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Original Research Article

Maternal resveratrol improves the intestinal health and weight gain of suckling piglets during high summer temperatures: The involvement of exosome-derived microRNAs and immunoglobin in colostrum

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ABSTRACT

Previous studies have shown that maternal resveratrol improved growth performance and altered the microbial composition of suckling piglets under hot summer conditions. However, it remains unclear how maternal resveratrol improves growth performance of suckling piglets during high summer temperatures. A total of 20 sows (Landrace × Large White; three parity) were randomly assigned to 2 groups (with or without 300 mg/kg resveratrol) from d 75 of gestation to d 21 of lactation during high ambient temperatures (from 27 to 30 °C). The results showed that maternal resveratrol supplementation increased total daily weight gain of piglets under hot summer conditions, which is consistent with previous studies. Furthermore, we found that maternal resveratrol improved the intestinal morphology and intestinal epithelial proliferation in suckling piglets. Dietary resveratrol supplementation affected the characteristics of exosome-derived microRNAs (miRNAs) in sow colostrum, as well as the genes targeted by differentially produced miRNAs. MiRNAs are concentrated in the tight junction pathway. As a result, the expression of intestinal tight junction proteins was increased in suckling piglets ($P < 0.05$). Notably, maternal resveratrol increased the intestinal secretory immunoglobulin A (sIgA) levels of suckling piglets via colostrum immunoglobin ($P < 0.05$), which could increase the abundance of beneficial microbiota to further increase the concentration of short chain fatty acids (SCFA) in suckling piglets' intestine ($P < 0.05$). Finally, our correlation analysis further demonstrated the positive associations between significantly differential intestinal microbiota, intestinal sIgA production and SCFA concentrations, as well as the positive relation between total daily weight gain and intestinal health of suckling piglets. Taken together, our findings suggested that maternal resveratrol could promote intestinal health to improve piglet growth during high summer temperatures, which might be associated with the immunoglobin and exosome-derived miRNAs in sows' colostrum.

1. Introduction

In recent years, research has suggested that resveratrol may serve as an excellent antioxidant, anti-inflammatory, and metabolic-regulating dietary supplement for sows (Meng et al., 2023). Previous studies have shown that dietary supplementation of sows with resveratrol during gestation and lactation can improve the average daily gain, litter weight, and weaning weight of the

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piglets (Meng et al., 2018, 2019). Similarly, we have previously found that supplementation of resveratrol during late gestation and lactation in sows increased the weight gain of the weaned litter of piglets during hot summer temperatures (Zhao et al., 2022). Furthermore, maternal resveratrol may improve growth performance by regulating the gut health of suckling pigs, as the gastrointestinal tract is critical for nutrient absorption and piglet development (Guilloteau et al., 2010; Yin et al., 2017). Meng et al. (2019) found that maternal resveratrol supplementation improved the intestinal health of suckling pigs during gestation and lactation by altering intestinal flora and reducing intestinal inflammation. In our previous study, we also demonstrated that maternal resveratrol supplementation can enhance the relative abundance of beneficial microbiota, such as *Lactobacillus*, particularly at high summer temperatures (Zhao et al., 2022). However, the mechanism by which maternal resveratrol regulates intestinal health to improve suckling piglet weight gain remains unknown.

Piglets have limited postnatal immunity and require the consumption of sow colostrum, which is rich in immunoglobulins and plays a critical role in promoting the maturation of the piglet immune system (Xiong et al., 2019). Nutritional interventions during the late stages of gestation and lactation in sows facilitate the establishment of the piglet immune system by increasing the levels of immunoglobulins present in colostrum (Li et al., 2021). Furthermore, exosomes are also abundant in mammalian milk, and milk-derived microRNAs (miRNAs) may influence the development of the immune system and gut health in offspring (Melnik et al., 2021; Miura et al., 2022; Sundaram et al., 2022). Previous studies have demonstrated the presence and high concentration of immune-related miRNAs in porcine milk exosomes, particularly in colostrum (Gu et al., 2012; Xie et al., 2019, 2020). Furthermore, protection of intestinal epithelial cells against infection with porcine epidemic diarrhea virus has been demonstrated by miRNAs derived from milk exosomes (Liang et al., 2023). These results suggest that colostrum-derived immunoglobulin and exosome-derived miRNAs may be beneficial for the intestinal health and growth performance of piglets during summer temperatures. However, to our knowledge, there are no data on the effect of maternal dietary resveratrol on the intestinal health and growth performance of suckling piglets during high summer temperatures by regulating colostrum immunoglobulin and exosome-derived miRNAs.

Therefore, we hypothesize that resveratrol supplementation during gestation and lactation may alter the intestinal health and weight gain of suckling piglet, as well as their association with colostrum immunoglobulin and exosome-derived miRNAs in sows exposed to high summer temperatures. Moreover, this study provided a better understanding of the effects of resveratrol supplementation on sows during gestation and lactation under hot summer conditions.

2. Materials and methods

2.1. Animal ethics statement

All animal experimental protocols used in this study were according to the guidelines for animal welfare and approved by the Animal Care and Use Committee of Guangdong Academy of Agricultural Sciences (GAASIAS-2020-010), China.

2.2. Animals and experimental design

According to our previous study (Zhao et al., 2022), 20 multiparous sows (Landrace × Large White; three parity) were assigned to two dietary treatments: a corn-soybean meal control diet, or a control diet with 300 mg/kg resveratrol (99% purity, Shanghai Aladdin Biochemical Technology Co. Ltd, China). The sows ($n = 10$) were fed the diets from d 75 of gestation to d 21 of lactation under summer conditions (ambient temperature from 27 to 30 °C). The body weight of each piglet was recorded at farrowing and weaning periods and the total daily gain of piglets during the lactation was calculated. The basal diet was formulated to meet the nutrient requirements of swine according to NRC (2012). The ingredients and nutritional levels of basal diet are shown in Table 1.

2.3. Feed chemical analyses

The chemical analyses of the basal diet at gestation phase and lactation phase were performed. The crude protein was calculated by multiplying nitrogen by the factor 6.25. The nitrogen was measured according to the method 984.13 of AOAC (2007). The ash was analyzed according to the method 942.05 of AOAC (2007). Neutral detergent fiber was determined using the procedure described by Van Soest et al. (1991). The gross energy content of the basal diet was analyzed by using an isoperibol calorimeter (Parr 6300 Calorimeter, Moline, IL, USA) with benzoic acid as a standard.

2.4. Sample collection

After oxytocin injection, the colostrum was collected from all mammary glands within 2 h of farrowing and then frozen at −80 °C for further analysis. The blood sample from the sows was collected from the ear vein on d 14 of lactation and centrifuged at 3,000 × *g*

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

Item	Diet during gestation	Diet during lactation
Ingredients		
Corn	75.85	67.85
Soybean meal	8.50	23.00
Alfalfa powder	12.50	–
Fishmeal	–	3.00
Soybean oil	–	2.00
Lysine hydrochloride	–	0.15
$CaHPO_4$	0.80	1.10
Limestone	0.70	1.20
NaCl	0.40	0.45
Choline chloride	0.25	0.15
Premix[1]	1.00	1.00
Total	100.00	100.00
Calculated nutrient levels		
DE, MJ/kg	3.15	3.48
NE, MJ/kg	2.33	2.53
CP	11.17	17.60
NDF	12.82	8.44
SID Lys	0.38	0.93
SID Met + Cys	0.35	0.52
SID Thr	0.33	0.54
SID Trp	0.10	0.19
Total Ca	0.62	0.90
Total P	0.43	0.63
STTD	0.26	0.41
Analyzed nutrient levels		
GE, kcal/kg	3321.45	3756.29
Ash	6.23	6.79
CP	13.23	16.99
NDF	13.04	12.00

DE = digestible energy; NE = net energy; CP = crude protein; NDF = neutral detergent fiber; SID = standardized ileal digestibility; STTD = standard total intestinal digestibility; GE = gross energy.

