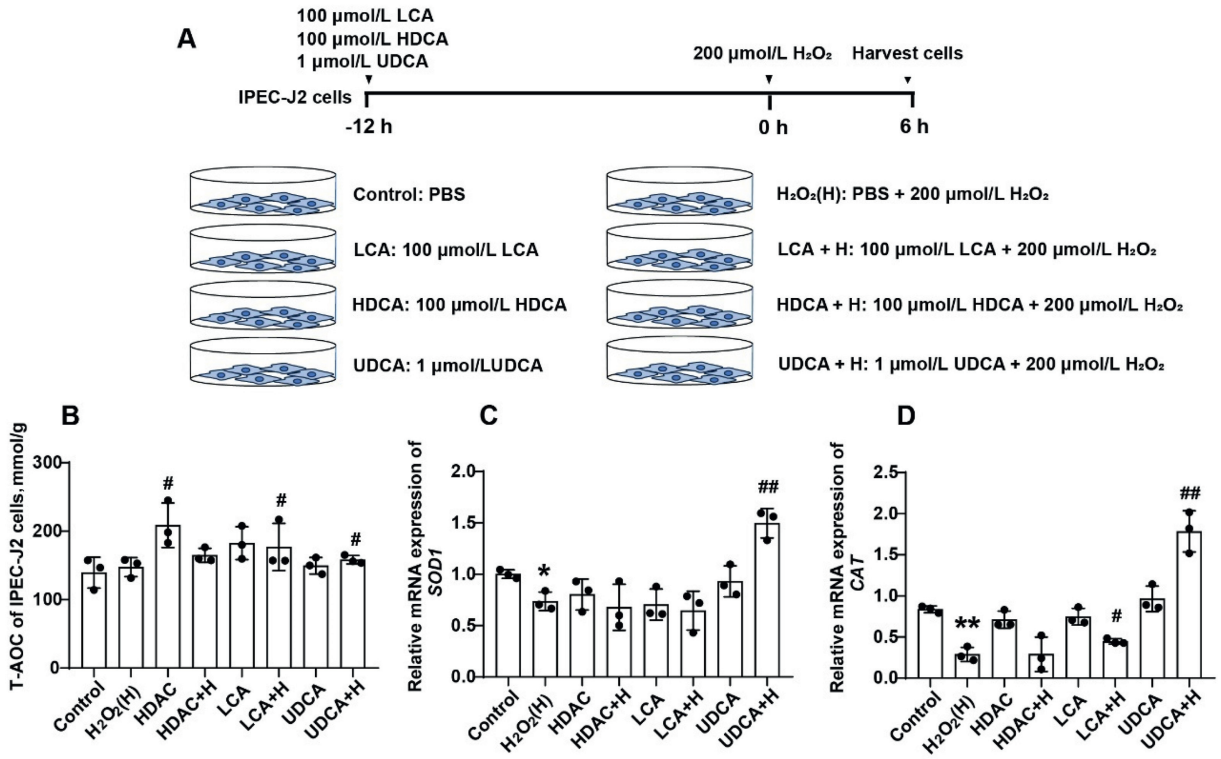

Fig. 4. The effects of LCA, UDCA, and HDCA on the antioxidant abilities of the IPEC-J2. (A) Schematic illustration. After incubation with 100 μmol/L LCA, 100 μmol/L HDCA, 1 μmol/L UDCA, and PBS for 24 h, respectively, IPEC-J2 cells then were stimulated with 200 μmol/L H_2O_2 for 6 h. (B) The concentration of T-AOC in IPEC-J2 cells. (C and D) The relative mRNA expression of *SOD1* (C) and *CAT* (D) of IPEC-J2 cells. Data are analyzed by one-way ANOVA with Dunnett's test and represented as mean ± SEM. The well in the plate represents the experimental unit, $n = 3$. *$0.01 < P < 0.05$, **$P < 0.01$, compared with control group; #$0.01 < P < 0.05$, ##$P < 0.01$, compared with H_2O_2 (H) group. H_2O_2 = hydrogen peroxide; IPEC-J2 = intestinal porcine epithelial cell-jejunum 2 cell line; PBS = phosphate buffered saline; LCA = lithocholic acid; UDCA = ursodeoxycholic acid; HDCA = hyodeoxycholic acid. T-AOC = total antioxidant ability; *SOD1* = superoxide dismutase-1; *CAT* = catalase.

