# Effect of dietary pyrroloquinoline quinone disodium in sows on intestinal health of the offspring

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# Abstract

Background: Pyrroloquinoline quinone (PQQ) is a putative essential nutrient and redox modulator in microorganisms, cell and animal models. The objective of this study was to investigate the effects of pyrroloquinoline quinone disodium (PQQ·Na2) in sows during gestation and lactation on intestinal health in the offspring.

Results: The activities of SOD and GSH-Px were significantly (P<0.05) increased by PQQ·Na2 supplementation, and MDA activity was decreased (P<0.05) in the plasma of piglets. CAT, SOD and GSH-Px activities were significantly (P<0.05) increased, and MDA activity was decreased (P<0.05) in the small intestine of piglets. The mRNA expression levels of SOD1, CAT and MGST1 in the jejunum were increased in newborn piglets (P<0.05), and the mRNA expression levels of HO1, SOD1, CAT, SOD2, GPX4, GPX1 and GCLC in the jejunum were increased in weaned piglets (P<0.05). The mRNA expression of ZO-1 was increased (P<0.05) in the jejunum of newborn piglets, and the mRNA expression of Occludin and ZO-1 was increased (P<0.05) in the jejunum of weaned piglets. The villous height of the duodenum and jejunum of weaned piglets was increased (P<0.05) by dietary PQQ·Na2. In weaned piglets, Bacteroidetes and Firmicutes were the most prevalent phyla in both the Con and PQQ·Na2 treatment groups, and the most prevalent genera were Alloprevotella and Bacteroides. At the phylum level, the abundance of Firmicutes was significantly increased (P<0.05), and the abundance of Proteobacteria was significantly decreased (P<0.05). At the genus level, the abundance of Alloprevotella was significantly increased (P<0.05), and the abundance of Actinobacillus and Escherichia was decreased (P<0.05).

Conclusions: In conclusion, dietary supplementation with PQQ·Na2 in sows during gestation and lactation had positive effects on intestinal health in offspring.


# Background

Weaning is a critical stage in young mammals owing to an increased susceptibility to gastrointestinal disorders, inflammation and diarrhea[1]. The postweaning period is characterized by an immediate and transient drop in food intake resulting in severe undernutrition and growth inhibition, which consequentially inhibits various aspects of small intestinal architecture and function and can further lead to gut-associated disorders and diarrhea[2–3]. In humans and animals, dietary substances such as prebiotics, sodium butyrate and plant extracts have been reported to control diarrhea caused by weaning and improve intestinal health[4]. Traditional measures of alleviating weaning-associated intestinal dysfunction have been mainly through dietary manipulation during weaning stages. However, recent studies have indicated that maternal dietary supplementation improves the immune status and gastrointestinal health of offspring. Zhong et al. reported that maternal consumption of methionine improved neonatal intestinal growth by promoting morphological development and upregulating the expression of genes responsible for nutrient metabolism[5]. In addition, supplementing the maternal diet with seaweed extract and fish oil is beneficial for the intestinal microflora, intestinal morphology and

immune status of weaned pigs[6]. Thus, there is a potential possibility that beneficial maternal dietary treatments could reduce or alleviate weaning-associated intestinal dysfunction and diarrhea.

Pyrroloquinoline quinone (PQQ) is a water soluble thermostable triglyceride-quinone[7]. PQQ, initially identified as a novel cofactor of various bacterial dehydrogenases[8], is an important animal nutrient. PQQ-deficient animals display multifarious illnesses[9]. PQQ has attracted considerable attention because of its importance for mammalian growth, development, reproduction and immune function[10]. PQQ is an effective antioxidant that protects mitochondria from oxidative stress-induced lipid peroxidation, protein carbonyl formation and mitochondrial respiratory chain inactivation[11]. Despite abiotic synthesis of PQQ in mammals, a minute quantity of PQQ has been found in human and rat tissues, at picomolar to nanomolar levels, and a particularly large amount has been found in human milk[12]. Owing to its versatile functions, PQQ disodium ($PQQ{\cdot}Na_2$) salt has been certified by authorities as a Natural Health Product in Canada, providing 20 mg of $PQQ{\cdot}Na_2$ salt per day as an antioxidant for the maintenance of good health[13]. On August 13, 2018, the European Commission issued regulations ((EU) 2018/1122) ratifying pyrroquinone sodium salt as a new type of food. Pyrroquinone sodium is defined as a dietary supplement by the European Commission.

The gastrointestinal microbiota has a significant impact on host health through regulating intestinal nutritional metabolism, maturing the immune system, and establishing the intestinal barrier[14]. Soon after birth, microbiota communities assemble in this ecosystem and have been shown to be host specific and broadly stable over time[15]. In most cases, the bacterial composition of the intestinal content is stable and protects the host from pathogens; however, many factors, such as dietary factors, can influence this composition. The weaning process for pigs involves an abrupt dietary shift from sow milk to a completely feed-based diet that produces abrupt taxonomic and functional shifts in the intestinal microbiota[16]. $PQQ{\cdot}Na_2$ has been reported to have an impact on the intestinal microbiota[17]. However, the impact of maternal dietary $PQQ{\cdot}Na_2$ supplementation on the intestinal microbiota in offspring is still unknown. Therefore, the objective of the present study was to explore the effects of maternal dietary $PQQ{\cdot}Na_2$ supplementation during gestation and lactation on the intestinal barrier functions and intestinal microbiota of offspring during weaning in piglets.

# Methods

# Animals and management

A total of 40 cross-bred (Landrace × Large White crossed with Duroc boar) multiparity gestation sows with an average parity of 4.3 were used in the study. Forty sows were allotted to 2 dietary treatments after breeding. One group was the control sows, which were fed a corn-soybean meal control diet (Con treatment, n = 20), and the other group was the treatment sows fed a control diet with 20 mg/kg $PQQ{\cdot}Na_2$ after breeding and through gestation and lactation (PQQ treatment, n = 20). $PQQ{\cdot}Na_2$ (purity, ≥ 98%) was synthesized by chemical reactions. It was diluted with corn starch to a concentration of 1 g/kg mixture

before being mixed into the diet. Based on the known range of PQQ in foods[18], we inferred that the concentration of PQQ in the basal diet was less than 0.01 mg/kg. The sows were kept in single crates (0.6 m × 2.0 m) from insemination to day 110 of gestation. On day 110 of gestation, sows were transported to the farrowing facility, where they were placed in individual farrowing crates (2.4 m × 2.4 m). Each crate had steel mesh floors with a heat lamp for newborn pigs. The crates were mounted over a solid concrete floor, and manure was removed manually each day. The farrowing room temperature was maintained at approximately 18 to 20 °C. Births were watched, but the observers interfered as little as possible in the farrowing process. The protocols used in this experiment were approved by the Northeast Agricultural University Institutional Animal Care and Use Committee. All animal experimental diets (Table 1) were formulated to meet or exceed the recommended nutrient requirements of the NRC (2012). The piglets were weaned at day 21 of lactation. The protocols used in this experiment were approved by the Northeast Agricultural University Institutional Animal Care and Use Committee. All animal care and treatment complied with the standards described in the "Laboratory Animal Management Regulations" (revised 2016) of Heilongjiang Province, China.