[1] The premix provided the following per kilogram of the diet: vitamin A, 10,000 IU; vitamin D, 1,400 IU; vitamin E, 40 mg; vitamin K, 3.0 mg; vitamin B, 10.50 mg; vitamin B_{12}, 0.04 mg; nicotinic acid, 45 mg; pantothenic acid, 20 mg; folic acid, 1.2 mg; biotin, 0.20 mg; choline chloride, 550 mg; Cu, 80 mg; Fe, 100 mg; Zn, 100 mg; Mn, 50 mg; I, 0.3 mg; and Se, 0.25 mg.

for 15 min to obtain the serum. The serum was stored at −20 °C until analyse for the detection of resveratrol and its derivatives. On d 21 of lactation, piglets from two groups ($n = 10$) were slaughtered and intestinal tissues (jejunum and ileum) and colonic contents were collected. Middle segments of the jejunal samples and posterior segments of the ileal samples (each 3 to 5 cm) were collected. Each segment of the intestinal samples was divided into two parts equally: one part was fixed with 4% paraformaldehyde for hematoxylin and eosin (H&E) staining and immunohistochemistry (IHC) staining, and the other part was stored at −80 °C for further analysis of immune and barrier function-related indices.

2.5. Determination of the concentrations of resveratrol and its derivates in serum and colostrum of sows

The concentrations of resveratrol and its derivates (oxyresveratrol and 3,4′,5-trimethoxy-trans-stilbene) in serum and colostrum from sows were determined and analyzed using UPLC–Orbitrap–MS/MS combined with chemometrics, referring to previous methods (Xin et al., 2018).

2.6. Morphological examination and immunohistochemistry

Following dehydration, paraffin embedding, sectioning, dewaxing, H&E staining, dehydration, and sealing, the intestine pieces were obtained (Xiong et al., 2022). For morphological examination, the images were captured using a microscope (Eclipse E100, Nikon, Tokyo, Japan with a Nikon DS-U3 imaging system). The intestinal villus height (VH) and crypt depth (CD) were assessed using Case Viewer software (3DHISTRCH Ltd., Budapest, Hungary) and the VH:CD ratio (VCR) was calculated. Five fields were chosen at random for observation and measurement in each slice. In addition, the antibody proliferating cell nuclear antigen (PCNA) (ab29, Abacm) and polymeric immunoglobulin receptor (P-IgR) (ab275020, Abacm) were used for IHC and then the images were captured as aforementioned. Positive results were shown in tan or yellow, and the color of the cell nuclei was in purple-blue. Three photographs were selected randomly for each section and the areas of positive products were measured using Image J software (National Institutes of Health, Bethesda, MD, USA).

2.7. Determination of short chain fatty acids (SCFA) concentrations

Colonic content samples from piglets were tested for SCFA (acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid and valeric acid) concentrations by gas chromatograph with a mass spectrometer detector (7890A and 5975C inert XL EI/CI mass spectrometric detector, Agilent Technologies, Santa Clara, CA, USA) described in the previous study (Liu et al., 2022b). Briefly, 1 g of colonic content was taken and mixed with 3 mL of ultrapure water, which was centrifuged at 2,500 × *g* for 30 min after vortex oscillation for 1 min. Subsequently, 1 mL of supernatant was transferred in a centrifuge tube and reconstituted by adding 200 μL of 42 mmol/L crotonic acid and 200 μL of 10% metaphosphoric acid solution, which was then mixed for 30 s thoroughly. The supernatant was obtained by centrifugation at 10,000 × *g* for 10 min at 4 °C and then mixed with ether in equal proportions and left to extract for 5 min. The ether layer was aspirated and injected into a brown injection bottle and stored at −20 °C for further analysis.

2.8. Evaluation of immune indices

The concentrations of secretory immunoglobulin A (sIgA) in intestinal tissue samples were detected using commercial enzyme-linked immunosorbent assay kits (Cusabio, Wuhan, China). The protein concentrations of samples were measured by a BCA protein assay kit (CWBIO, China) for the calculation of sIgA concentrations per milligram of protein.

2.9. Quantitative real-time PCR (qPCR)

The reagent box of tissue RNA purification kit (EZBioscience, China) was used to extract the total RNA from intestinal tissue samples in accordance with the manufacturer's protocol. The RNA concentration was detected using a NanoDrop-ND1000 spectrophotometer (Thermo Fisher Scientific, USA). After reverse transcription with 4× Color Reverse Transcription Kit (EZBioscience, China), qRT-PCR was performed on a QuantStudio 6 RealTime PCR System (Thermo Fisher, USA) with 2× Color SYBR Green (EZBioscience, China), which conditions were 95 °C for 10 min followed by 40 cycles of amplification (95 °C for 15 s and 60 °C for 1 min). The mRNA expression of the target genes relative to reference gene (β-actin) were computed by $2^{-\Delta\Delta CT}$ method. The primers used in this study were created by Sangon Biotech Co. Ltd (Shanghai, China). The sequences are shown in Table 2.

Table 2
Primer sequences used in this study.

Gene	Sequences (5′→3′)		GenBank accession
Beta-actin	Forward	CTCCATCATGAAGTGCGACG	XM_003124280.5
	Reverse	CCTGCTTGCTGATCCACATC	
P-IgR	Forward	CCAAGGACTACAAGGGCAGA	NM_214159.1
	Reverse	TGCTGTGTAGACGTGGACAT	
J-chain	Forward	ATCATCCGCTCTGCTGAAGA	XM_003356961.3
	Reverse	TCTGGGTGGCAGTAACGATT	
IL-4	Forward	CACAGCGAGAAAGAACTCGTG	NM_214123.1
	Reverse	ATGCACGTGTGGTGTCTGTA	
IL-5	Forward	GCTGAGCCAGACAAGACTCCT	XM_013995115.2
	Reverse	TGAAATCATCAAGTTCCCATCGC	
IL-6	Forward	AATCTGGGTTCAATCAGGAGACCT	NM_214399.1
	Reverse	GGTTAGGGGTGGTGGCTTTG	
IL-10	Forward	AGAGGGGTGTCTACAAAGCC	NM_214041.1
	Reverse	AGAGGTACAGCAGGGTTTCC	
TGFβ1	Forward	AATGCTGGAAAGCGGCAAC	NM_214015.2
	Reverse	GGAATCATTGCTGTATTTCTGGTA	
TGFβ2	Forward	AAGCCAGAGTGGCTGAACAA	XM_021064293.1
	Reverse	TCCCAGGTTCCTGTCTTTATGG	
TGFβ3	Forward	TACATCGACGGCAAGAACCT	NM_214198.1
	Reverse	CATCCTCACTGTCCACACCT	
TGFβR1	Forward	GGACTCAGCTTTGGTTGGTG	NM_001038639.1
	Reverse	TGCCAGTCCTAAGTCTGCAA	
TGFβR2	Forward	GGATGACTTGGCCAACAGTG	XM_021071493.1
	Reverse	TGCTTTCAACACAGGGATGC	
BAFF	Forward	GATGCGGAGGAAACAGTCAC	XM_005668532.3
	Reverse	AACCCGTTTCTTTGACCACG	
VAPC1	Forward	GGCTAACTTCTTCTGGCTGC	XM_013981510.2
	Reverse	TGAGGAGTTGATGGTGTCCC	
VAPC2	Forward	GGACGACGTTCTGTACTCCA	NM_001195117.1
	Reverse	CAGGAGGGTGTGCAGATACA	
iNOS	Forward	TCCAAGTCTTGCCTAGGAGC	XM_013981169.2
	Reverse	CTCATCTCCCGTCAGCTGAT	
RALDH	Forward	ATCTTTGGCCCTGTTCAGGA	XM_021094606.1
	Reverse	AACAGTCCCAGCTTGCATTG	
APRIL	Forward	TTCTGCATCTCGTTCCCACT	XM_021066031.1
	Reverse	TAGAGTCTCCTGCCTTCCCT	
Occludin	Forward	ATGGCTGCCTTCTGCTTCAT	XM_005672525.3
	Reverse	TCACTTTCCCGTTGGACGAG	
Claudin-1	Forward	GTGACAACATTGTGACGGCC	NM_001244539.1
	Reverse	TGGAAGGCGAAGGTTTTGGA	
ZO-1	Forward	TGAGGAAGAAGCACACGACC	XM_021098896.1
	Reverse	TCTGACCGCTGATCAGGAGA	

P-IgR = polymeric immunoglobulin receptor; *IL* = interleukin; *TGF-β* = transforming growth factor-β; *TGF-βR* = transforming growth factor-β receptor; *BAFF* = B cell-activating factor; *VAPC* = vasoactive intestinal peptide receptor; *iNOS* = inducible nitric oxide synthase; *RALDH* = retinal dehydrogenases; *APRIL* = a proliferation-inducing ligand; *ZO-1* = zonula occludens protein 1.

