



## Original Research Article

# Dietary dimethylglycine sodium salt supplementation alleviates redox status imbalance and intestinal dysfunction in weaned piglets with intrauterine growth restriction



Kaiwen Bai <sup>a</sup>, Luyi Jiang <sup>b</sup>, Qiming Li <sup>a</sup>, Jingfei Zhang <sup>a</sup>, Lili Zhang <sup>a</sup>, Tian Wang <sup>a,\*</sup>

<sup>a</sup> College of Animal Science and Technology, Nanjing Agricultural University, Nanjing, China

<sup>b</sup> College of Animal Science, Zhejiang University, Hangzhou, China

## ARTICLE INFO

## Article history:

Received 15 June 2021

Received in revised form

28 November 2021

Accepted 15 December 2021

Available online 25 January 2022

## Keywords:

Intrauterine growth restriction

Weaned piglet

Jejunum

Redox status

Mitochondrial function

Dimethylglycine sodium salt

## ABSTRACT

There are few studies on the mechanism of redox status imbalance and intestinal dysfunction in intrauterine growth restricted (IUGR) newborn piglets. Here, we investigated the mechanism of jejunum dysfunction in weaned piglets with IUGR and the mechanism through which dimethylglycine sodium salt (DMG-Na) supplementation improving the imbalance of their redox status. In this work, a total of 10 normal birth weight (NBW) newborn piglets and 20 IUGR newborn piglets were obtained. After weaning at 21 d, they were assigned to 3 groups ( $n = 10/\text{group}$ ): NBW weaned piglets fed standard basal diets (NBWC); one IUGR weaned piglets fed standard basal diets (IUGRC); another IUGR weaned piglets from the same litter fed standard basal diets plus 0.1% DMG-Na (IUGRD). The piglets in these 3 groups were sacrificed at 49 d of age, and the blood and jejunum samples were collected immediately. The growth performance values in the IUGRC group were lower ( $P < 0.05$ ) than those in the NBWC group. Jejunum histomorphological parameters, inflammatory cytokines, and digestive enzyme activity as well as serum immunoglobulin were lower ( $P < 0.05$ ) in the IUGRC group than those in the NBWC group. Compared with these in the NBWC group, the redox status of serum, jejunum, and mitochondria and the expression levels of jejunum redox status-related, cell adhesion-related, and mitochondrial function-related genes and proteins were suppressed in the IUGRC group ( $P < 0.05$ ). However, compared with those in the IUGRC group, the growth performance values, jejunum histomorphological parameters, inflammatory cytokines, digestive enzyme activity, serum immunoglobulin, redox status of serum, jejunum, and mitochondria, and the expression levels of jejunum redox status-related, cell adhesion-related, and mitochondrial function-related genes and proteins were improved ( $P < 0.05$ ) in the IUGRD group. In conclusion, dietary DMG-Na supplementation alleviates redox status imbalance and intestinal dysfunction in IUGR weaned piglets mainly by activating the sirtuin 1 (SIRT1)/peroxisome proliferator-activated receptor $\gamma$ coactivator-1 $\alpha$  (PGC1 $\alpha$ ) pathway, thereby improving their unfavorable body state.

© 2022 Chinese Association of Animal Science and Veterinary Medicine. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co. Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

\* Corresponding author.

E-mail address: [tianwangnjau@163.com](mailto:tianwangnjau@163.com) (T. Wang).

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



Production and Hosting by Elsevier on behalf of KeAi

## 1. Introduction

Intrauterine growth restricted (IUGR) newborns are defined as small-sized individuals with weights below the 10th-centile for their gestational age or the population mean minus 2 standard deviations of a population-based nomogram, and their occurrence is an important problem in animal husbandry (Dong et al., 2014; Li et al., 2021). This growth restriction exerts a permanent stunting effect on the efficiency of nutrient utilization in posterity and impairs their long-term health (Xu et al., 2016; Zhang et al., 2019). The small intestine is crucial for the digestion, absorption, and

metabolism of dietary nutrients. However, little is known regarding the postnatal effects of IUGR on the small intestinal function, which is very important because of its role in postnatal immune system maturation to prevent the body from environmental microorganisms. The alteration of small intestinal functions may result in diseases easily after birth, and slow gastrointestinal growth may, in turn, contribute to slow growth rates in IUGR newborns. In addition, reduction redox status of the gastrointestinal tract may compromise the health of IUGR neonates throughout the postnatal stage and later in adulthood (He et al., 2011; Dong et al., 2014). Oxidative damage was caused by a disturbance of the antioxidant systems and free radical generation systems, which enhanced the reactive oxygen species (ROS) levels, and finally, mediate tissue and mitochondrial damage. Mitochondria are the main cellular sites where provide energy production, and compromised mitochondrial function is associated with numerous diseases, like intrauterine growth restriction (Dillin et al., 2002; Cheng et al., 2020).