Table 1
Composition and nutrient levels of diets

| Item | Gestation |
|---|---|
| Ingredient, % | |
| Corn | 67.5 |
| Soybean meal | 16 |
| Wheat bran | 13.5 |
| Dicalcium phosphate | 1 |
| Limestone | 1.1 |
| Salt | 0.4 |
| Premix[1] | 0.5 |
| Nutritional composition[2], % | |
| Net energy, MJ/kg | 9.62 |
| Crude protein (CP) | 15.58 |
| Calcium | 0.71 |
| Total phosphorus | 0.60 |
| Available phosphorus | 0.31 |
| SID Lysine | 0.54 |
| [1]The premix provides following for per kg diet: vitamin A, 8,000 U; vitamin D3, 2,000 U; vitamin E, 50 U; vitamin K3, 1.5 mg; vitamin B1, 1.6 mg; vitamin B6, 1.5 mg; vitamin B12, 15 μg; niacin, 20 mg; D-pantothenic acid, 15 mg; Zn (ZnO), 100 mg; Fe ($FeSO_4 \cdot 7H_2O$), 80 mg; Cu ($CuSO_4 \cdot 5H_2O$), 20 mg; Mn ($MnSO_4 \cdot H_2O$), 25 mg; I (KI), 0.3 mg; Se ($NaSeO_3 \cdot 5H_2O$), 0.2 mg. | |
| [2]Nutrient levels were calculated values | |

# Sample and data collection

Eight litters per treatment were randomly selected for sample collection at weaning (day 21). Newborn piglets (n = 8 per treatment, 1 piglet per litter) and weaning piglets (n = 8 per treatment, 1 piglet per litter), regardless of sex, were randomly selected and slaughtered by an intra-arterial injection of pentobarbital (200 mg/kg) after general anesthesia. Then, fresh feces of weaning piglets were collected from each pen and stored immediately at − 80 °C for microbiota analysis. For blood collection, microcentrifuge tubes were coated with 10 mL of heparin sodium salt (20 U/mL in phosphate-buffered saline). Whole blood was collected from the submandibular site as described previously and maintained on ice for 30 min. The

samples were centrifuged at 3000 × g and 4 °C for 10 min. After slaughter, their small intestines were dissected and freed from the mesenteric attachment, and all chyme was immediately removed. The jejunum segments were rinsed in a chilled sodium chloride solution (0.9 g/L saline) and divided into three segments of equal length. One part was frozen in liquid $N_2$ and stored at − 80 °C until RNA extraction. The second part was fixed in 10% formalin for morphological analysis. The third part was sealed into pouches and preserved at − 20 °C until used for the evaluation of antioxidant status. The contents of the caecum were immediately frozen in liquid $N_2$ and stored at − 80 °C until analysis for microbial DNA could be performed.

# Evaluation of antioxidant enzyme activity and the concentration of nitric oxide

Superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) enzyme activities in the plasma and small intestine were determined using commercially available kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) in accordance with this study. The results of the measurements were expressed as U/mL in plasma and as U/mg protein in the small intestine. Lipid peroxidation in the plasma and small intestine was determined by measuring the amounts of malondialdehyde (MDA) through the thiobarbituric acid method using commercially available kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The results of the measurements were expressed as nmol/mL in plasma and as nmol/mg protein in the small intestine.

# Morphological analysis and Brush border enzymes activities

Cross-sectional jejunal samples from the formalin-preserved segments were cut into 2 mm sections and fixed by standard paraffin embedding. Samples were sectioned at 5 mm and stained with hematoxylin and eosin. Each slide was divided into three single segments, and the microstructures of the jejunum were analyzed using an optical microscope (Nikon Eclipse E400).

Other sections (10 cm) of the jejunum were dissected from each rat and rinsed with phosphate-buffered saline (PBS) with a pH of 7.2 to measure the specific activity of mucosal enzymes. The mucosa was carefully scraped off with a glass slide, placed in Eppendorf tubes and immediately frozen in liquid $N_2$ for later measurement. Mucosa samples were thawed and flushed with isotonic saline at 4 °C. Exactly 0.5 g of mucosa was homogenized for 2 min in a chilled sodium chloride solution (0.9 g/L saline) at a ratio of 1:9 (w/v), followed by centrifugation at 3000 rpm for 10 min. The supernatant was diluted with a sodium chloride solution (0.9 g/L saline) at a ratio of 1:5 (w/w) for the determination of lactase (β-galactosidase; EC 3.2.1.23) and sucrase (sucrose-α-glucosidase; EC 3.2.1.48) activities and later diluted 1:50 (w/w) for the determination of maltase (β-glucosidase; EC 3.2.1.20).

# Quantitative real-time PCR (qRT-PCR) validation

Total RNA was extracted from approximately 100 mg of frozen jejunal tissues. The concentration of RNA was estimated based on its absorbance at 260 nm, which was determined using a spectrophotometer. The RNA quality was determined by checking its integrity through agarose gel electrophoresis and by

confirming that the A260 nm/A280 nm absorbance ratio was between 1.8 and 2.0. Total RNA from each sample was converted into cDNA using a Prime Script® RT reagent kit (TaKaRa® Bio Catalog, Dalian, China) according to the manufacturer's instructions and used for reverse transcription polymerase chain reactions (RT-PCRs). β-actin was used as a reference gene, was stable, and did not change in response to the different treatments. The real-time PCR process was in accordance with the description in a previous study. All PCRs were performed in triplicate (Table 2). The relative gene expression levels in the intestines and placenta were determined using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001)[19].

Table 2

Primers used for Real-time PCR

| Gene | Primer sequence(5'to3') | Product size (bp) | Genbank |
|---|---|---|---|
| Occludin | F:GAGTACATGGCTGCTGCTGA<br>R:AACAAGGTGGCCTCTGTCTC | 173 | NM_001163647.2 |
| ZO-1 | F:AGCTGCCTCTCAACAGAAAG<br>R:GTCTCTGGGCACTGTGTGAG | 217 | XM_005659811.1 |
| Claudin 1 | F:CTGAACACCACTTTGCAAGC<br>R:ATCCGCATCTTCTGCACCTC | 137 | NM_001244539.1 |
| Slc2a1 | F: GCCTGAGACCAGTTGAAAGCAC<br>R: CTGCTTAGGTAAAGTTACAGGAG | 155 | XM_021096908.1 |
| Slc2a3 | F: TGCACGGGCTTTGTGCCGATG<br>R: AAGGAGGTGAAGATTAGGAA | 132 | XM_021092391.1 |
| SOD1 | F: TCCATGTCCATCAGTTTGGA<br>R: AGTCACATTGCCCAGGTCTC | 131 | NM_001190422.1 |
| GAPDH | F: ATGGTGAAGGTCGGAGTGAA<br>R: CCGTGGGTGGAATCATACTG | 155 | NM_001206359.1 |
| NQO1 | F: CCAGCAGCCCGGCCAATCTG<br>R: AGGTCCGACACGGCGACCTC | 160 | NM_001159613.1 |
| HO1 | F: AGGCTGAGAATGCCGAGTTC<br>R: TGTGGTACAAGGACGCCATC | 90 | NM_001004027.1 |
| TXNRD1 | F: CTTTACCTTATTGCCCGGGT<br>R: GTTCACCGATTTTGTTGGCC | 162 | NM_214154.2 |
| GCLC | F: CTTGCCTCTTGCTGTGTGAT<br>R: CCACTCATGTGCCTCGATGT | 159 | XM_001926378.4 |
| MGST1 | F: TTGGCGCGCGAATCTACCACA<br>R:TCCTCGGCTCCCTTCCCACTTA | 239 | NM_214300.1 |
| UGT1A1 | F: GATCCTTTCCTGCAACGCAT<br>R: GGAAGGTCATGTGATCTGAG | 313 | XM_001927673 |
| IL-6 | F: AGCAAGGAGGTACTGGCAGA<br>R: GTGGTGGCTTTGTCTGGATT | 257 | NM_001252429.1 |
| IL-8 | F: ACTTCCAAACTGGCTGTTGC<br>R:GGAATGCGTATTTATGCACTGG | 120 | NM_213867.1 |