2.10. Western blot analysis

Frozen jejunal tissue samples were lysed thoroughly in RIPA buffer (Beyotime, China) containing 1% phenylmethanesulfonyl fluoride at 4 °C (0.1 g sample/mL RIPA buffer) and then centrifuged at 14,000 × *g* at 4 °C for 5 min, following the measuring of extracted protein samples' concentrations using a BCA protein assay kit (CWBIO, China). The extracted protein samples were then diluted and denatured, and then separated by SDS-PAGE, followed by transferring the protein to nitrocellulose membranes. The membranes were incubated with the primary antibodies against zonula occludens-1 (ZO-1) (HuaBio, China, cat. no. ER41204), occludin (HuaBio, China, cat. no. R1510-33), junctional adhesion molecule 1 (JAM-1) (HuaBio, China, cat. no. ET1610-90), claudin-1 (HuaBio, China, cat. no. RT1141) and the internal reference antibody β-actin (HuaBio, China, cat. no. R1102-1) at 4 °C overnight after blocking with 5% BSA (Beyotime, China). Following the incubation with the appropriate secondary antibodies, enhanced chemiluminescent solution (NCM Biotech, China) was added to the membranes to visualize the protein bands in a ChemiDoc XRS imaging system (Bio-Rad, Hercules, CA, USA). Image J software (National Institutes of Health, Bethesda, MD, USA) was used to measure the optical density of each band, which represented the abundance of each target protein. The abundance of each target protein relative to reference protein (β-actin) represented the relative expressions of each target protein in samples.

2.11. MicroRNA sequencing and bioinformatic analysis

The methods of colostrum exosome-derived miRNAs isolation, sequencing and bioinformatic analysis were according to a previous study (Liu et al., 2022a). Briefly, exosomes were isolated from colostrum by ultracentrifugation. Subsequently, the total exosome RNA isolation kit (Thermo Scientific) was used to extract the total RNA in accordance with the manufacturer's instructions, following the preparation of small RNA library in line with the protocol of Tru Seq Small RNA Sample Prep Kits (Illumina). Then, we used differentially expressed gene sequencing (DEGseq) (Wang et al., 2010) to calculate the expression of miRNAs according to microarray-plot (Yang et al., 2002), aiming to screen the differential miRNAs. The Q value was used to perform multiple hypothesis testing corrections for each gene with *P*-value. Differential miRNAs were considered significant with two or more matching differences and a Q-value of less than 0.001. Finally, Kyoto encyclopedia of genes and genomes (KEGG) (Kanehisa et al., 2008) was used to identify the pathway of significant enrichment in target genes with differential miRNAs compared among the whole genomic background. The formula is as follows:

$$P = 1 - \sum_{i=0}^{m-1} \frac{\binom{M}{i}\binom{N-M}{n-i}}{\binom{N}{n}},$$

where P = *P*-value; N = the number of genes with pathway annotations among all genes; n = the number of target genes with differential miRNAs in N; M = the number of genes annotated as a specific pathway in all genes; m = the number of target genes with differential small RNA annotated as a particular pathway. A pathway that Q value less than 0.05 was defined as the pathway significantly enriched in the differential expressed genes. Volcano map of differential miRNAs and KEGG enrichment bubble diagram were plotted by https://www.bioinformatics.com.cn, an online platform for data analysis and visualization.

2.12. Statistical analysis

The Student's *t*-test was used to analyze the difference between the two groups by using GraphPad Prism 8. The association among intestinal significantly differential microbiota, intestinal sIgA production and SCFA concentrations, and the relationship between total daily weight gain and indices of intestinal health in suckling piglets during high summer temperatures were analyzed by applying the Pearson procedure of GraphPad Prism 8. Data were displayed as means ± standard error of the mean (SEM). $P < 0.05$ represented a statistically significant difference, and $0.05 \leq P < 0.1$ represented a trend.

3. Results

3.1. Concentrations of resveratrol and its derivates in the serum and colostrum of sows in the resveratrol group

To determine the role of maternal resveratrol in sucking piglets, we explored the metabolism of resveratrol in sows. The concentrations of resveratrol and its derivates in the serum and colostrum of sows supplemented with resveratrol were detected. The results showed that resveratrol in the serum (74.9 ± 21.64 ng/mL) and colostrum (69.9 ± 12.07 ng/mL), but the derivates of resveratrol could not be detected (Table 3). These results indicate that resveratrol cannot be metabolized to its derivates in sows and the effect of maternal resveratrol on sucking piglets is mainly through resveratrol instead of its derivates.

3.2. Maternal supplementation with resveratrol improved the intestinal health and increased the weight gain of suckling piglets during high summer temperatures

Our previous study found that maternal resveratrol supplementation improved litter gain at weaning during high summer temperatures (Zhao et al., 2022). Similarly, we found that maternal supplementation with resveratrol significantly enhanced total daily gain of suckling piglets ($P < 0.05$) (Fig. 1A). Due to the importance of the gut in the regulation of piglet growth and development (Guilloteau et al., 2010; Yin et al., 2017), we next detected intestinal morphology and intestinal epithelial proliferation in suckling piglets. We examined the morphology of the jejunum (Fig. 1B) and ileum (Fig. 1C), and evaluated the VH and CD of the jejunum (Fig. 1D) and ileum (Fig. 1E). The results showed that maternal resveratrol supplementation significantly reduced CD and enhanced VCR in suckling piglets' jejunum ($P < 0.05$). Similarly, maternal resveratrol supplementation increased VH,

Table 3
Concentrations of resveratrol and its derivates in the serum and colostrum of sows in resveratrol group (ng/mL).

Item	Serum	Colostrum
Resveratrol	74.9 ± 21.64	69.9 ± 12.07
Oxyresveratrol	N.D	N.D
3,4′,5-Trimethoxy-trans-stilbene	N.D	N.D

N.D = non-detected.
The data are shown as means ± SEM.