Dimethylglycine sodium salt (DMG-Na) can improve body immunity and relieve oxidative damage by scavenging excess free radicals (Feng et al., 2018). DMG-Na is beneficial in improving the redox status by acting as an important substrate for synthesizing glutathione (Friesen et al., 2007). Studies revealed that DMG-Na can improve the offspring growth performance through relieving their oxidative damage induced ROS excessive generation (Hariganesh and Prathiba, 2000; Friesen et al., 2007). It has been reported that DMG-Na could improve the utilization of oxygen to protect the body against excessive free radical generation and enhance their immune status (Bai et al., 2019). In the present study, it was found that decreased sirtuin 1 (SIRT1) activity (via decreasing of its substrate peroxisome proliferator-activated receptor-coactivator-1 $\alpha$  [PGC1 $\alpha$ ]) could damage the redox status and suppress the mitochondrial function of the jejunum in IUGR weaned piglets. We also provide a novel insight into the SIRT1/PGC1 $\alpha$  pathway-mediated effects of DMG-Na on the jejunum redox status and mitochondrial function in IUGR weaned piglets.

## 2. Materials and methods

This trial was conducted according to the Chinese guidelines for animal welfare and experimental protocols for animal care and authorized by the Nanjing Agricultural University Institutional Animal Care and Use Committee (SYXK[Su]2017-0027).

### 2.1. Experimental design and sample collection

This experiment was conducted on a trial pig farm that was owned by the Yangzhou Fangling Agricultural and Pastoral Co., Ltd. (Jiangsu, China). A total of 80 healthy pregnant multiparous sows (Landrace  $\times$  Yorkshire) with similar expected farrowing dates (less than 3 d) and parity (the second or third) were preselected during gestation. The sows were fertilized by the pool of Duroc boars and fed the same gestating diet that met the National Research Council (NRC, 2012) nutrient requirements. At farrowing, 10 sows that had 11 to 13 live-born piglets and met the selection criteria for IUGR were chosen. In this study, 10 normal birth weight (NBW) newborn piglets (1.53  $\pm$  0.04 kg) and 20 IUGR newborn piglets (0.76  $\pm$  0.06 kg) were selected from 10 sows according to the method described by Wang et al. (2005), respectively. All newborn piglets were naturally weaned at 21 d of age, and one NBW weaned piglet and 2 IUGR weaned piglets were collected from each sow. They were allocated to 3 groups with 10 piglets per group. NBWC: NBW weaned piglets fed standard basal diets (Appendix Table 1); IUGRC: one IUGR weaned piglets fed standard basal diets; IUGRD: another IUGR weaned piglets from the same sow fed standard basal diets plus 0.1% DMG-Na (99.9% of purity, obtained from Qilu Sheng Hua Pharmaceutical Co.,

Ltd., Shandong, China). They were housed individually in plastic floored pens (1 m  $\times$  0.6 m) in an environmentally controlled room (temperature of 28 °C and free access to water). At 49 d of age, all the piglets were weighed after feed deprivation for 12 h to measure average daily weight gain (ADG), average daily feed intake (ADFI), and weight gain-to-feed intake (G:F) ratio to evaluate the feed conversion. The blood was withdrawn from the precaval vein, and the serum was separated by centrifugation at 3,500  $\times$  g for 15 min at a temperature of 4 °C, and then stored at a temperature of -80 °C for further study. After that, the piglets were anesthetized via electrical stunning and sacrificed by exsanguination. Subsequently, the jejunum samples were harvested immediately, one (1 cm  $\times$  1 cm  $\times$  1 cm) sample embedded into 1% (vol/vol) glutaraldehyde solution and another (1 cm  $\times$  1 cm  $\times$  1 cm) sample embedded into 4% buffered formaldehyde for the morphological measurements, the rest sample stored at -80 °C for further study.

### 2.2. Evaluation of histomorphology of the jejunum

This assay was carried out according to the previous method described by Dong et al. (2014). Jejunum samples that fixed in 1% (vol/vol) glutaraldehyde solution were examined using a Philips 420 transmission electron microscope (Philips, Amsterdam, The Netherlands) at 80 kV.

The jejunum samples fixed in 4% buffered formaldehyde were dried using a graded series of xylene and ethanol and then embedded into paraffin for histological processing according to the method described before (Dong et al., 2014). Ten slides of the middle site of each sample were obtained, and the images were acquired. Villus length, villus width, and crypt depth were metered, and the villus area was calculated using the following formula:

$$\text{Villus area} = \pi \times \left( \frac{\text{Villus width}}{2} \right) \sqrt{\left( \frac{\text{Villus width}}{2} \right)^2 + \text{Villus length}^2}$$

### 2.3. Measurement of immunoglobulin in serum and jejunum

Serum immune globulin A (IgA), immune globulin G (IgG), and immune globulin M (IgM) concentrations were tested by corresponding ELISA assay kits bought from Nanjing Jiancheng Bioengineering Institute according to a previously described method (Lebacqz-Verheyden et al., 1972).