GPx4, glutathione peroxidase 4; GPx1, glutathione peroxidase 1; CAT, catalase; SOD3, superoxide dismutase 3; SOD2, superoxide dismutase 2; SOD1, superoxide dismutase 1; NQO1, NAD(P)H quinone dehydrogenase 1; HO1, heme oxygenase 1; CYP1A1, cytochrome P450 family 1 subfamily A member 1; TXNRD1, thioredoxin reductase 1; GCLM, glutamate-cysteine ligase modifier; MGST1, microsomal glutathione S-transferase 1; UGT1A1, UDP glucuronosyl- transferase family 1 memberA1; IL-1, interleukin 1; IL-6, interleukin 6; IL-8, interleukin 8; TNF-α, tumor necrosis factor α

| Gene | Primer sequence(5'to3') | Product size (bp) | Genbank |
|---|---|---|---|
| IL-1α | F: CTGAAGAAGAGACGGTTGAG<br>R:GCACTGGTGGTT GATGAC | 162 | NM_214029 |
| IL-1β | F: GTTCTCTGAGAAATGGGAGC<br>R: CTGGTCATCATCACAGAAGG | 143 | NM_214055.1 |
| IL-2 | F: TTGCACTCATGGCAAACGGT<br>R: ATTCTGTAGCCTGCTTGGGC | 177 | NM_213861.1 |
| TNF-α | CATGAGCACTGAGAGCATGA<br>CGATAACTTCGAAGTGCAGT | 170 | NM_214022.1 |
| SOD3 | F: ACGCTGCTCTGTGCTTACCT<br>R: CTGCCAGATCTCCGTCACTT | 135 | NM_001078688.1 |
| GPX4 | F: CACCCTCTGTGGAAGTGGAT<br>R: TCACCACACAGCCGTTCTTA | 112 | NM_214407.1 |
| GPX1 | F: AAATGCTCACCCGCTCTTC<br>R: GTCATTGCGACACACTGGAG | 118 | NM_214201.1 |
| CAT | F: ACGCCTGTGTGAGAACATTG<br>R: GTCCAGAAGAGCCTGAATGC | 124 | NM_214301.2 |
| SOD2 | F: TGGAGGCCACATCAATCATA<br>R: TTTCGAAGGAACCAAAGTCG | 113 | NM_214127.2 |

GPx4, glutathione peroxidase 4; GPx1, glutathione peroxidase 1; CAT, catalase; SOD3, superoxide dismutase 3; SOD2, superoxide dismutase 2; SOD1, superoxide dismutase 1; NQO1, NAD(P)H quinone dehydrogenase 1; HO1, heme oxygenase 1; CYP1A1, cytochrome P450 family 1 subfamily A member 1; TXNRD1, thioredoxin reductase 1; GCLM, glutamate-cysteine ligase modifier; MGST1, microsomal glutathione S-transferase 1; UGT1A1, UDP glucuronosyl- transferase family 1 memberA1; IL-1, interleukin 1; IL-6, interleukin 6; IL-8, interleukin 8; TNF-α, tumor necrosis factor α

# 16S rDNA sequencing: DNA extractions, PCR amplification and sequencing

DNA from fecal samples was extracted using a Stool DNA Kit (Omega, Inc., USA) according to the manufacturer's instructions. Total DNA was eluted in 50 μL of elution buffer by a modification of the procedure described by the manufacturer (QIAGEN) and stored at − 80 °C until measurement via PCR amplification. The V3– V4 region of the bacterial 16S rRNA was amplified with primers (F: 5'-ACTCCTACGGGAGGCAGCAG-3'; R: 5'-GGACTACHVGGGTWTCTAAT-3'). PCR analysis was performed in triplicate using 25 mL reactions containing 25 ng of template DNA, 12.5 μL of PCR Premix, 2.5 μL of each primer, and PCR-grade water to equalize the final volumes. PCRs were performed under the following conditions: initial denaturation at 98 °C for 30 s; 35 cycles of denaturation at 98 °C for 10 s, annealing at 54 °C for 30 s, and extension at 72 °C for 45 s; and a final extension at 72 °C for 10 min. The PCR products were confirmed with 2% agarose gel electrophoresis. The PCR products were purified with AMPure XT beads (Beckman Coulter Genomics, Danvers, MA, USA) and quantified by Qubit (Invitrogen, CA, USA). Amplicon pools were prepared for sequencing, and the size and quantity of the amplicon library

were assessed on an Agilent 2100 bioanalyzer (Agilent, USA) and with a Library Quantification Kit for Illumina (Kapa Biosciences, Woburn, MA, USA). A PhiX control library (Illumina) was combined with the amplicon library (expected at 30%). The libraries were sequenced via 300PE MiSeq runs, and one library was sequenced with both protocols using standard Illumina sequencing primers, eliminating the need for a third (or fourth) index read.

Samples were sequenced on an Illumina MiSeq platform according to the manufacturer's recommendations, provided by LC-Bio. Paired-end reads were assigned to samples based on their unique barcodes and truncated by removing the barcode and primer sequences. Paired-end reads were merged using FLASH. Quality filtering of the raw tags was performed under specific filtering conditions to obtain high-quality clean tags according to FastQC (V 0.10.1). Chimeric sequences were filtered using Vsearch software. Sequences were assigned to OTUs at 97% similarity. Representative sequences were chosen for each OTU, and taxonomic data were then assigned to each representative sequence using the Ribosomal Database Project (RDP) classifier. To estimate alpha diversity, we calculated the Chao1 (species richness estimator) and Shannon and Simpson indices (diversity indices)[20].

# Statistical analysis

All data analyses were performed with SPSS 19.0 software (IBM-SPSS Inc., Chicago, Illinois, USA). The data were analyzed by one-way analysis of variance (ANOVA), and multiple comparisons were analyzed with Tukey's test in SPSS. Each individual sow and her litter were defined as an experimental unit. The results are presented as the mean values and the standard errors of the mean (SEMs). In all statistical tests used, $P < 0.05$ was considered significantly different.

## Results

# Antioxidant status

The effects of dietary PQQ·$Na_2$ supplementation during gestation and lactation on the antioxidant capacity in the plasma of piglets are shown in Table 3. The activities of SOD and GSH-Px were significantly ($P < 0.05$) increased by PQQ·$Na_2$ supplementation of both newborn piglets and weaned piglets. The MDA activity was significantly ($P < 0.05$) decreased by PQQ·$Na_2$ supplementation of weaned piglets. The results of the antioxidant status in the small intestine of piglets are presented in Table 4. The CAT activity in the duodenum and jejunum of newborn piglets was increased ($P < 0.05$) by PQQ·$Na_2$ supplementation. PQQ·$Na_2$ dietary supplementation increased the CAT activity in the jejunum of weaned piglets. The SOD activity in the duodenum and jejunum of weaned piglets was increased ($P < 0.05$) by PQQ·$Na_2$ supplementation. The GSH-Px activity was increased ($P < 0.05$) and the MDA activity was decreased ($P < 0.05$) by PQQ·$Na_2$ supplementation in small intestine of weaned piglets.

Table 3

Effects of dietary PQQ·$Na_2$ in sows on antioxidant capacity in plasma of piglets

| Item | Con | | PQQ | | P-value |
|---|---|---|---|---|---|
| | Mean | SEM | Mean | SEM | |
| Newborn piglets | | | | | |
| SOD, U/mL | 87.35[b] | 5.32 | 104.28[a] | 3.45 | 0.015 |
| CAT, U/mL | 2.32 | 0.22 | 2.33 | 0.14 | 0.967 |
| GSH-Px, U/mL | 207.81[b] | 10.37 | 251.58[a] | 14.80 | 0.030 |
| MDA, nmol/mL | 2.57 | 0.22 | 2.58 | 0.17 | 0.977 |
| Weaned piglets | | | | | |
| SOD, U/mL | 98.34[b] | 3.41 | 116.23[a] | 4.86 | 0.011 |
| CAT, U/mL | 2.41 | 0.10 | 2.40 | 1.12 | 0.932 |
| GSH-Px, U/mL | 384.80[b] | 14.74 | 446.43[a] | 15.92 | 0.013 |
| MDA, nmol/mL | 3.45[a] | 0.17 | 2.86[b] | 0.12 | 0.014 |

All of the values are expressed as the means and pooled SEM, n = 8; [a, b] Means without a common letter differ ($P < 0.05$). Con, control treatment, PQQ, PQQ·$Na_2$ treatment; SOD, superoxide dismutase; CAT, catalase; GSH-Px, glutathione peroxidase; MDA, malondialdehyde.