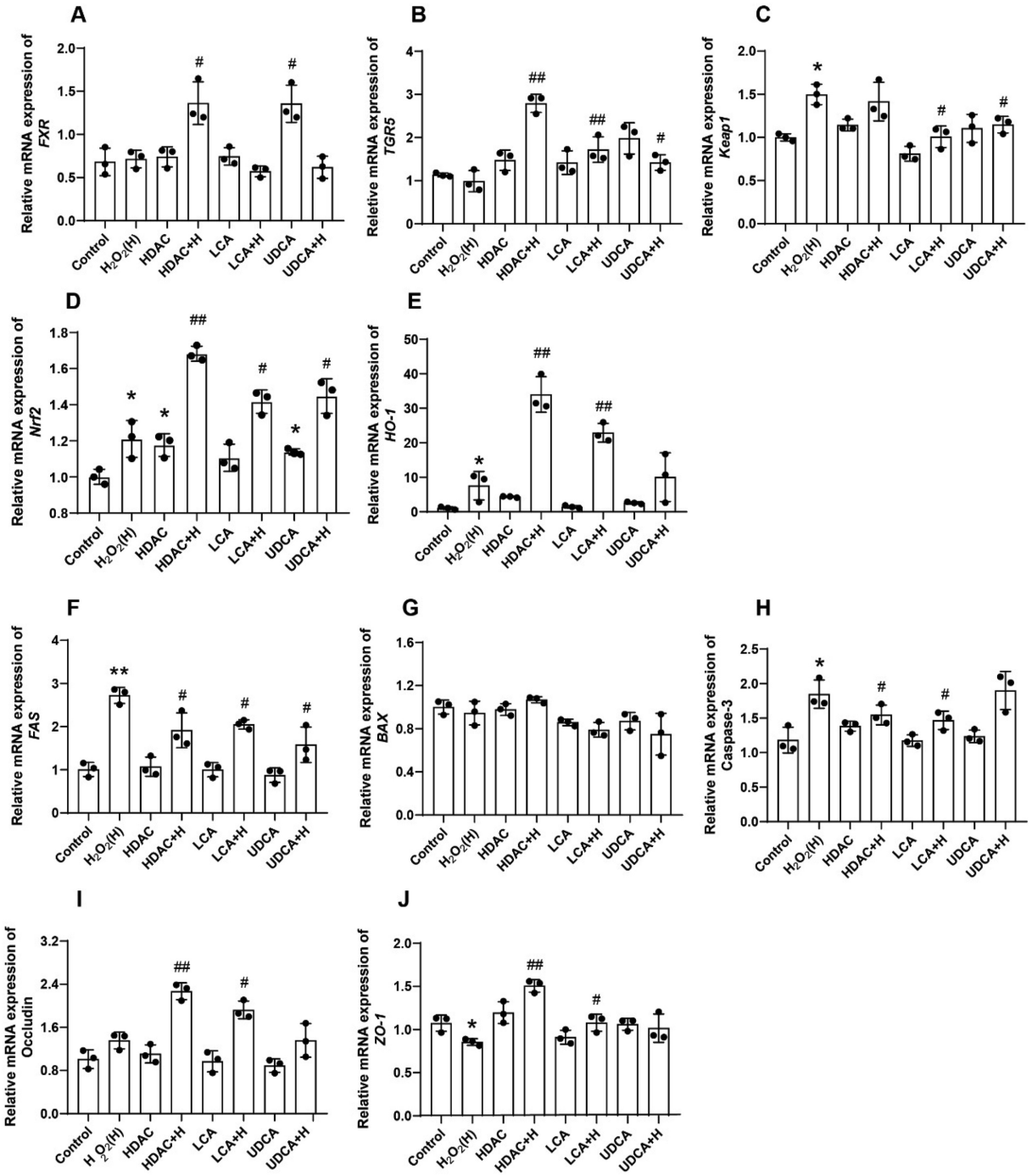

Fig. 5. The effects of LCA, UDCA, and HDCA on the antioxidant activities, cell apoptosis, and tight junctions of IPEC-J2 cells. The relative mRNA expression of *FXR* (A), *TGR5* (B), *Keap1* (C), *Nrf2* (D), *HO-1* (E), *FAS* (F), *BAX* (G), caspase-3 (H), occludin (I), and *ZO-1* (J) in IPEC-J2 cells. Data are analyzed by one-way ANOVA with Dunnett's test and represented as mean ± SEM. The well in the plate represents the experimental unit, $n = 3$. Group control means cells treated with PBS; group H_2O_2(H) means cells treated with PBS and H_2O_2; Group HDCA means cells treated with HDCA; group HDCA + H means cells treated with HDCA and H_2O_2; group LCA means cells treated with LCA; group LCA + H means cells treated with LCA and H_2O_2; group UDCA means cells treated with UDCA; group UDCA + H means cells treated with UDCA and H_2O_2. *$0.01 < P < 0.05$, **$P < 0.01$, compared with control group; #$0.01 < P < 0.05$, ##$P < 0.01$, compared with H_2O_2(H) group. PEC-J2 = intestinal porcine epithelial cell-jejunum 2 cell line; PBS = phosphate buffered saline; H_2O_2 = hydrogen peroxide; LCA = lithocholic acid; UDCA = ursodeoxycholic acid; HDCA = hyodeoxycholic acid; *FXR* = farnesoid X receptor; *TGR5* = takeda G protein-coupled receptor 5; *FAS* = fas cell surface death receptor; *Keap1* = Kelch-like ECH associated protein 1; *Nrf2* = nuclear factor E2-related factor 2; *BAX* = B cell leukemia/lymphoma 2-associated X; *ZO-1* = zonula occludens-1; *HMOX1* = heme oxygenase 1.

have also demonstrated that maternal prebiotic supplementation (oligofructose and inulin) can influence offspring health by regulating BAs metabolism in mice models. For instance, a maternal oligofructose diet improved insulin resistance in offspring by enriching bile acid-modifying bacteria, *Clostridium cluster XI* (Klancic et al., 2020). Additionally, maternal inulin supplementation has been shown to alleviate hepatotoxic effects caused by prenatal methamphetamine exposure, restoring the antioxidant system and reducing oxidative stress by normalizing cecal microbiota and BAs metabolism (Li et al., 2024).