Jejunum samples were homogenized in 0.9% sodium chloride buffer on ice and then centrifuged at 2,800  $\times$  g for 15 min at a temperature of 4 °C. The supernatant was used to measure the secreted immunoglobulin A (sIgA) concentration using an ELISA assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) (Lebacqz-Verheyden et al., 1972).

### 2.4. Concentrations of jejunum inflammatory cytokine

The concentrations of the inflammatory cytokines in the jejunum, including interleukin (IL)-1 $\beta$ , IL-6, IL-8, and tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), were calculated by ELISA assay kits according to their manufacturer's instructions (Elabscience Biotechnology Co. Ltd, Wuhan, China).

### 2.5. Concentrations of jejunum digestive enzyme

The jejunum samples were homogenized with a hand-held homogenizer in 1 mL of cold PBS (pH = 7.4, 0.01 mol/L). The homogenate was centrifuged at 500  $\times$  g for 10 min at a temperature of

4 °C, and the supernatants were collected. The activity of the digestive enzymes sucrase, maltase, lactase, amylase, lipase, and chymotrypsin were determined according to the manufacturer's instructions of Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China).

## 2.6. Measurement of redox status

Jejunum samples were homogenized in 0.9% sodium chloride solution on ice and then centrifuged at  $3,500 \times g$  for 15 min at a temperature of 4 °C. Both serum and jejunum supernatant solution were used to measure the levels of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione (GSH), and malondialdehyde (MDA) according to the manufacturer's instructions of corresponding assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Protein content was tested with a bicinchoninic acid (BCA) protein assay kit purchased from Nanjing Jiancheng Bioengineering Institute.

## 2.7. Measurement of jejunum mitochondrial redox status

Jejunum mitochondria was isolated using the mitochondria isolation kit (Solarbio, Beijing, China). The levels of protein content, manganese superoxide dismutase (MnSOD), GSH-Px, GSH, and  $\gamma$ -glutamylcysteine ligase ( $\gamma$ -GCL) were calculated according to the manufacturer's instructions with the corresponding assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

The ROS concentration was measured using a ROS assay kit bought from the Nanjing Jiancheng Institute of Bioengineering. Briefly, the mitochondria were incubated with 10  $\mu\text{mol/L}$  of dichlorodihydrofluorescein diacetate (DCFH-DA) and 10  $\text{mmol/L}$  of DNA stain Hoechst 33,342 at 37 °C for 30 min. Then the DCFH fluorescence of the mitochondria was measured at an emission wavelength of 500 nm and an excitation wavelength of 525 nm with a fluorescence reader (SpectraMax Gemini EM; Molecular Devices, Franklin Lakes, NJ, USA). The results were expressed as the mean DCFH-DA fluorescence intensity over that of the control. The mitochondrial membrane potential (MMP) level was calculated using an MMP assay kit bought from the Nanjing Jiancheng Institute of Bioengineering. Briefly, the mitochondria were loaded with  $1 \times \text{JC-1}$  dye at 37 °C for 20 min, and then analyzed, after washing, by a fluorescence microscope. The MMP was calculated as the increase in ratio of green and red fluorescence. The results were calculated as the ratio of the fluorescence of aggregates (red) to that of the monomers (green). The number of apoptotic cells and necrotic cells was tested by an Alexa Fluor 488 Annexin V/dead Cell Apoptosis kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA) and carried out according to their manufacturer's instructions. Protein carbonyls (PC) and 8-hydroxy-2-deoxyguanosine (8-OHdG) in the jejunum sample were calculated using their respective ELISA assay kits and carried out following the manufacturer's instructions (Nanjing Jiancheng Institute of Bioengineering, Nanjing, China) (Berlett and Stadtman, 1997; Valavanidis et al., 2009; Bai et al., 2011).