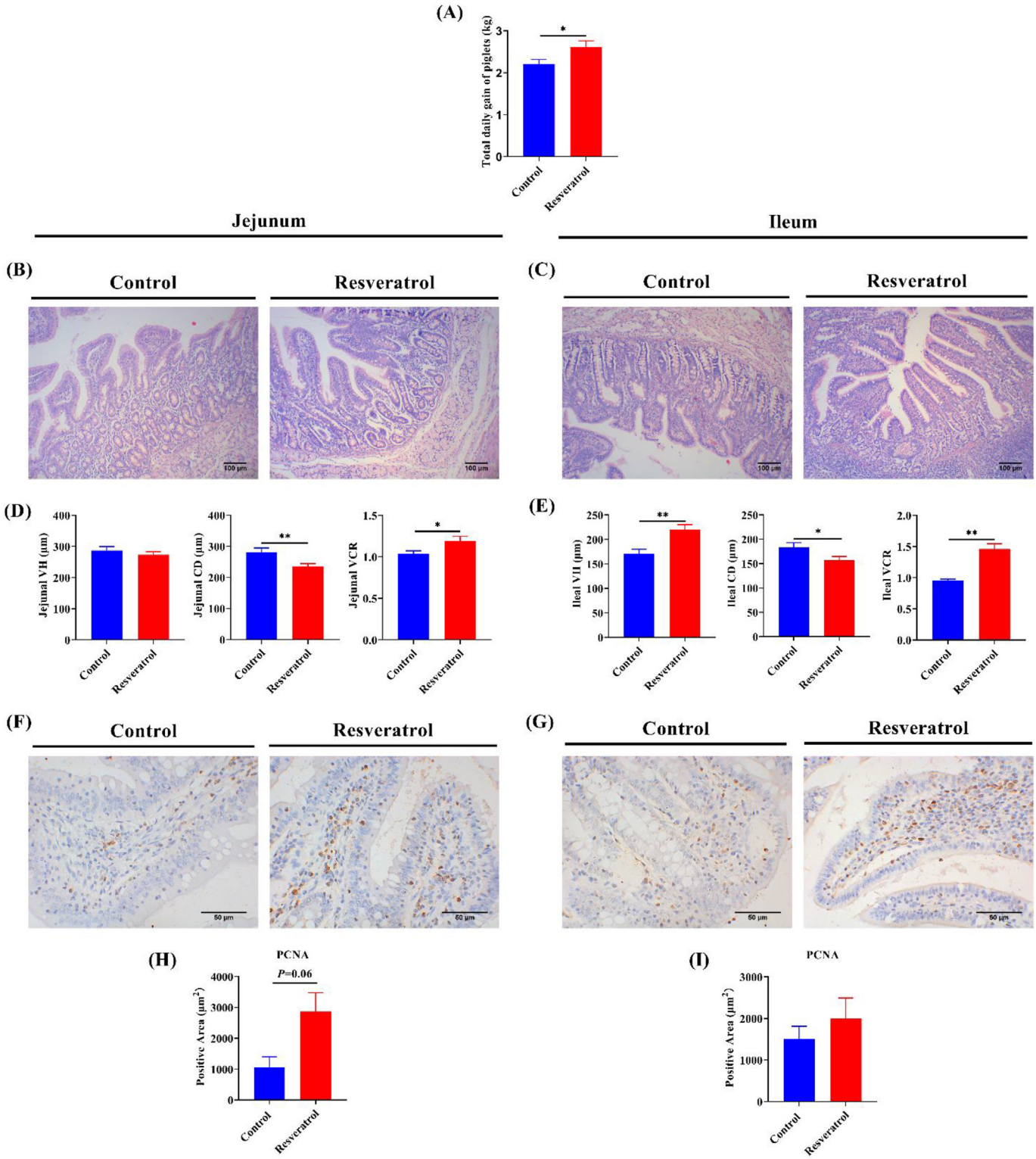

Fig. 1. Maternal resveratrol supplementation increases the weight gain and intestinal health of suckling piglets. (A) Total daily gain of piglets. (B and C) H&E staining of jejunum and ileum. (D and E) VH, CD, and VCR of jejunum and ileum. VH = villous height; CD = crypt depth; VCR = villous height to crypt depth ratio. (F and G) Proliferating cell nuclear antigen (PCNA) IHC staining of jejunum and ileum. IHC = immunohistochemistry. (H and I) The positive area of PCNA in the jejunum and ileum, respectively. All data were expressed as means ± SEM of at least three independent experiments. *$P < 0.05$, **$P < 0.01$.

decreased CD, and enhanced VCR in suckling piglets' ileum ($P < 0.05$). Moreover, maternal resveratrol supplementation relatively increased jejunal proliferation marker PCNA expression ($P = 0.06$) (Fig. 1F and H), showing that maternal resveratrol may boost intestinal epithelial growth in suckling piglets. In conclusion, these results suggest that maternal resveratrol supplementation could improve the intestinal health, and thereby increase suckling piglets' growth.

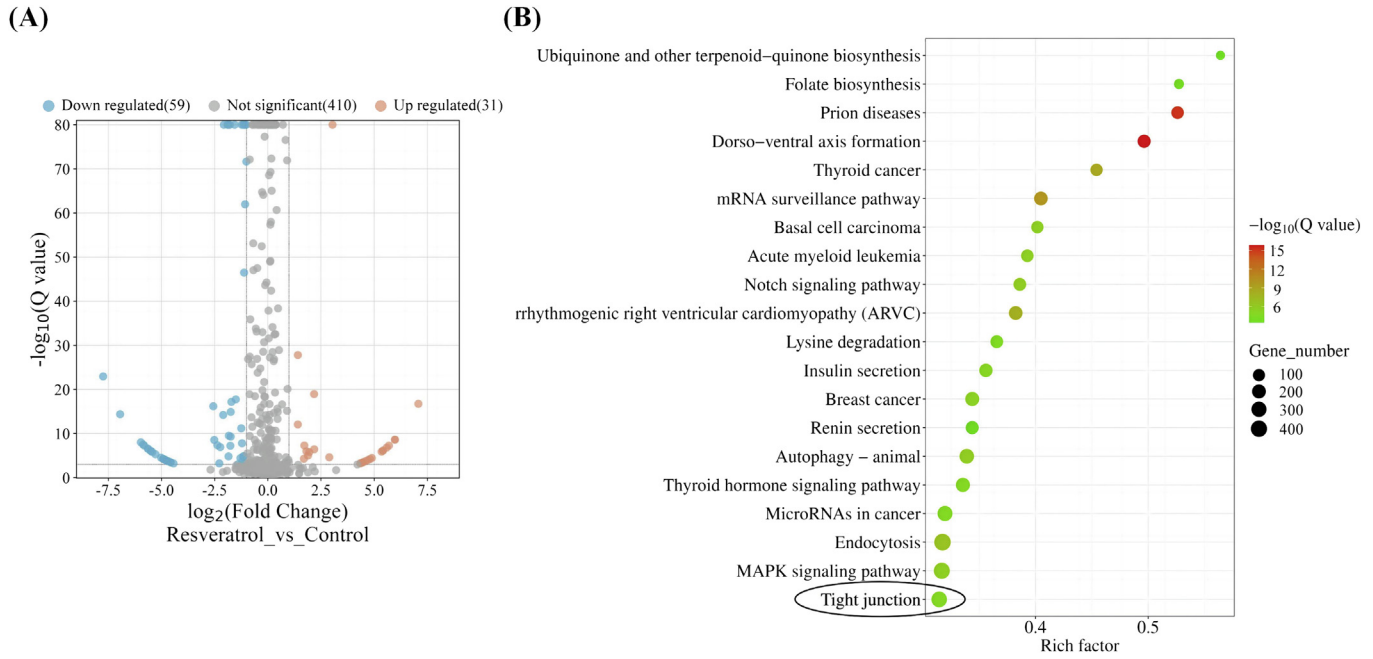

Fig. 2. Resveratrol supplementation affects the features of exosome-derived miRNAs in colostrum of sows. (A) Volcano map of differential miRNAs. (B) KEGG enrichment bubble diagram of differential miRNAs target genes. miRNAs = microRNAs; KEGG = Kyoto encyclopedia of genes and genomes.