Table 4

Effects of dietary PQQ·$Na_2$ in sows on antioxidant capacity in intestine of piglets

| Item | Con | | PQQ | | P-value |
|---|---|---|---|---|---|
| | Mean | SEM | Mean | SEM | |
| Newborn piglets | | | | | |
| Duodenum | | | | | |
| SOD, U/mL | 30.57 | 3.32 | 37.41 | 3.40 | 0.115 |
| CAT, U/mL | 66.22$^{b}$ | 3.94 | 79.97$^{a}$ | 3.96 | 0.027 |
| GSH-Px, U/mL | 49.61 | 2.36 | 47.20 | 2.18 | 0.403 |
| MDA, nmol/mL | 4.42 | 0.16 | 4.46 | 0.14 | 0.885 |
| Jejunum | | | | | |
| SOD, U/mL | 38.49 | 2.64 | 38.59 | 2.44 | 0.979 |
| CAT, U/mL | 92.96$^{b}$ | 3.81 | 107.94$^{a}$ | 4.60 | 0.025 |
| GSH-Px, U/mL | 62.22 | 2.49 | 59.93 | 2.24 | 0.506 |
| MDA, nmol/mL | 4.25 | 0.22 | 4.27 | 0.20 | 0.961 |
| Ileum | | | | | |
| SOD, U/mL | 38.59 | 2.29 | 38.82 | 2.08 | 0.946 |
| CAT, U/mL | 89.62 | 4.61 | 95.87 | 3.72 | 0.308 |
| GSH-Px, U/mL | 60.52 | 2.90 | 62.07 | 2.35 | 0.685 |
| MDA, nmol/mL | 4.89 | 0.17 | 5.00 | 0.21 | 0.718 |
| Weaned piglets | | | | | |
| Duodenum | | | | | |
| SOD, U/mL | 54.35$^{b}$ | 2.96 | 65.69$^{a}$ | 4.10 | 0.042 |
| CAT, U/mL | 76.94 | 2.27 | 74.67 | 2.54 | 0.840 |
| GSH-P, U/mL | 74.33$^{b}$ | 4.03 | 86.79$^{a}$ | 3.59 | 0.024 |
| MDA, nmol/mL | 7.44$^{a}$ | 0.24 | 6.50$^{b}$ | 0.27 | 0.046 |

All of the values are expressed as the means and pooled SEM, n = 8; $^{a, b}$ Means without a common letter differ ($P < 0.05$). Con, control treatment, PQQ, PQQ·$Na_2$ treatment; SOD, superoxide dismutase; CAT, catalase; GSH-Px, glutathione peroxidase; MDA, malondialdehyde.

| Item | Con | | PQQ | | P-value |
|---|---|---|---|---|---|
| | Mean | SEM | Mean | SEM | |
| Jejunum | | | | | |
| SOD, U/mL | 51.85[b] | 3.02 | 61.82[a] | 3.08 | 0.037 |
| CAT, U/mL | 110.74[b] | 4.41 | 131.10[a] | 6.34 | 0.020 |
| GSH-Px, U/mL | 92.02[b] | 4.89 | 112.25[a] | 6.10 | 0.014 |
| MDA, nmol/mL | 6.21[a] | 0.18 | 5.43[b] | 0.23 | 0.019 |
| Ileum | | | | | |
| SOD, U/mL | 48.31 | 2.31 | 49.86 | 2.88 | 0.682 |
| CAT, U/mL | 101.86 | 3.77 | 113.89 | 4.51 | 0.060 |
| GSH-Px, U/mL | 93.35[b] | 3.96 | 107.29[a] | 4.20 | 0.030 |
| MDA, nmol/mL | 5.72[a] | 0.20 | 5.04[b] | 0.24 | 0.027 |

All of the values are expressed as the means and pooled SEM, n = 8; [a, b] Means without a common letter differ ($P < 0.05$). Con, control treatment, PQQ, PQQ·$Na_2$ treatment; SOD, superoxide dismutase; CAT, catalase; GSH-Px, glutathione peroxidase; MDA, malondialdehyde.

# Gene expression of antioxidant status

The mRNA expression levels of antioxidant in the jejunum of newborn piglets are presented in Fig. 1a. The mRNA expression levels of SOD1, CAT and MGST1 in the jejunum were increased ($P < 0.05$) by dietary PQQ·$Na_2$ supplementation of newborn piglets. The mRNA expression levels of antioxidant in the jejunum of weaned piglets are presented in Fig. 1b. The mRNA expression levels of HO1, SOD1, CAT, SOD2, GPX4, GPX1 and GCLC in the jejunum were increased ($P < 0.05$) by dietary PQQ·$Na_2$ supplementation of weaned piglets.

# Gene expression of concentrations of cytokines

Immune responses in the intestinal mucosa are partly controlled by cytokine release in response to environmental stimuli. We therefore investigated the gene expression of cytokines in the jejunum. The results are shown in Fig. 2. The mRNA expression of pro-inflammatory cytokines including IL-1β in the jejunal of newborn piglets were decreased ($P < 0.05$). The IL-α, IL-2 and IL-8 cytokines were decreased ($P < 0.05$) by dietary PQQ·$Na_2$ in the jejunal of weaned piglets.

# Gene expression of tight junction proteins and glucose transporterin

Figure 3 shows that dietary supplementation with PQQ·$Na_2$ influenced the expression of tight junction proteins in the jejunum of piglets. The mRNA expression of ZO-1 was increased ($P < 0.05$) in the jejunal of newborn piglets. The mRNA expression of Occludin and ZO-1 were increased ($P < 0.05$) in the jejunal of weaned piglets. Figure 4 shows that dietary supplementation with PQQ·$Na_2$ influenced the expression of glucose transporterin in the jejunum of piglets. The mRNA expression of SLC2A3 in the jejunal of newborn piglets was increased ($P < 0.05$) by dietary PQQ·$Na_2$. The mRNA expression of SLC2A1 and SLC2A3 in the jejunal of weaned piglets was increased ($P < 0.05$) by dietary PQQ·$Na_2$.

# Intestinal morphology and brush border enzymes activities of piglets

The effects of dietary PQQ·$Na_2$ supplementation during gestation and lactation on villous height, crypt depth and villous height/crypt depth (V/C) in the small intestine of weaned piglets are shown in Table 5. The villous height of duodenum and jejunum of weaned piglets was increased ($P < 0.05$) by dietary PQQ·$Na_2$. The results of brush border enzymes activities are shown in Table 6. The activity of lactase in the jejunal of weaned piglets exhibited an increasing tendency, but it was not significant ($P = 0.078$).

Table 5

Effects of dietary PQQ·$Na_2$ supplementation in small intestine of weaned piglets

| Item | Con | | PQQ | | P-value |
|---|---|---|---|---|---|
| | Mean | SEM | Mean | SEM | |
| Weaned piglets | | | | | |
| Duodenum (μm) | | | | | |
| Villous height | 262.28[b] | 41.25 | 324.88[a] | 50.11 | 0.040 |
| Crypt depth | 124.83 | 15.62 | 132.74 | 8.45 | 0.666 |
| V/C | 2.22 | 0.23 | 2.48 | 0.17 | 0.394 |
| Jejunum (μm) | | | | | |
| Villous height | 299.35[b] | 23.82 | 432.69[a] | 41.73 | 0.020 |
| Crypt depth | 122.12 | 9.63 | 144.75 | 14.12 | 0.072 |
| V/C | 2.46 | 0.158 | 3.05 | 0.36 | 0.140 |
| Ileum (μm) | | | | | |
| Villous height | 486.52 | 35.20 | 504.62 | 44.01 | 0.729 |
| Crypt depth | 184.50 | 12.06 | 192.52 | 26.34 | 0.308 |
| V/C | 2.69 | 0.25 | 2.86 | 0.22 | 0.749 |

All of the values are expressed as the means and pooled SEM, n = 8; [a, b] Means without a common letter differ ($P < 0.05$). Con, control treatment, PQQ, PQQ·$Na_2$ treatment, V/C, Villous height/Crypt depth.