The adenosine triphosphate (ATP) content in the jejunum sample was determined using an ATP Assay Kit (Solarbio, Beijing, China) following the manufacturer's instructions. The mitochondrial DNA (mtDNA) copy number of the jejunum sample was determined using a real-time fluorescence quantitative polymerase chain reaction (PCR) kit (Tli RNaseH Plus; Takara Bio, Inc., Otsu, Japan). In brief, the 20  $\mu\text{L}$  PCR mixture consisted of 10  $\mu\text{L}$  of SYBR Premix Ex Taq ( $2 \times$ ), 0.4  $\mu\text{L}$  of upstream primer, 0.4  $\mu\text{L}$  of downstream primer, 0.4  $\mu\text{L}$  of ROX dye ( $50 \times$ ), 6.8  $\mu\text{L}$  of ultra-pure water, and 2  $\mu\text{L}$  of cDNA template. The sequence of the Mt D-loop gene upstream primer was 5'-AGGACTACGGCTTGAAGC-3', and that of

the downstream primer was 5'-CATCTTGGCATCTTCAGTGCC-3'; the length of the target fragment was 198 bp. The sequence of the  $\beta$ -actin upstream primer was 5'-TTCTTGGGTATGGAGTCTG-3' and that of the downstream primer was 5'-TAGAAGCATTGCGGTGG-3'; the length of the target fragment was 150 bp. The amplification of each jejunum sample was performed in triplicate. The fold-expression of each gene was calculated according to the  $2^{-\Delta\Delta\text{Ct}}$  method (Mohamed et al., 2010), in which  $\beta$ -actin was used as an internal standard.

## 2.8. Quantitative real-time PCR analysis

Quantitative real-time PCR (qPCR) was conducted according to the method described before (Mohamed et al., 2010). Total RNA from each jejunum samples was extracted using a Trizol Reagent kit (TaKaRa, Dalian, China) and then reverse-transcribed with a kit (Perfect Real Time, SYBR PrimeScript, TaKaRa) following the manufacturer's instructions. The mRNA expression levels of genes listed in Appendix Table 2 were quantified via real-time qPCR using SYBR Premix Ex Taq II (Tli RNaseH Plus, TaKaRa) with an ABI 7300 Fast Real-Time PCR detection system (Applied Biosystems, Foster City, CA, USA). The  $\beta$ -actin was acted as an internal standard. The qRT-PCR amplification efficiency was calculated according to specific gene standard curves generated from serial dilutions. The gene expression levels of the target genes were analyzed by the  $2^{-\Delta\Delta\text{Ct}}$  method (Mohamed et al., 2010) after verifying that the primers were amplified with an efficiency of approximately 100%, and data for IUGRC and IUGRD groups were compared with the data for the NBWC group.

## 2.9. Western blot analysis

Total protein was isolated from the 3 jejunum samples per group with a radioimmunoprecipitation assay lysis buffer containing protease inhibitor cocktail (Beyotime Institute of Biotechnology, Jiangsu, China). The nuclear protein in the jejunum samples was carried out using a Nuclear Protein Extraction Kit (Beyotime Institute of Biotechnology, Jiangsu, China). The concentrations of total cellular protein and nuclear protein in the jejunum samples were measured by the bicinchoninic acid protein assay kit (Beyotime Institute of Biotechnology, Jiangsu, China). Antibodies against related proteins were all purchased from Cell Signaling Technology (Danvers, MA, USA). Thereafter, equal quantities of protein were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel and then transferred onto polyvinylidene difluoride membranes. After that, the membranes were incubated with blocking buffer (5% bovine serum albumin in Tris-buffered saline containing 1% Tween 20) for 1 h at room temperature and probed with the primary antibody (1:1,000) against nuclear factor erythroid 2-related factor 2 (Nrf2, # 12721 S), heme oxygenase 1 (HO1, # 82206 S), superoxide dismutase (SOD, # 37385 S), glutathione peroxidase (GSH-Px, # 3286 S), Sirt1 (# 9475 S), PGC1 $\alpha$  (# 2178 S), cytochrome C (Cyt C, # 11940S), estrogen-related receptor  $\alpha$  (ERR $\alpha$ , # 13826 S), mitochondrial transcription factor A (mtTFA, # 8076 S), nuclear respiratory factor 1 (NRF1, # 46743 S), mitochondrial mitofusin 2 (Mfn2, # 9482 S), dynamin-related protein 1 (Drp1, # 8570 S), mitochondrial fission 1 (Fis1, # 84580 S), Tubulin (# 2125 S) overnight at 4 °C. Then, the membranes were washed by Tris-buffered saline with 0.05% Tween-20 and incubated with a suitable secondary antibody (goat anti-rabbit IgG, 1:2,000, # 7074 S) for 1 h at room temperature. Finally, the blots were detected using enhanced chemiluminescence reagents (ECL-Kit, Beyotime, Jiangsu, China), followed by autoradiography. Photographs of the membranes were taken using the Luminescent Image Analyzer

LAS-4000 system (Fujifilm Co.) and quantified with ImageJ 1.42 q software (NIH, Bethesda, MD, USA).

### 2.10. Statistical analysis

Data are shown as the mean  $\pm$  SEM and were analyzed by a one-way analysis of variance procedure using SAS (version 9.1; SAS Institute, Inc., Cary, NC, USA). This was followed by Tukey's test when significant differences were expressed. Statistical significance was considered at  $P < 0.05$ .