The activities of FXR and TGR5 may contribute to the BAs-induced oxidative reductions. FXR is a common receptor in tissues, and unconjugated BAs act as high-affinity ligand agonists of FXR (Campbell et al., 2020). Previous studies suggested that the rank order in the activation of FXR by BAs was CDCA > DCA > LCA > CA (Jia et al., 2018). Notably, we demonstrated that the order of secondary BA to activate FXR in the intestinal oxidative stress model was UDCA = HDCA > LCA. *TGR5*, expressed throughout the gastrointestinal tract, is involved in regulating gut homeostasis functions, including gut hormone secretion, gut motility, gut barrier, and immunity (Sorrentino et al., 2020). Moreover, TGR5 has the potential to activate the antioxidant response by targeting the Nrf2/HO-1 (Deng et al., 2019). Previous studies suggested that the activation order for TGR is LCA > DCA > CDCA > CA (Chen et al., 2011; Rezen et al., 2022). In this study, however, we found that the order for TGR5 activation under an oxidative stress condition was HDCA > LCA > UDCA. These results suggested that the BAs receptor activation can be altered under different conditions.

5. Conclusions

In summary, this study provides new insights into how the maternal diet modulates early piglet health. Specifically, the maternal inulin diet promoted sow feed intake and milk production, thereby enhancing the growth of piglets during suckling. Additionally, the maternal inulin diet increased the relative abundance of *Ruminococcus* and the production of secondary BAs in weanling piglets. These secondary BAs alleviated intestinal oxidative stress by regulating the TGR5/FXR-Keap1/Nrf2 pathway.

CRediT authorship contribution statement

Dongdong Lu: Writing – original draft, Validation, Formal analysis, Conceptualization. **Cuiping Feng:** Writing – review & editing, Funding acquisition, Conceptualization. **Yu Pi:** Software, Methodology. **Hao Ye:** Software, Methodology. **Yujun Wu:** Software, Methodology. **Bingxu Huang:** Writing – review & editing. **Jinbiao Zhao:** Writing – review & editing. **Dandan Han:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Nicoline Soede:** Writing – review & editing. **Junjun Wang:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Data availability

Data will be available on request.

Declaration of competing interest

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

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Appendix supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aninu.2024.11.016.

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