Table 6

Effects of dietary PQQ·$Na_2$ supplementation on brush border enzymes activities in the jejunal of piglets

| Item | Con | | PQQ | | P-value |
|---|---|---|---|---|---|
| | Mean | SEM | Mean | SEM | |
| Newborn piglets | | | | | |
| Lactase (U/mL) | 1.68 | 0.22 | 1.54 | 0.18 | 0.700 |
| Sucrase (U/mL) | 7.74 | 0.61 | 8.21 | 0.64 | 0.603 |
| Maltase (U/mL) | 19.80 | 0.89 | 18.34 | 0.76 | 0.236 |
| Weaning piglets | | | | | |
| Lactase (U/mL) | 4.70 | 0.48 | 6.18 | 0.75 | 0.078 |
| Sucrase (U/mL) | 19.79 | 0.93 | 21.47 | 0.96 | 0.229 |
| Maltase (U/mL) | 40.84 | 1.92 | 42.63 | 1.68 | 0.496 |

All of the values are expressed as the means and pooled SEM, n = 8; $^{a, b}$ Means without a common letter differ ($P < 0.05$). Con, control treatment, PQQ, PQQ·$Na_2$ treatment.

# Summary of microbiota analysis

The intestinal microbiota exerts profound influences on the intestinal immune system and health status. To further understand these effects, we performed 16S rDNA gene sequencing to measure microbiota in the feces of weaned piglets with a total of 10 samples with 5 biological replicates in each group. Across all samples, 472,760 quality sequences were of sufficient quality for analysis. On average, 35,413 sequences per sample with a read length greater than 400 bp were obtained (Supplementary table 1). The top 15 phyla and genera in terms of relative abundance of the fecal bacteria are shown in Fig. 5. In weaned piglets, Bacteroidetes and Firmicutes were the most prevalent phyla in both Con and PQQ·$Na_2$ treatments, followed by Proteobacteria, Fusobacteria and Verrucomicrobia (Fig. 5a); the most prevalent genera were Alloprevotella and Bacteroides, followed by Prevotella and Fusobacterium (Fig. 5b).

# Differences in microbiota composition

Alpha diversity measurements of the fecal microbiota community, including the Shannon, Simpson, and Chao1 indices, were not affected by maternal PQQ·$Na_2$ supplementation (Table 7). At the phylum level, the abundance of Firmicutes was significantly increased ($P < 0.05$) and the abundance of Proteobacteria was significantly decreased ($P < 0.05$) (Table 8). At the genus level, the abundance of Alloprevotella was significantly increased ($P < 0.05$) and the abundance of Actinobacillus and Escherichia was decreased ($P < 0.05$) (Table 9).

Table 7
Effects of dietary PQQ·$Na_2$ supplementation in sows on alpha diversity in fecal microbiota of weaned piglets

| Item | Con | | PQQ | | P-value |
|---|---|---|---|---|---|
| | Mean | SEM | Mean | SEM | |
| Shannon index | 4.13 | 0.17 | 4.06 | 0.19 | 0.275 |
| Simpson index | 0.04 | 0.01 | 0.05 | 0.01 | 0.750 |
| Chao1 | 396.98 | 18.83 | 383.12 | 22.16 | 0.646 |
| ace | 393.38 | 18.01 | 381.15 | 23.41 | 0.690 |

Table 8
Effects dietary PQQ·$Na_2$ in sows on fecal microbiota composition of weaned piglets at phylum level

| Item (%) | Con | | PQQ | | P-value |
|---|---|---|---|---|---|
| | Mean | SEM | Mean | SEM | |
| Bacteroidetes | 45.50 | 3.13 | 48.11 | 3.13 | 0.577 |
| Firmicutes | $34.66^{b}$ | 0.98 | $38.42^{a}$ | 0.83 | 0.026 |
| Proteobacteria | $13.05^{a}$ | 1.95 | $5.55^{b}$ | 0.80 | 0.012 |
| Fusobacteria | 3.41 | 1.02 | 4.82 | 1.75 | 0.366 |
| Verrucomicrobia | 0.71 | 0.63 | 1.57 | 1.02 | 0.299 |
| Spirochaetes | 1.14 | 0.38 | 1.24 | 0.37 | 0.926 |
| Synergistetes | 0.17 | 0.03 | 0.07 | 0.31 | 0.275 |
| Actinobacteria | 0.20 | 0.05 | 0.15 | 0.06 | 0.394 |
| Cyanobacteria | 0.19 | 0.07 | 0.22 | 0.10 | 0.73 |
| Tenericutes | 0.20 | 0.03 | 0.22 | 0.08 | 0.677 |

Table 9
Effects dietary PQQ·$Na_2$ in sows on fecal microbiota composition of weaned piglets at genus level

| Item (%) | Con | | PQQ | | P-value |
|---|---|---|---|---|---|
| | Mean | SEM | Mean | SEM | |
| Bacteroidetes | 9.09 | 0.66 | 9.24 | 0.56 | 0.887 |
| Alloprevotella | $7.62^{b}$ | 0.71 | $11.41^{a}$ | 1.09 | 0.027 |
| Prevotella | 5.90 | 0.95 | 7.34 | 1.42 | 0.532 |
| Fusobacterium | 4.16 | 0.86 | 4.82 | 1.26 | 0.682 |
| Eubacterium | 1.87 | 0.61 | 2.78 | 0.37 | 0.061 |
| Actinobacillus | $4.59^{a}$ | 0.81 | $1.58^{b}$ | 0.80 | 0.040 |
| Escherichia | $3.89^{a}$ | 0.87 | $0.47^{b}$ | 0.31 | 0.010 |

# Discussion

The use of PQQ in nutrition is increasingly being discussed in the literature[13, 21]. Zhang et al reported that dietary PQQ·$Na_2$ supplementation during gestation and lactation of female rats can significantly increase the duodenum villous height, jejunum villous height, the activities of maltase and sucrase, the number of Lactobacillus spp. and the expression of ZO-1 and Claudin mRNA in weaned rats[17]. Yin et al suggested that the expression of the jejunal tight junction protein ZO-1 was significantly higher in pigs with PQQ·$Na_2$ supplementation[22]. The major objective of the present study was to investigate the influence of maternal dietary PQQ·$Na_2$ supplementation during gestation and lactation on intestinal health in piglets.

Unlike in humans, the weaning process for pigs involves an abrupt dietary shift from sow milk to a completely feed-based diet, which consequentially inhibits the intestinal architecture and function, resulting in gut associated disorders, such as inflammation and diarrhea[23]. Weaning stress disrupted free-radical metabolism and antioxidative system and then caused serious oxidative stress[24]. Weaning pigs are easily attacked by oxidative stress owning to its imbalance and immature antioxidant system in the intestine[25]. In this context, it seems like a feasible way to overcome impaired antioxidant status and poor immune response in piglets by using dietary antioxidant compounds after weaning[26–27]. Thus, dietary antioxidant concentrations need to be added in diets, especially to prevent excessive oxidative stress during weaning. In this study, the activities of SOD and GSH-Px were significantly increased and MDA was decreased by PQQ·$Na_2$ supplementation in the plasma and small intestine of piglets. SOD is known to serve a protective function for eliminating reactive free radicals and, therefore, it is an important

antioxidant defense for almost all cells exposed to oxygen. GSH forms an essential part of the nonenzymatic antioxidants[28]. As other sulfhydryl-containing products, GSH also shows regulatory and protective roles in the body, owning to its establishment of the defenses of the body against tissue injury due to chemicals through its ROS scavenging, cell viability and membrane-stabilizing effects[29]. MDA is an end product of free-radical chain reactions and lipid peroxidation[30], so it is frequently used to measure lipid peroxide levels, and it correlates well with the degree of lipid peroxidation. Yin et al shown that dietary PQQ·$Na_2$ increased the activities of SOD, GSH-Px and CAT, but reduced the concentration of MDA in the small intestine of piglets, and this is consistent with our study results. HO1 is conducive to the catalysis of heme to carbon monoxide, free ferrous iron and biliverdin[31]. GCLM is the modifier subunit of glutamate cysteine ligase, an important antioxidant that contributes to the maintenance of cellular redox status, which catalyzes the rate-limiting reaction in the de novo synthesis of glutathione[32]. Furthermore, the increased mRNA expression of the family of glutathione peroxidases (GPX), including GPX1 and GPX4, is in accord with enzyme activity in placenta. Organisms with exogenous toxins have a certain ability to detoxify. In biological detoxification systems, the glutathione S-transferase (GST) and UDP-glucuronosyltransferase (UGT) families play an extremely important role in biological detoxification systems, which are regulated by Nrf2 and ARE[33]. In this study dietary PQQ·$Na_2$ supplementation in the diet of sows improved the antioxidant status of intestine. The mRNA expression levels of SOD1, CAT and MGST1 in the jejunum were increased of newborn piglets and the mRNA expression levels of HO1, SOD1, CAT, SOD2, GPX4, GPX1 and GCLC in the jejunum were increased of weaned piglets. Accumulated evidences have shown that it has been practiced to enhance the antioxidant system, relieve stress, and regulate the dynamic balance of intestinal microbiota in livestock husbandry through supplementing natural antioxidants such as vitamins E and C, tea polyphenols, and probiotics[25].