## 3. Results

### 3.1. Growth performance

The piglets in the IUGRC group showed lower ( $P < 0.05$ ) initial body weight (IBW), final body weight (FBW), ADG, and ADFI in comparison with the NBWC group. The FBW, ADG, and G:F ratio were improved ( $P < 0.05$ ) in the piglets in the IUGRD group than those in the IUGRC group (Table 1).

### 3.2. Evaluation of histomorphology of the jejunum

The jejunum microvilli in the IUGRC group were shorter and less frequent than those in the NBWC group. In addition, autophagosomes and mitochondrial swelling were observed in the IUGRC group but not in the NBWC group (Fig. 1A). Compared with the NBWC group, the jejunum villi in IUGRC group were shorter and of different lengths (Fig. 1B). The IUGRD group's jejunum villi, internal microvilli, and internal structure were improved relative to those in the IUGRC group (Fig. 1A and B).

Histomorphological parameters (villus length, villus width, crypt depth, and villus area) of the jejunum were lower ( $P < 0.05$ ) in the IUGRC group than those in the NBWC group. Compared with the IUGRC group, the IUGRD group revealed improvement ( $P < 0.05$ ) in the histomorphological parameters of the jejunum (Table 2).

### 3.3. Concentrations of jejunum inflammatory cytokines

The IUGRC group revealed a decrease ( $P < 0.05$ ) of serum IgA, IgG, IgM, and jejunum sIgA levels compared with the NBWC group. Serum IgA, IgG, IgM, and jejunum sIgA levels were all improved ( $P < 0.05$ ) in the IUGRD group compared to these in the IUGRC group (Table 3).

**Table 1**

Dietary dimethylglycine sodium salt (DMG-Na) supplementation improves the growth performance in intrauterine growth restricted (IUGR) weaned piglets<sup>1</sup>.

Item	Groups		
	NBWC <sup>2</sup>	IUGRC <sup>3</sup>	IUGRD <sup>4</sup>
ADG, g	333.21 $\pm$ 37.65 <sup>a</sup>	283.57 $\pm$ 31.29 <sup>b</sup>	351.79 $\pm$ 58.04 <sup>a</sup>
ADFI, g/d	607.13 $\pm$ 38.33 <sup>a</sup>	532.14 $\pm$ 34.19 <sup>b</sup>	546.42 $\pm$ 37.39 <sup>b</sup>
G:F ratio	0.55 $\pm$ 0.04 <sup>b</sup>	0.53 $\pm$ 0.06 <sup>b</sup>	0.64 $\pm$ 0.05 <sup>a</sup>
IBW, kg	7.71 $\pm$ 0.58 <sup>a</sup>	5.80 $\pm$ 0.60 <sup>b</sup>	6.02 $\pm$ 0.57 <sup>b</sup>
FBW, kg	17.04 $\pm$ 1.22 <sup>a</sup>	13.74 $\pm$ 1.12 <sup>c</sup>	15.87 $\pm$ 2.02 <sup>b</sup>

ADG = average daily weight gain; ADFI = average daily feed intake; IBW = initial body weight; FBW = final body weight; G:F ratio = weight gain-to-feed intake ratio.  
<sup>a, b, c</sup> Within a row, means with different superscripts were significantly different ( $P < 0.05$ ).

<sup>1</sup> Data are shown as mean  $\pm$  standard error of mean,  $n = 10$  piglets per group.

<sup>2</sup> NBWC = normal birth weight weaned piglets fed standard basal diets.

<sup>3</sup> IUGRC = one IUGR weaned piglets fed standard basal diets.

<sup>4</sup> IUGRD = another IUGR weaned piglets from the same litter fed standard basal diets plus 0.1% DMG-Na.

### 3.4. Concentrations of jejunum inflammatory cytokine

Increased ( $P < 0.05$ ) concentrations of IL-1 $\beta$ , IL-6, and TNF- $\alpha$ , and decreased ( $P < 0.05$ ) concentrations of IL-8 were observed in group IUGRC compared with those in group NBWC. The IUGRD group showed an improvement ( $P < 0.05$ ) in IL-1 $\beta$ , IL-6, IL-8, and TNF- $\alpha$  levels in comparison with those in the IUGRC group (Table 4).

### 3.5. Concentrations of digestive enzyme

Intestinal digestive enzyme (amylase, lipase, trypsin, maltase, and lactase) activity of the jejunum showed a decrease ( $P < 0.05$ ) in the IUGRC group compared with those in the NBWC group. The IUGRD group showed increased ( $P < 0.05$ ) activity of the jejunum digestive enzymes (amylase, lipase, trypsin, maltase, and lactase) compared with that in the IUGRC group (Table 5).