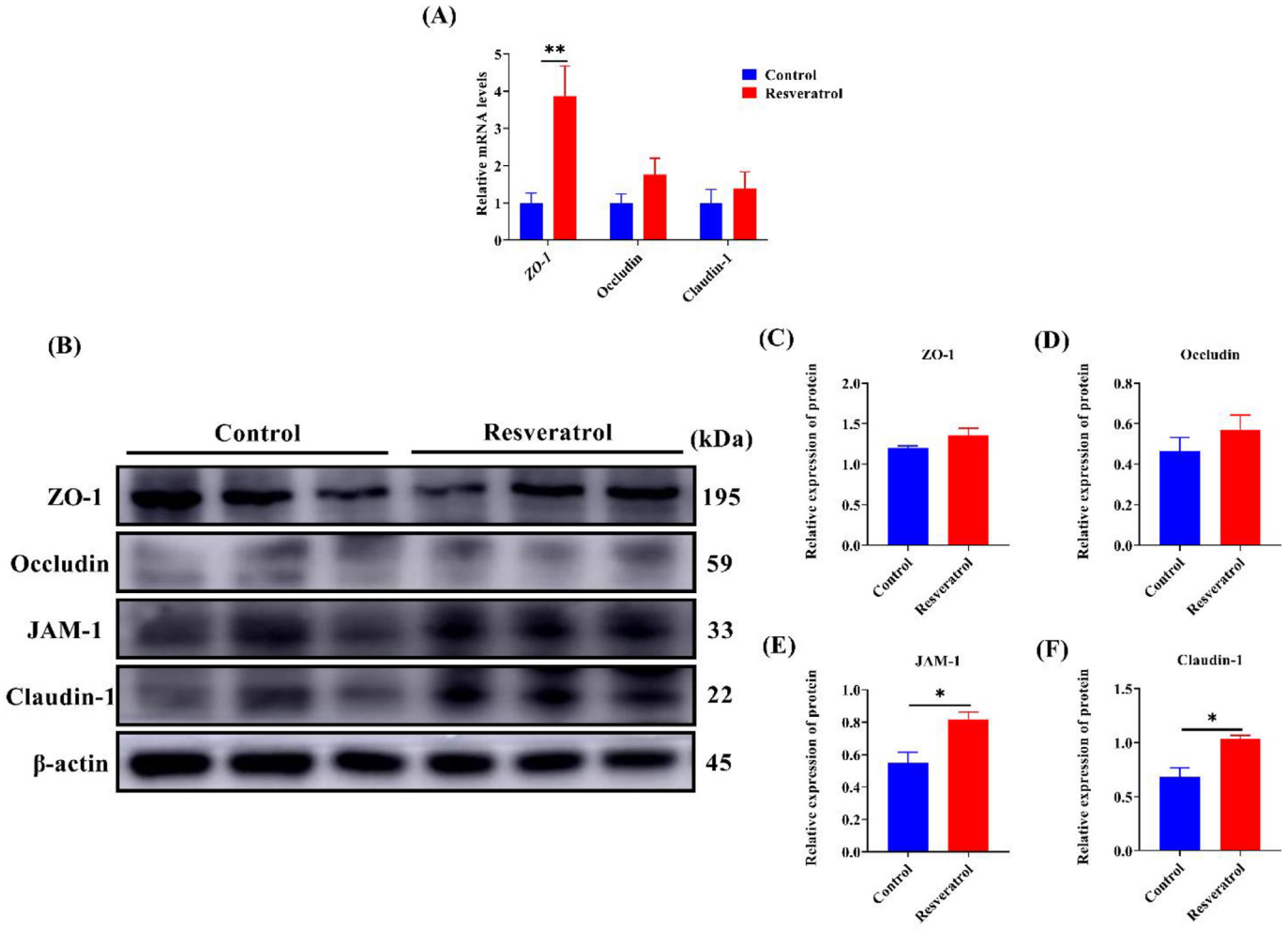

Fig. 3. Maternal resveratrol supplementation increases the expression of tight junction proteins in the jejunum of suckling piglets. (A) Relative mRNA expression of tight junction proteins. (B–F) Relative protein expression of tight junction proteins. ZO-1 = zonula occludens protein 1; JAM 1 = junctional adhesion molecule 1. All data were expressed as means ± SEM of at least three independent experiments. *$P < 0.05$, **$P < 0.01$.

3.3. Maternal supplementation with resveratrol increased the expression of tight junction proteins in the intestine of suckling piglets via colostrum exosome-derived miRNAs of sows during high summer temperatures

Previous studies have shown that porcine milk exosomes and miRNAs are beneficial for the intestinal health of suckling piglets (Chen et al., 2016; Xie et al., 2019, 2020; Zeng et al., 2021a). We hypothesized that resveratrol supplementation influences the intestinal health of suckling piglets by regulating colostrum exosome-derived miRNAs in sows. Therefore, we used DEGseq to identify differential miRNAs from sow colostrum exosomes. As shown in Fig. 2, supplementation with resveratrol resulted in 31 upregulated miRNAs and 59 downregulated miRNAs in the differential miRNAs from sows' colostrum exosomes compared with control group (Fig. 2A; Supplementary File: The list of differential miRNAs between the resveratrol group and control group). Notably, KEGG analysis revealed that the target genes of the differential miRNAs were enriched in several signaling pathways, especially the tight junction pathway, which is essential for intestinal health and homeostasis (Buckley and Turner, 2018) (Fig. 2B and Fig. S1). Furthermore, the differential miRNAs were enriched in most tight junction pathway genes (e.g., *ZO*, occludin, *JAM*, and claudin) (Fig. S1).

We next detected the intestinal tight junction proteins in suckling piglets. As shown in Fig. 3, maternal resveratrol supplementation significantly increased the mRNA expression of *ZO-1* (Fig. 3A) ($P < 0.01$) as well as the protein levels of JAM-1 (Fig. 3E) and claudin-1 (Fig. 3F) in the jejunum ($P < 0.05$). In conclusion, these results indicate that maternal resveratrol supplementation improves the intestinal tight junction proteins of suckling piglets via colostrum exosome-derived miRNAs of sows.

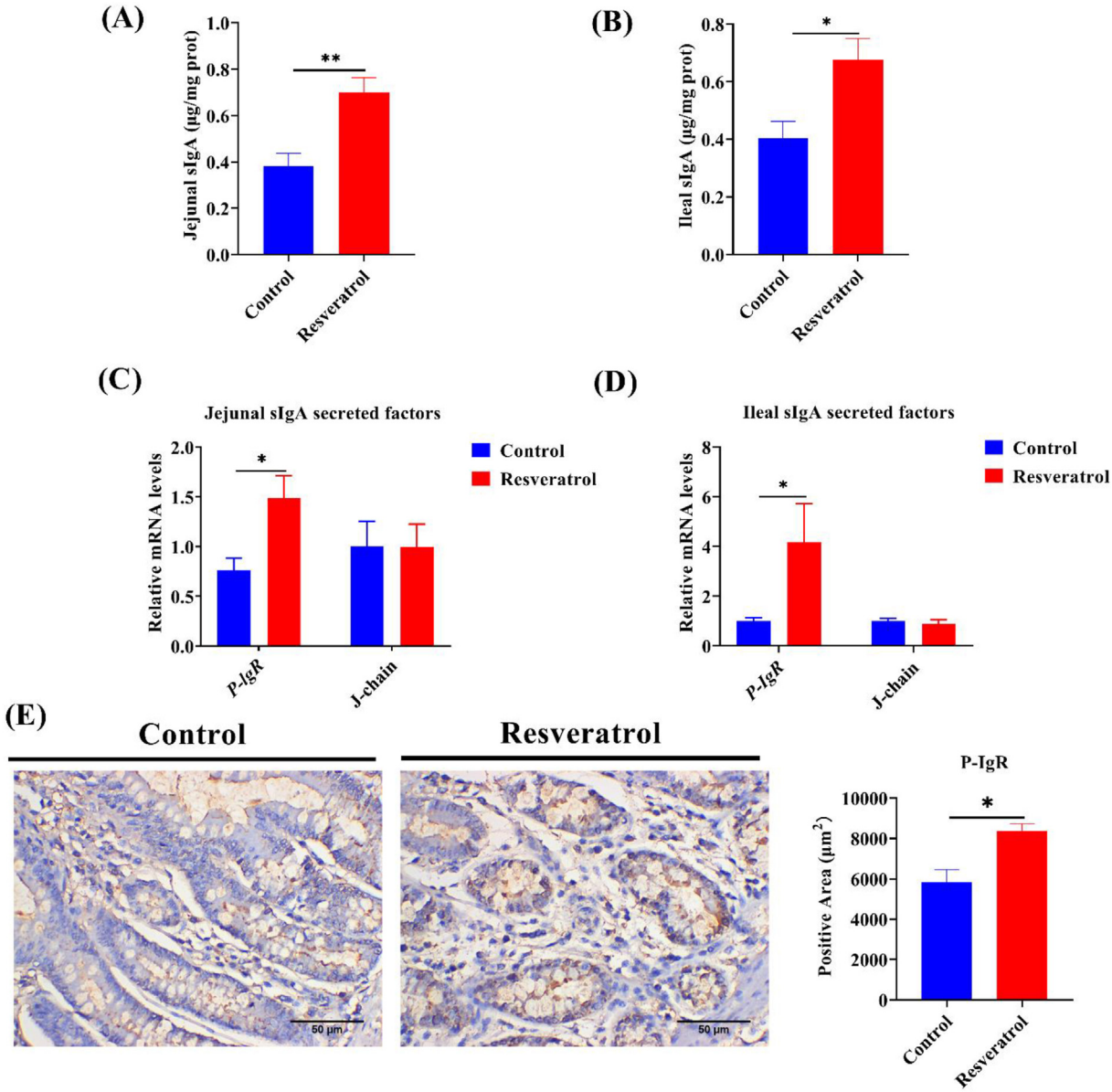

Fig. 4. Maternal resveratrol supplementation promotes the intestinal sIgA production of suckling piglets. (A–B) The sIgA concentration in the jejunum and ileum. sIgA = secretory immunoglobulin A. (C–D) The mRNA expression of sIgA secreted factors in the jejunum and ileum. (E) The P-IgR IHC staining of jejunum. P-IgR = polymeric immunoglobulin receptor; IHC = immunohistochemistry. All data were expressed as means ± SEM of at least three independent experiments. *$P < 0.05$, **$P < 0.01$.

3.4. Maternal supplementation with resveratrol promotes the intestinal sIgA production of suckling piglets through colostrum immunoglobulins of sows during high summer temperatures

Our previous study has shown that dietary resveratrol supplementation significantly increased the contents of colostrum immunoglobulins in sows (Zhao et al., 2022). Maternal diets influence the makeup of cytokines like immunoglobulins in colostrum and milk, which can then be passed on to piglets and alter their immune system development (Rooke and Bland, 2002; Nguyen et al., 2007). Previous studies have shown that immunoglobulins can be polymerized to secretory immunoglobulins, e.g., sIgA, which can be carried across intestinal epithelial cells into gut secretions by P-IgR (Rogier et al., 2014; Turula and Wobus, 2018; Wang et al., 2020). Thus, we hypothesize that maternal resveratrol supplementation could increase the intestinal sIgA synthesis of suckling piglets through the transfer of colostrum immunoglobulins. As expected, we found that maternal supplementation with resveratrol significantly increased the sIgA secretion in the jejunum (Fig. 4A) and ileum (Fig. 4B) ($P < 0.05$). Furthermore, maternal resveratrol supplementation significantly enhanced *P-IgR* gene expression in the jejunum (Fig. 4C) and ileum (Fig. 4D), and P-IgR protein abundance in the jejunum (Fig. 4E) ($P < 0.05$).