The gastrointestinal tract acts as the primary immune organ because it must respond to pathogenic stimuli and maintain resistance to co-immunization and dietary antigens. Intestinal immunity is also closely related to other intestinal functions. Previous studies have shown that reduced the level of expression of inflammatory cytokine genes may help to establish a more stable ecosystem and interfere with the growth of certain bacterial populations. The balance between pro-inflammatory and anti-inflammatory cytokines is all-important in the health of weaned piglets[34]. Jiro Omata et al reported that PQQ is not included in parenteral nutrient solutions for clinically treated because it has not been proven to be an essential nutrient for the body. However, in patients receiving parenteral nutrition, it is possible to prevent gastrointestinal mucosal immune disorders exists by supplementation of PQQ[35]. Cytokines play a critical role in the immune response and inflammation and can be important mediators to prevent susceptibility to infection and some gastrointestinal dysfunctions happening[36]. Both in vitro and in vivo studies showed that a negative contribution to gut integrity and epithelial function, including permeability to macromolecules and transport of nutrients and ions partly derives from the uncontrolled synthesis of pro-inflammatory cytokines[37].

Weaning in pigs is associated with an early and transient response in gene expression of inflammatory cytokines in the gut[38]. It has been shown that pro-inflammatory cytokines such as IL-1β and IL-2

mediate the host inflammatory response to prevent infection[39]; IL-1β also can increase the tight junction permeability[40]. The intestinal expression of pro-inflammatory cytokine genes, such as IL-1β, IL-6, and TNF-α, has been reported to be up-regulated in weaned piglets[38]. In this study, the data suggest that dietary supplementation with 20 mg/kg PQQ·$Na_2$ in sows modulated immune responsivity by inhibiting the expression of pro-inflammatory cytokines in weaned piglets. Hu et al[41] reported that dietary supplementation with 50 mg/kg low-molecularweight chitosan which has antioxidant activity significantly reduced the jejunal mucosal expression of pro-inflammatory cytokines. Yin et al suggested that the concentrations of IFN-γ, IL-1β and IL-2 were decreased in pigs as the PQQ·$Na_2$ supplementation was increased and this is consistent with our study results[22].

Parenteral nutrition can be useful to prevent and reverse malnutrition and to maintain the nutritional status of patients who are unable to receive adequate enteral nutrition. During weaning, the morphology of intestinal tract often changes greatly, characterized by a decrease in the height of the villi and an increase in the depth of the crypt. This finding suggests that owning to nutrient absorption, the surface area present on the villi is so small that the increase in immature fluff formed in the crypt may reduce the overall growth performance of the animal. In this study, the villous height of duodenum and jejunum of weaned piglets was increased by dietary PQQ·$Na_2$. Variations in villous height and the villous height: crypt ratio are both good indicators of ameliorative nutrient digestion and absorption capacity of the small intestine. Previous studies have shown that lower villus height: crypt depth is associated with microbial challenges and the composition of animal feed[42]. Hedemann et al reported that the growth of many pathogens can be inhibited by the increases in villus length[43]. The intestinal epithelial layer plays a role of a barrier to the infiltration of harmful intestinal contents into the bloodstream. In the jejunum, when the microvilli become more sparse and shorter, the permeability of the intestinal epithelial layer, the inflammatory response and the inflammatory response all increases[44]. Therefore, we have further investigations of the gene expression of tight junction proteins and cytokine concentrations in the jejunum of piglets. The balance between pro-inflammatory and anti-inflammatory cytokines is of great importance for the health of weaned piglets[34]. Tight junction proteins (Claudin 1, ZO-1 and Occludin) have an enormous importance in intestinal barrier integrity and permeability. They can prevent the paracellular diffusion of intestinal bacteria and other antigens across the epithelium by sealing the paracellular space between epithelial cells[45]. Friedman JA, et al reported that continuous exposure to PQQ improved barrier dysfunction[46]. Yin et al suggested that with the increase of PQQ·$Na_2$, the expression of the jejunal tight junction protein ZO-1 was significantly higher in pigs[22]. In this study, the mRNA expression of Occludin and ZO-1 were increased in the jejunal of weaned piglets with the PQQ·$Na_2$ supplementation. Zhang et al[17] reported that dietary PQQ·$Na_2$ supplementation during gestation and lactation of female rats can significantly increase the expression of ZO-1 and Claudin mRNA in the jejunal mucosa of weaned rats which is consistent with our study results.

The mammalian intestinal microbiota is consist of trillions of microbes that facilitate host health, including colonization resistance against gastrointestinal disorders[47]. There is a far-reaching implication for the gastrointestinal microbiota of newborn animals on host health through regulating

intestinal nutritional metabolism, maturating immune system, and establishing the gut barrier[48]. Microbial dysbiosis, characterized aberrations in the structure and function of gut microbiota, is a key factor that impact several bowel diseases and inflammatory intestinal disorders[49]. In the present study, we analyzed the fecal microbiota in weaning by 16S rDNA sequencing. At the at the phylum level, Firmicutes and Bacteroidetes were predominant in weaned piglets, which is in agreement with that of Bian et al[50]. Firmicutes was the most predominant phylum in weaned piglets, because Firmicutes are predominant bacteria in intestinal tract, including the Clostridia and Bacilli class and the Lactobacteriales, and capable of oxidising organic sugars via fermentation to produce large amounts of lactic acid[51–52]. In this study, the Firmicutes level was increased and the Proteobacteria level was decreased. Earlier studies have shown that Firmicutes, Bacteroidetes and Proteobacteria were the most dominant phyla in pigs[53–54] which was similar with our study. At the genus level, we observed that the Escherichia was significantly decreased. Post-weaning diarrhoea, induced by enterotoxigenic Escherichia coli, is one of reasons causing poor growth performance and swine disease[55]. Recent studies have also shown that enterotoxigenic Escherichia coli infection could impair intestine and induce the inflammatory response in weaned piglets[56–57]. In this study, the results shown that dietary PQQ·$Na_2$ in sows could decreased the Escherichia in weaned piglets in order to improve the intestinal health.

# Conclusions

In conclusion, our results have shown that dietary 20 mg/kg PQQ·$Na_2$ in sows could improve the antioxidant status, including increasing CAT, SOD and GSH-Px activity and decreasing MDA activity in the small intestine and plasma of piglets. The mRNA expression levels of SOD1, CAT and MGST1 in the jejunum were increased in newborn piglets, and the mRNA expression levels of HO1, SOD1, CAT, SOD2, GPX4, GPX1 and GCLC in the jejunum were increased in weaned piglets. Dietary supplementation with 20 mg/kg PQQ·$Na_2$ in sows modulated immune responsivity by inhibiting the expression of proinflammatory cytokines in weaned piglets. The villous height of the duodenum and jejunum of weaned piglets was increased by dietary PQQ·$Na_2$, and the mRNA expression of Occludin and ZO-1 was increased in the jejunum of weaned piglets with PQQ·$Na_2$ supplementation. Firmicutes and Bacteroidetes were predominant in weaned piglets at the phylum level. Dietary supplementation with 20 mg/kg PQQ·$Na_2$ in sows increased the Firmicutes level and decreased the Escherichia abundance in weaned piglets. Based on the results from the current study, dietary supplementation with 20 mg/kg PQQ·$Na_2$ improved the antioxidant status, villous height, intestinal barrier function and intestinal microflora in offspring.