### 3.6. Measurement of redox status

The IUGRC group showed a lower ( $P < 0.05$ ) antioxidant enzymes activity (SOD, GSH-Px, GSH, GR, and CAT) and higher ( $P < 0.05$ ) MDA content in the serum (Table 6) and jejunum (Table 7) compared with those in the NBWC group. The IUGRD group revealed an increased ( $P < 0.05$ ) antioxidant enzymes activity (SOD, GSH-Px, GSH, GR, and CAT) and decreased ( $P < 0.05$ ) MDA content in the serum (Table 6) and jejunum (Table 7) compared with these in the IUGRC group.

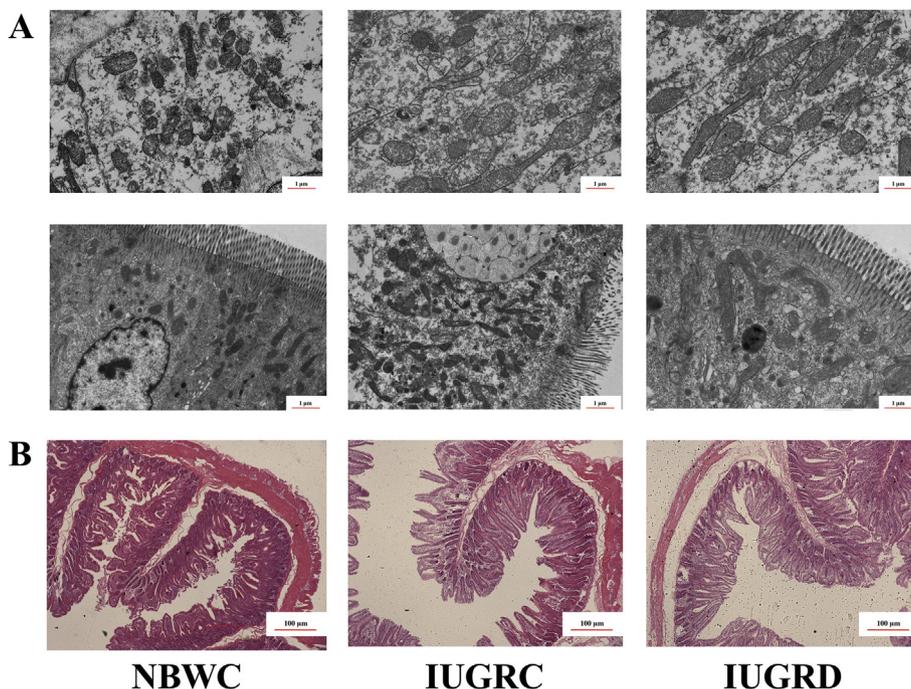
### 3.7. Measurement of mitochondrial redox status

The IUGRC group showed a lower ( $P < 0.05$ ) level of jejunum mitochondrial MnSOD, GSH-Px, GSH, GR, and  $\gamma$ -GCL activity relative to their levels in the NBWC group. The IUGRD group showed higher ( $P < 0.05$ ) levels of jejunum mitochondrial MnSOD, GSH-Px, GSH, GR, and  $\gamma$ -GCL activity in comparison with the IUGRC group (Table 8).

Compared with the NBWC group, higher ( $P < 0.05$ ) levels of ROS, PC, 8-OHdG, apoptosis cells, and necrosis cells, and lower ( $P < 0.05$ ) levels of MMP, ATP, and mtDNA were shown in group IUGRC. Decreased ( $P < 0.05$ ) levels of ROS, PC, 8-OHdG, apoptosis cells, and necrosis cells, and increased ( $P < 0.05$ ) levels of MMP, ATP, and mtDNA were revealed in the IUGRD group than those in the IUGRC group (Table 9).