Next, the mRNA expression of sIgA-related factors, such as Th2 cytokines, transforming growth factor (TGF)-related factors, and cell proliferation-related factors, was then evaluated. For Th2 cytokines, resveratrol dramatically increases *IL-10* mRNA expression in the jejunum ($P < 0.05$) (Fig. 5A), while has little effect on the

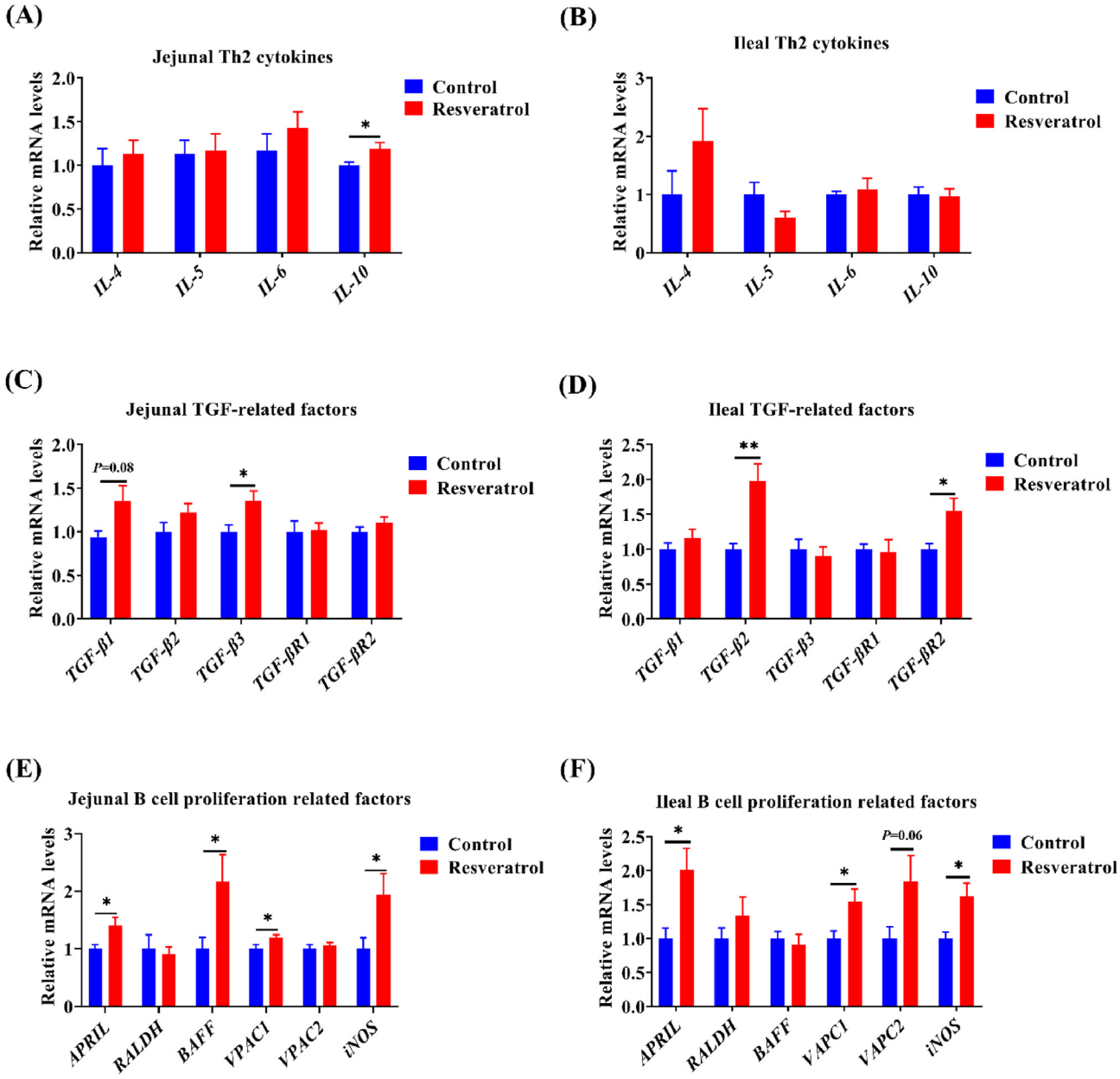

Fig. 5. Maternal resveratrol supplementation increases the mRNA expression of sIgA related factors in the intestine of suckling piglets. (A–B) The mRNA expression of Th2 cytokines in the jejunum and ileum. *IL* = interleukin. (C–D) The mRNA expression of TGF-related factors in the jejunum and ileum. *TGF-β* = transforming growth factor-β; *TGF-βR* = transforming growth factor-β receptor. (E–F) The mRNA expression of B cell proliferation related factors in the jejunum and ileum. *APRIL* = a proliferation-inducing ligand; *RALDH* = retinal dehydrogenases; *BAFF* = B cell-activating factor; *VAPC* = vasoactive intestinal peptide receptor; *iNOS* = inducible nitric oxide synthase. All data were expressed as means ± SEM of at least three independent experiments. *$P < 0.05$, **$P < 0.01$.

expression of ileal Th2 cytokines ($P > 0.05$) (Fig. 5B). For TGF-related factors, maternal resveratrol has higher *TGF-β3* ($P < 0.05$) and rising trend of *TGF-β1* mRNA expressions in the jejunum ($P = 0.08$), and higher *TGF-β2* and *TGF-βR2* mRNA expressions in the ileum ($P < 0.05$) (Fig. 5D). Also, maternal resveratrol significantly enhanced *APRIL*, *BAFF*, *VAPC1* and *iNOS* mRNAs expressions in the jejunum ($P < 0.05$) (Fig. 5E), and markedly increased *APRIL*, *VAPC1* and *iNOS* mRNAs in the ileum ($P < 0.05$) (Fig. 5F).

Collectively, these results suggest that maternal resveratrol supplementation increased sow colostrum immunoglobulins as well as the expression of various sIgA-related factors such as TGF-related factors and P-IgR in the intestine of suckling piglets, which promoted the intestinal sIgA production of suckling piglets.

3.5. Maternal supplementation with resveratrol increased the concentration of SCFA in the colonic content of suckling piglets through immunoglobulin-microbiota axis during high summer temperatures

Our previous study has shown that dietary resveratrol supplementation significantly increases the relative abundance of probiotics (Zhao et al., 2022), especially *Alloprevotella* in suckling piglets, which is associated with SCFA synthesis (Yang et al., 2019; Zhang et al., 2020). Therefore, we hypothesize that dietary resveratrol supplementation may also increase the concentration of SCFA in suckling piglets. As expected, maternal resveratrol supplementation significantly increased the level of isovaleric acid in the colonic content ($P < 0.05$) (Fig. 6E), and relatively increased the levels of acetic acid ($P = 0.05$) (Fig. 6A), propionic acid ($P = 0.08$) (Fig. 6B), isobutyric acid ($P = 0.08$) (Fig. 6C), butyric acid ($P = 0.07$) (Fig. 6D) and valeric acid ($P = 0.09$) (Fig. 6F) in the colonic content. Our previous study also found that the relative abundance of *Lactobacillus* and *Alloprevotella* were positively correlated with colostrum IgM content (Zhao et al., 2022). Therefore, these results indicate that maternal resveratrol supplementation increases the concentration of SCFA in the colonic content of suckling piglets through immunoglobulin-microbiota axis.