# Abbreviations

PQQ·$Na_2$:Pyrroloquinoline quinone disodium; SOD:Superoxide dismutase; GSH-Px:Glutathione peroxidase; MDA:Malondialdehyde; CAT:Catalase; MGST1:Microsomal glutathione S-transferase 1; HO1:Heme oxygenase 1; SOD1:Superoxidedismutase 1; SOD2:Superoxidedismutase 2; SOD3:Superoxide dismutase 3; GPX:Glutathione peroxidases; GPX4:Glutathione peroxidases 4; GPX1:Glutathione

peroxidases 1; NQO1:NAD(P)H quinone dehydrogenase 1; CYP1A1:Cytochrome P450 family 1 subfamily A member 1; TXNRD1:Thioredoxin reductase 1; GCLC:Glutamate-cysteine ligase catalytic; PQQ:Pyrroloquinoline quinone; PBS:Phosphate-buffered saline; RT-PCRs:Reverse transcription polymerase chain reactions; RDP:Ribosomal Database Project; ANOVA:Analysis of variance; SEMs:Standard errors of the mean; IL-1:Interleukin 1; IL-1β:Interleukin 1β; IL-2:Interleukin 2; IL-6:Interleukin 6; IL-8:Interleukin 8; TNF-α:Tumor necrosis factor α; SLC2A1:Solute carrier family 2 member 1; SLC2A3:Solute carrier family 2 member3; GCLM:Glutamate-cysteine ligase modifier; GST:Glutathione S-transferase; UGT:UDP-glucuronosyltransferase; UGT1A1:UDP glucuronosyl- transferase family 1 memberA1; Nrf2:Nuclear factor, erythroid 2 like 2; IFN-γ:Interferon-gamma; V/C:Villous height/Crypt depth

# Declarations

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# Authors' contributions

All authors participated in the development of the study concept and design; C. W., B. Z. and H. Z. were responsible for the execution of the study; W. Y., Q. M. and S. B. were involved in the animal experiments, analysis and data collection; C. W. and B. Z. were responsible for the statistical analysis; C. W. wrote the draft of the manuscript; B. Z., A. S. and S. B. reviewed and revised the manuscript. None of the authors has any conflicts of interest to declare. All authors read and approved the final manuscript.

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# Availability of data and materials

The datasets produced and/or analyzed during the current study are available from the corresponding author on reasonable request.

# Ethics approval and consent to participate

The protocols used in this experiment were approved by the Northeast Agricultural University Institutional Animal Care and Use Committee. All animal experimental procedures were approved by the Ethical and

Animal. Welfare Committee of Heilongjiang Province, China.

## Consent for publication

Not applicable.

## Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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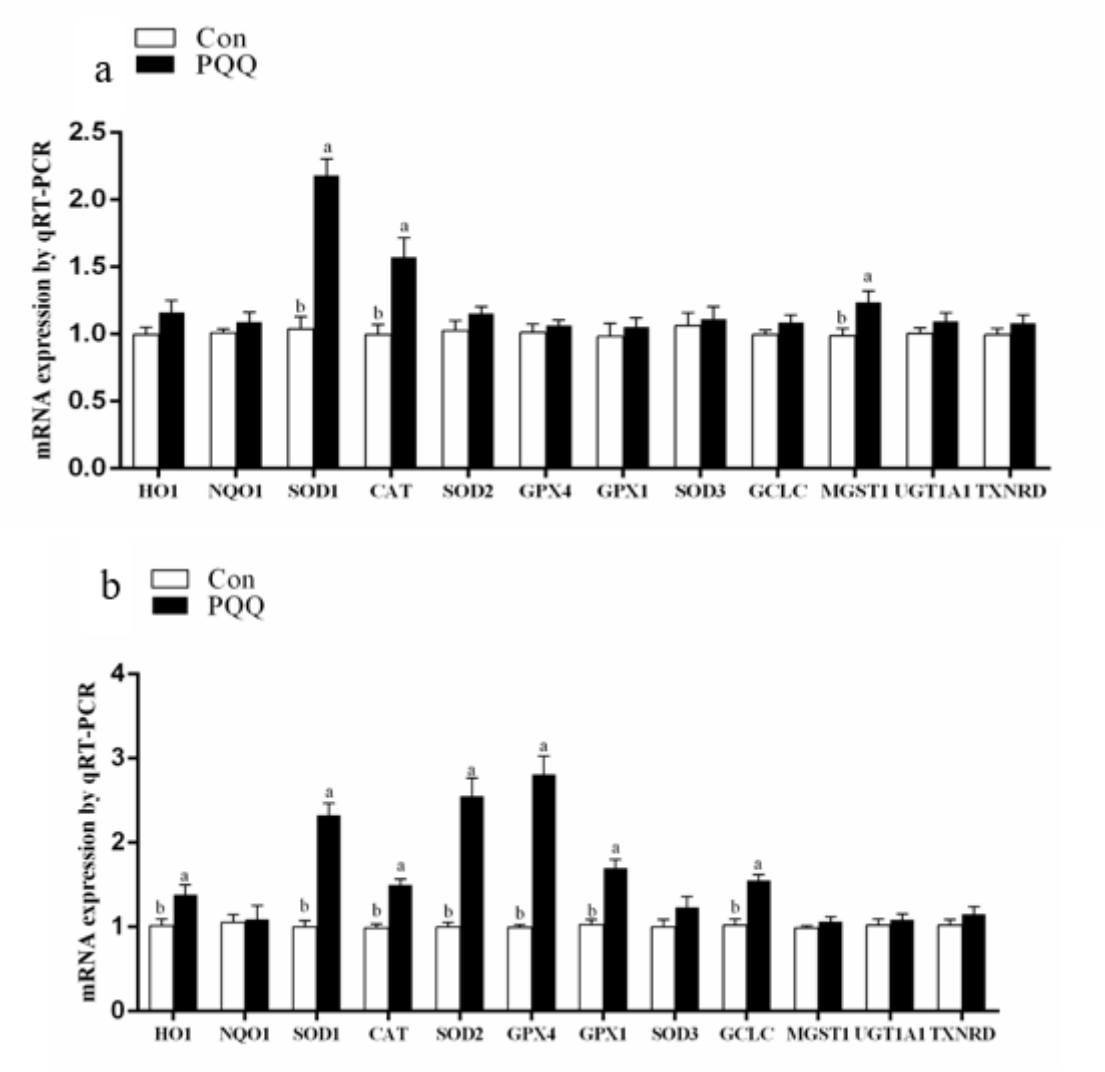
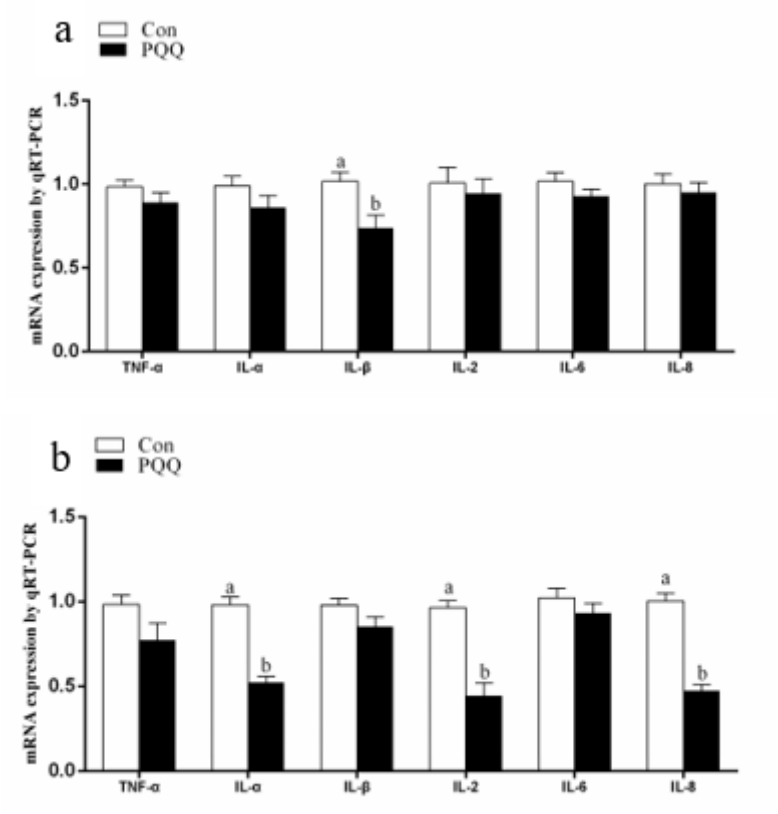
# Figures