### 3.8. Quantitative real-time PCR analysis

The redox status-related gene expression (*Nrf2*, *HO1*, *Cu/ZnSOD*, *GSH-Px*, *MnSOD*,  $\gamma$ -glutamylcysteine ligase c [ $\gamma$ -GCLc],  $\gamma$ -glutamylcysteine ligase m [ $\gamma$ -GCLm], thioredoxin 2 [*Trx2*], thioredoxin reductase 2 [*Trx-R2*], peroxiredoxin 3 [*Prx3*], *Sirt1*, *PGC1 $\alpha$* ), cell adhesion-related (occluding [*OCLN*], claudin2 [*CLDN2*], claudin3 [*CLDN3*], zonula occludens-1 [*ZO1*]) genes expression (Fig. 2A), and mitochondrial function-related gene expression (lipid oxidation enzymes malonyl-CoA decarboxylase [*MCD*], medium-chain acyl-CoA dehydrogenase [*MCAD*], mitochondrial proteins succinate dehydrogenase [*SDH*], uncoupling protein 2 [*UCP2*], cyclooxygenase 2 [*COX2*], citrate synthase [*CS*], cyclooxygenase 1 [*COX1*], *Cyt C*, *ERR $\alpha$* , major histocompatibility complex I [*MHC1*], *mtTFA*, NADH dehydrogenase (ubiquinone) iron-sulfur protein 2 [*Ndufa2*], *NRF1*, uncoupling protein 1 [*UCP1*],  $\gamma$  DNA polymerases catalytic subunit [*POLG1*],  $\gamma$  DNA polymerases accessory subunit [*POLG2*], single-strand DNA binding protein 1 [*SSBP1*], *Drp1*, *Fis1*, *Mfn2*) (Fig. 2B and C) levels of the jejunum deteriorated ( $P < 0.05$ ) in IUGRC group relative to the NBWC group. Compared with the IUGRC group, redox status-related gene expression, cell adhesion-related gene



**Fig. 1.** Dietary dimethylglycine sodium salt (DMG-Na) supplementation improves the jejunum microvilli integrity and their structure in intrauterine growth restricted (IUGR) weaned piglets. (A) Jejunum mitochondrial swelling and microvilli. Scale bars represent 1 μm; (B) jejunum histological morphology (villus length, villus width, crypt depth, and villus area). Scale bars represent 100 μm, n = 10 piglets per group. NBWC, NBW weaned piglets fed standard basal diets; IUGRC, one IUGR weaned piglets fed standard basal diets; IUGRD, another IUGR weaned piglets from the same litter fed standard basal diets plus 0.1% DMG-Na.

**Table 2**  
Dietary dimethylglycine sodium salt (DMG-Na) supplementation improves the jejunum villi length in intrauterine growth restricted (IUGR) weaned piglets <sup>1</sup>.

Item	Groups		
	NBWC <sup>2</sup>	IUGRC <sup>3</sup>	IUGRD <sup>4</sup>
Villus length, μm	636.77 ± 7.98 <sup>a</sup>	568.38 ± 7.15 <sup>b</sup>	609.18 ± 8.26 <sup>a</sup>
Crypt depth, μm	132.67 ± 3.38 <sup>a</sup>	110.78 ± 2.92 <sup>c</sup>	121.85 ± 3.65 <sup>b</sup>
Villus width, μm	126.66 ± 3.52 <sup>a</sup>	108.38 ± 4.05 <sup>b</sup>	119.58 ± 3.47 <sup>a</sup>
Villus area, mm <sup>2</sup>	0.127 ± 0.008 <sup>a</sup>	0.097 ± 0.007 <sup>b</sup>	0.115 ± 0.006 <sup>a</sup>

<sup>a, b, c</sup> Within a row, means with different superscripts were significantly different (*P* < 0.05).

- <sup>1</sup> Data are shown as mean ± standard error of mean, n = 10 piglets per group.
- <sup>2</sup> NBWC = normal birth weight weaned piglets fed standard basal diets.
- <sup>3</sup> IUGRC = one IUGR weaned piglets fed standard basal diets.
- <sup>4</sup> IUGRD = another IUGR weaned piglets from the same litter fed standard basal diets plus 0.1% DMG-Na.

**Table 3**  
Dietary dimethylglycine sodium salt (DMG-Na) supplementation improves the serum immunoglobulin and jejunum sIgA levels in intrauterine growth restricted (IUGR) weaned piglets <sup>1</sup>.

Item	Groups		
	NBWC <sup>2</sup>	IUGRC <sup>3</sup>	IUGRD <sup>4</sup>
IgA, μg/mL	12.39 ± 0.15 <sup>a</sup>	7.95 ± 0.07 <sup>b</sup>	11.37 ± 0.09 <sup>a</sup>
IgG, μg/mL	8.54 ± 0.08 <sup>a</sup>	5.96 ± 0.06 <sup>b</sup>	7.69 ± 0.08 <sup>a</sup>
IgM, μg/mL	5.78 ± 0.07 <sup>a</sup>	4.04 ± 0.09 <sup>b</sup>	5.18 ± 0.06 <sup>a</sup>
sIgA, μmol/g	18.37 ± 0.76 <sup>a</sup>	12.66 ± 0.70 <sup>b</sup>	16.48 ± 0.62 <sup>a</sup>

IgA = immune globulin A; IgG = immune globulin G; IgM = immune globulin M; sIgA = secreted immunoglobulin A.

- <sup>a, b</sup> Within a row, means with different superscripts were significantly different (*P* < 0.05).
- <sup>1</sup> Data are shown as mean ± standard error of mean, n = 10 piglets per group.
- <sup>2</sup> NBWC = normal birth weight weaned piglets fed standard basal diets.
- <sup>3</sup> IUGRC = one IUGR weaned piglets fed standard basal diets.
- <sup>4</sup> IUGRD = another IUGR weaned piglets from the same litter fed standard basal diets plus 0.1% DMG-Na.