3.6. Spearman's correlation analysis of significantly differential intestinal microbiota, intestinal sIgA production and SCFA concentrations of suckling piglets during high summer temperatures

The results from Fig. 7A–C showed the correlation analyses of significantly differential intestinal microbiota (data from Zhao et al., 2022) and intestinal sIgA production, significantly differential intestinal microbiota and intestinal SCFA concentration, as well as intestinal sIgA production and intestinal SCFA concentration, respectively. Bacilli (class), Lactobacillales (order), Lactobacillaceae (family) and *Lactobacillus* (genus), were positively ($P < 0.05$) associated with the sIgA concentrations and *P-IgR* gene expression in the jejunum and ileum (Fig. 7A). As shown in Fig. 7B, Bacilli (class), Lactobacillales (order), Lactobacillaceae (family) and *Lactobacillus* (genus) were positively ($P < 0.05$) associated with the concentrations of acetic acid, propionic acid, butyric acid and isovaleric acid. Moreover, *Alloprevotella* (genus) was positively ($P < 0.05$) associated with the concentrations of acetic acid, butyric acid and isovaleric acid. As shown in Fig. 7C, positive associations were observed among intestinal sIgA concentration and SCFA content: the jejunal sIgA concentration with the concentrations of acetic acid, butyric acid and isovaleric acid ($P < 0.05$); the jejunal *P-IgR* gene expression with the concentrations of acetic acid and isovaleric acid ($P < 0.05$); the ileal sIgA concentration with the

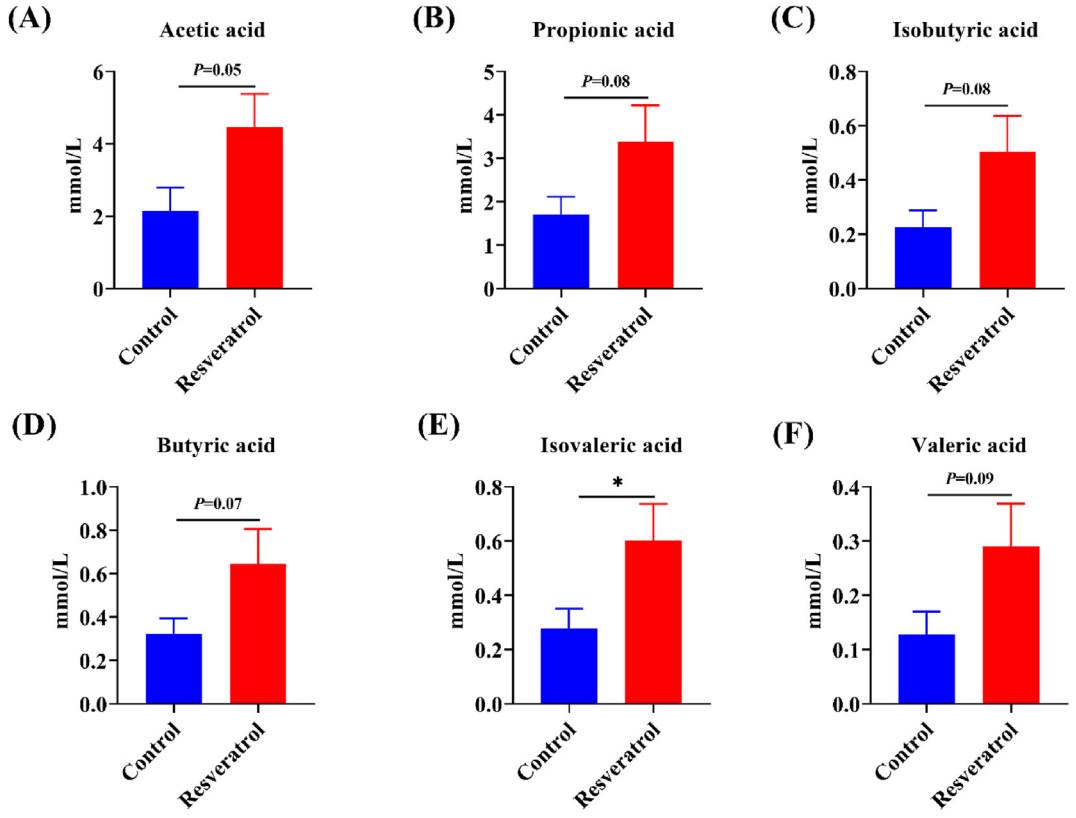

Fig. 6. Maternal resveratrol supplementation promotes the SCFA production in the colonic content of suckling piglets. (A) The level of acetic acid. (B) The level of propionic acid. (C) The level of isobutyric acid. (D) The level of butyric acid. (E) The level of isovaleric acid. (F) The level of valeric acid. SCFA = short chain fatty acids. All data were expressed as means ± SEM of at least three independent experiments. *$P < 0.05$.

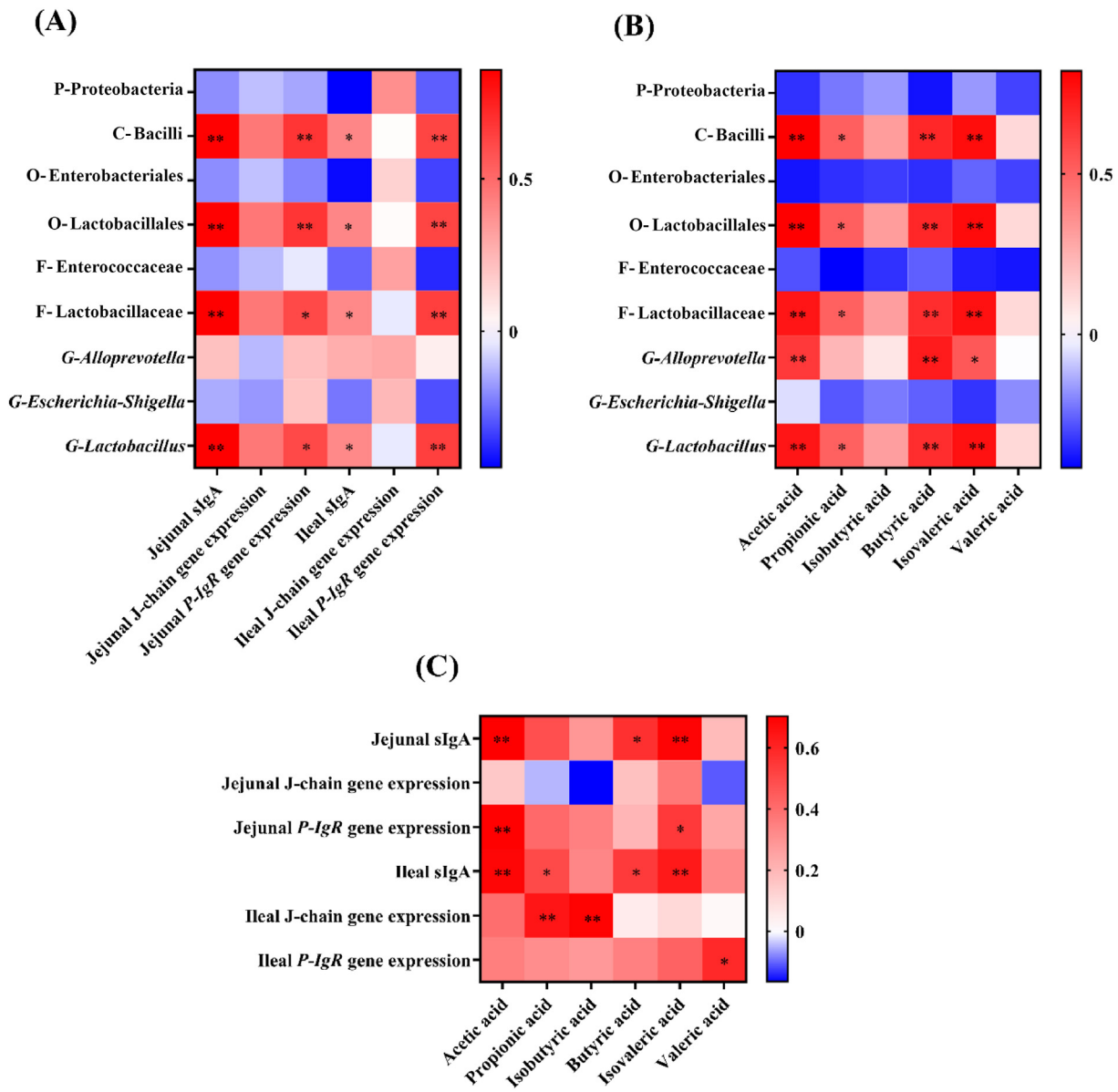

Fig. 7. Spearman's correlation analysis among intestinal differential microbiota with data from Zhao et al. (2022), intestinal sIgA concentration and SCFA content in suckling piglets. (A) Correlation analysis of intestinal differential microbiota with sIgA concentration. (B) Correlation analysis of intestinal differential microbiota with SCFA content. (C) Correlation analysis of intestinal sIgA concentration with SCFA content. P- = phylum; C- = class; O- = order; F- = family; *G*- = genus; sIgA = secretory immunoglobulin A; P-IgR = polymeric immunoglobulin receptor; SCFA = short chain fatty acids. In the heatmap of the correlation coefficient, the red represents positive correlations and the blue represents negative correlations. *$P < 0.05$, **$P < 0.01$.

concentrations of acetic, propionic, butyric and isovaleric acid ($P < 0.05$); the ileal J-chain gene expression with the concentrations of propionic acid and isobutyric acid concentrations ($P < 0.01$); and the ileal *P-IgR* gene expression with the concentration of valeric acid ($P < 0.05$). These results further evidenced the relationships among immunoglobulin, microbiota and SCFA in the intestine of piglets.