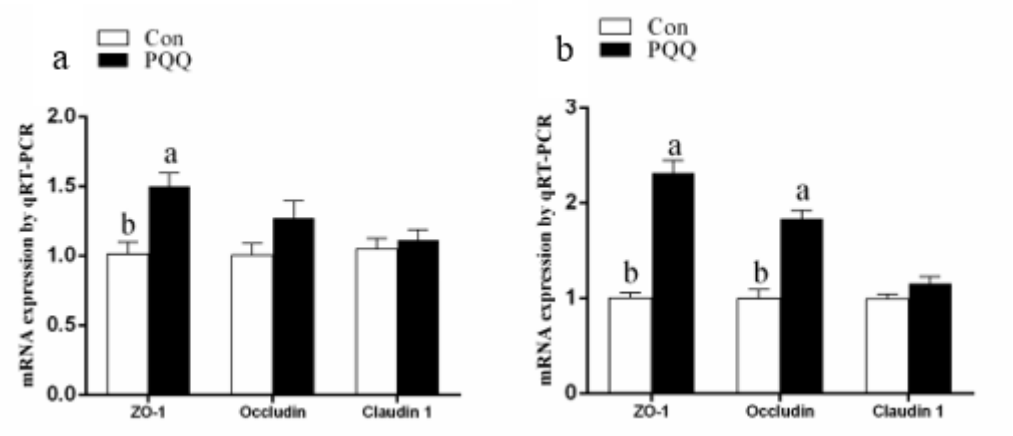

## Figure 1

Effects of dietary PQQ·Na2 supplementation during gestation and lactation on gene expression of antioxidant status in the jejunum of piglets. a, the gene expression of antioxidant status in the jejunum of newborn piglets; b, the gene expression of antioxidant status in the jejunum of weaned piglets. Con, control treatment, PQQ, PQQ·Na2 treatment. All values are expressed as means ± SEM (n=8). a,b,c Mean values within a column with unlike superscript letters were significantly different (P<0.05).



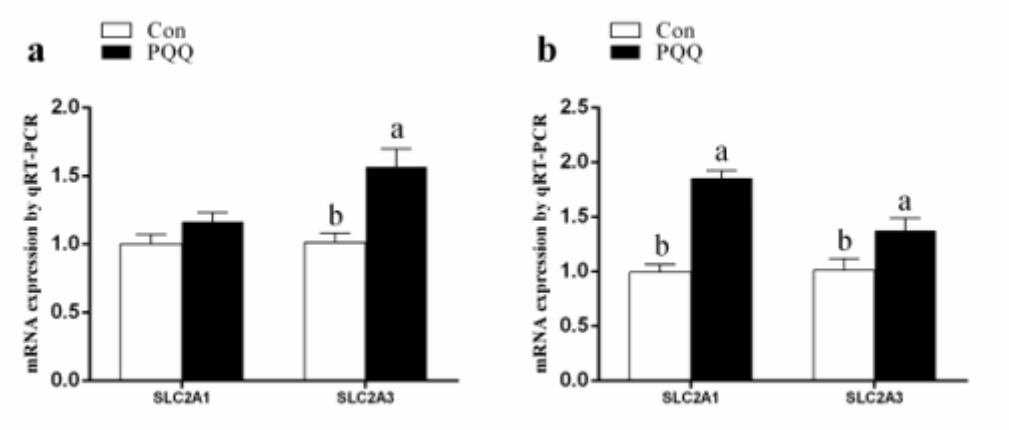

## Figure 2

Effects of dietary PQQ·Na2 supplementation during gestation and lactation on gene expression of concentrations of cytokines in the jejunum of piglets. a, the gene expression of concentrations of cytokines in the jejunum of newborn piglets; b, the gene expression of concentrations of cytokines in the jejunum of weaned piglets. Con, control treatment, PQQ, PQQ·Na2 treatment. All values are expressed as means ± SEM (n=8). a,b,c Mean values within a column with unlike superscript letters were significantly different (P<0.05).



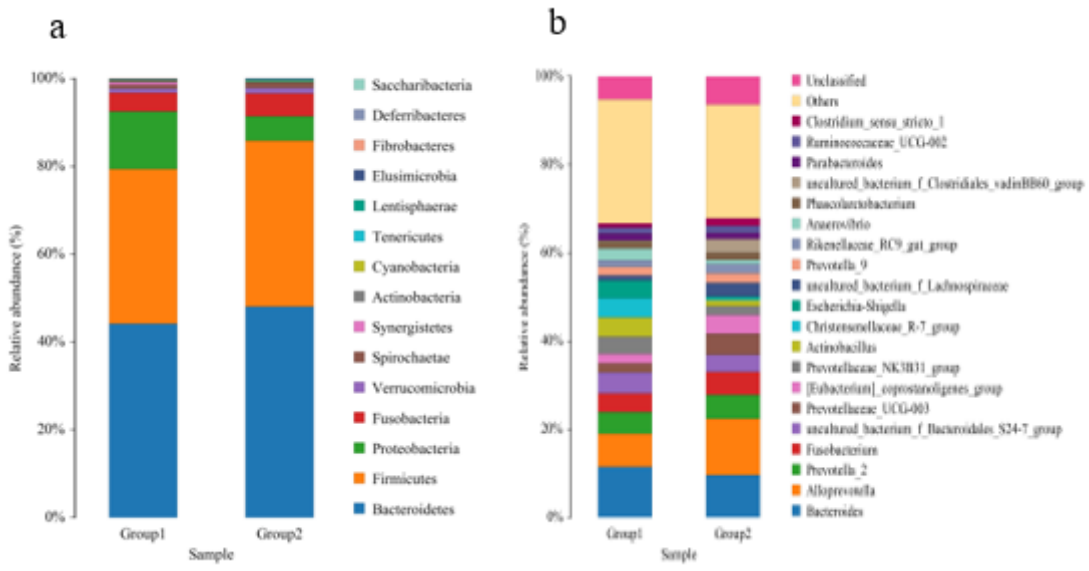

## Figure 3

Effects of dietary PQQ·Na2 supplementation during gestation and lactation on gene expression of tight junction proteins in the jejunum of piglets. a, the gene expression of tight junction proteins in the jejunum of newborn piglets; b, the gene expression of tight junction proteins in the jejunum of weaned piglets. Con, control treatment, PQQ, PQQ·Na2 treatment. All values are expressed as means ± SEM (n=8). a,b,c Mean values within a column with unlike superscript letters were significantly different (P<0.05).




## Figure 4

Effects of dietary PQQ·Na2 supplementation during gestation and lactation on gene expression of glucose transporterin the jejunum of piglets. a, the gene expression of glucose transporterin in the jejunum of newborn piglets; b, the gene expression of glucose transporterin in the jejunum of weaned piglets. Con, control treatment, PQQ, PQQ·Na2 treatment. All values are expressed as means ± SEM (n=8). a,b,c Mean values within a column with unlike superscript letters were significantly different (P<0.05).




## Figure 5

Fecal microbiota composition of weaned piglets. a, The phyla of fecal bacteria in weaned piglets. b, The genera of fecal bacteria in weaned piglets. Group 1, control treatment, Group 2, PQQ·Na2 treatment.

# Supplementary Files

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