3.7. Spearman's correlation analysis between total daily weight gain and intestinal health indices of suckling piglets during high summer temperatures

The results of the analysis of total daily weight gain with indices of intestinal health such as intestinal morphology, tight junctions, sIgA production and SCFA content in suckling piglets are shown in Fig. 8. The results showed that total daily gain was positively associated with ileal VCR, jejunal sIgA concentration, ileal *P-IgR* gene expression, isovaleric acid and valeric acid content of the colon in suckling piglets ($P < 0.05$).

4. Discussion

This present study demonstrated that maternal resveratrol improved the intestinal health and weight gain of suckling piglets through immunoglobin and exosome-derived microRNAs in sow colostrum during high summer temperatures. The living environment and maternal transmission, such as colostrum, promoted the growth performance of suckling piglets during lactation. High ambient temperatures damaged the intestinal integrity and barrier function of piglets, resulting in poor growth (Pearce et al.,

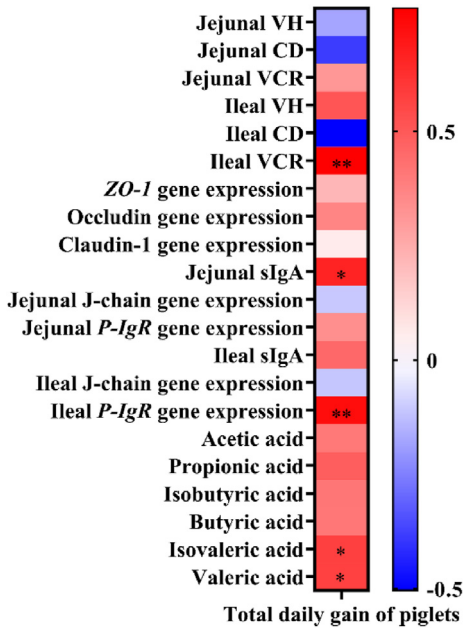

Fig. 8. Spearman's correlation analysis of total daily gain with intestinal morphology, tight junctions, sIgA concentration and SCFA content in suckling piglets. VH = villous height; CD = crypt depth; VCR = villous height to crypt depth ratio; *ZO-1* = zonula occludens protein 1; sIgA = secretory immunoglobulin A; *P-IgR* = polymeric immunoglobulin receptor; SCFA = short chain fatty acids. In the heatmap of the correlation coefficient, the red represents positive correlations and the blue represents negative correlations. $^{*}P < 0.05$, $^{**}P < 0.01$.

2013). During the perinatal period, sows are particularly susceptible to high temperatures, resulting in heat stress (Sasaki et al., 2018). Heat stress would reduce the feed intake of sows, which further decrease the production and quality of milk (Renaudeau et al., 2003; Silva et al., 2009). Due to the colostrum and milk are the guarantee for suckling piglets intestinal development and growth during lactation (Declerck et al., 2017; Devillers et al., 2011; Mach et al., 2015), heat stress would result in nutritional imbalance in sows and also have detrimental effects on the intestinal health and growth of suckling piglets (Guo et al., 2020). Therefore, nutritional strategies are used to alleviate heat stress in sows and improve the development and growth of piglets during high summer temperatures (Cottrell et al., 2015; Li et al., 2021, 2022). In the present study, we found that maternal resveratrol supplementation improved the intestinal health and further increased the total daily gain of piglets during high summer temperatures. In addition, our study also found that maternal resveratrol improved the intestinal morphology, such as the increase of ileal VH and VCR in suckling piglets under hot summer conditions, which is consistent with the study of Meng et al. (2019). Maternal resveratrol may increase intestinal VH to enhance the efficiency of intestinal absorption for nutrients in suckling piglets, thereby improve the growth of suckling piglets. However, whether maternal resveratrol supplementation could improve the efficiency of intestinal absorption of suckling piglets needs further investigation.

Our previous study found that maternal resveratrol supplementation increased the colostrum immunoglobulins (IgA, IgG and IgM) (Zhao et al., 2022). The colostrum immunoglobulins contribute to the development of intestinal sIgA and are essential for mucosal immunity and intestinal homeostasis (Mantis et al., 2011; Geuking et al., 2012; Corthésy, 2013). Various factors, e.g., Th2 cytokines and TGF-related factors, are associated with sIgA secretion (Mantis et al., 2011; Bemark et al., 2012; Pabst, 2012). Previous studies showed that functional amino acids, e.g., glutamine and leucine, increased the intestinal sIgA secretion and expression of sIgA-related proteins to improve intestinal health of mice (Ren et al., 2016; Song et al., 2020; Wu et al., 2016). Notably, resveratrol can also increase intestinal sIgA secretion in mice (Song et al., 2022). Similarly, we found that maternal resveratrol supplementation significantly increased the intestinal sIgA secretion and expression of sIgA-related factors, e.g., P-IgR, and TGF-related factors, in piglets in the present study. In addition, our previous study demonstrated that maternal resveratrol supplementation increased the relative abundance of probiotics such as *Lactobacilli* in suckling piglets (Zhao et al., 2022), which is consistent with Hsu et al. (2020). Our results also showed that maternal resveratrol increase the production of SCFA (metabolic product of microbes) in the colonic contents of suckling piglets. These results indicate that maternal resveratrol may improve the intestinal health of piglets through the immunoglobulin-microbes-SCFA axis. However, the underlying mechanisms of the interactions among immunoglobulin, microbes and SCFA in the intestine of piglets remain unclear and require further investigation.

Our findings show that maternal resveratrol improved intestinal tight junctions in suckling piglets by regulating colostrum exosome-derived miRNAs in sows, providing a better understanding of the effects of resveratrol supplementation on sows and piglets. Furthermore, pig milk small extracellular vesicles enhanced P-IgR expression to stimulate intestine sIgA production via suppression of its derived miR-221-5p (Zeng et al., 2021b). In addition to the tight junction pathway, our KEGG analysis revealed that the target genes of the colostrum exosome-derived miRNAs were abundant in some intestinal immune network genes that involved in IgA synthesis, such as *TGF-β* and *APRIL* (Fig. S2). These results suggest that colostrum exosome-derived miRNAs in sows could influence the production of intestinal sIgA of suckling piglets.

In this study, we determined the concentrations of resveratrol and its derivatives in the serum and colostrum of sows in the resveratrol group. Previous studies have shown that dietary resveratrol has beneficial effects on the intestinal health of piglets by strengthening the intestinal barrier (Cao et al., 2019; Wang et al., 2022). Therefore, it is also possible that resveratrol in colostrum directly influences the intestinal health of piglets. The direct effect of resveratrol on the intestinal health of nursing pigs needs to be further investigated using a piglet model fed with milk containing resveratrol.

5. Conclusion

In conclusion, the results of this study suggested that maternal supplementation with resveratrol from late gestation to lactation (from d 75 of gestation to d 21 of lactation) during high ambient temperatures could improve intestinal health and weight gain of suckling piglets, which might be associated with the exosome-derived miRNAs and immunoglobulins in sow colostrum.

Author contributions

Hao Xiao and **Zongyong Jiang** conceived the project. **Kaiguo Gao** and **Xiaolu Wen** performed the sample preparation. **Changming Hong** and **Yujian Huang** analyzed and interpreted the data, prepared the original draft. **Changming Hong**, **Yujian Huang**, **Guan Yang**, **Li Wang**, **Xuefen Yang**, **Zongyong Jiang** and **Hao Xiao** revised this paper. All authors contributed to the article and approved the final version.

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.

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Appendix supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aninu.2024.01.002.

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