**Table 4**  
Dietary dimethylglycine sodium salt (DMG-Na) supplementation improves the concentrations of jejunum inflammatory cytokines in intrauterine growth restricted (IUGR) weaned piglets (ng/g protein) <sup>1</sup>.

Item	Groups		
	NBWC <sup>2</sup>	IUGRC <sup>3</sup>	IUGRD <sup>4</sup>
IL-1β	30.29 ± 2.96 <sup>b</sup>	50.27 ± 3.03 <sup>a</sup>	33.77 ± 2.83 <sup>b</sup>
IL-6	54.51 ± 3.75 <sup>b</sup>	72.61 ± 3.12 <sup>a</sup>	56.29 ± 3.54 <sup>b</sup>
IL-8	22.32 ± 1.16 <sup>a</sup>	15.33 ± 0.54 <sup>b</sup>	20.79 ± 1.15 <sup>a</sup>
TNF-α	14.06 ± 1.05 <sup>b</sup>	24.83 ± 1.11 <sup>a</sup>	17.15 ± 0.96 <sup>b</sup>

IL-1β = interleukin 1β; IL-6 = interleukin 6; IL-8 = interleukin 8; TNF-α = tumor necrosis factor α.

<sup>a, b</sup> Within a row, means with different superscripts were significantly different (*P* < 0.05).

- <sup>1</sup> Data are shown as mean ± standard error of mean, n = 10 piglets per group.
- <sup>2</sup> NBWC = normal birth weight weaned piglets fed standard basal diets.
- <sup>3</sup> IUGRC = one IUGR weaned piglets fed standard basal diets.
- <sup>4</sup> IUGRD = another IUGR weaned piglets from the same litter fed standard basal diets plus 0.1% DMG-Na.

expression, and mitochondrial function-related gene expression levels of the jejunum were improved (*P* < 0.05) in IUGRD group (Fig. 2A–C).

### 3.9. Western blot analysis

Compared with the NBWC group, the IUGRC group showed lower (*P* < 0.05) protein levels of SOD, GSH-Px, Sirt1, PGC1α, Cyt C, ERRα, mtTFA, NRF1, Mfn2, Drp1, and Fis1, along with higher (*P* < 0.05) protein levels of Nrf2 and HO1 (Fig. 3A and B). The IUGRD group presented higher (*P* < 0.05) protein levels of SOD, GSH-Px, Sirt1, Cyt C, ERRα, mtTFA, NRF1, Mfn2, Drp1, and Fis1, and lower (*P* < 0.05) protein levels of Nrf2 and HO1 compared with IUGRC group (Fig. 3A and B).

**Table 5**

Dietary dimethylglycine sodium salt (DMG-Na) supplementation improves the digestive enzyme activity in intrauterine growth restricted (IUGR) weaned piglets (U/mg protein)<sup>1</sup>.

Item	Groups		
	NBWC <sup>2</sup>	IUGRC <sup>3</sup>	IUGRD <sup>4</sup>
Amylase	6.28 ± 0.08 <sup>a</sup>	4.51 ± 0.03 <sup>b</sup>	6.09 ± 0.03 <sup>a</sup>
Lipase	0.25 ± 0.01 <sup>a</sup>	0.17 ± 0.01 <sup>b</sup>	0.22 ± 0.01 <sup>a</sup>
Trypsin	0.52 ± 0.03 <sup>a</sup>	0.38 ± 0.01 <sup>b</sup>	0.50 ± 0.01 <sup>a</sup>
Sucrase	73.58 ± 4.41	70.71 ± 3.52	72.82 ± 4.03
Maltase	388.82 ± 6.98 <sup>a</sup>	307.46 ± 5.18 <sup>b</sup>	378.83 ± 6.26 <sup>a</sup>
Lactase	147.98 ± 1.71 <sup>a</sup>	106.20 ± 1.99 <sup>b</sup>	138.74 ± 1.83 <sup>a</sup>

<sup>a, b</sup> Within a row, means with different superscripts were significantly different ( $P < 0.05$ ).

<sup>1</sup> Data are shown as mean ± standard error of mean,  $n = 10$  piglets per group.

<sup>2</sup> NBWC = normal birth weight weaned piglets fed standard basal diets.

<sup>3</sup> IUGRC = one IUGR weaned piglets fed standard basal diets.

<sup>4</sup> IUGRD = another IUGR weaned piglets from the same litter fed standard basal diets plus 0.1% DMG-Na.

**Table 6**

Dietary dimethylglycine sodium salt (DMG-Na) supplementation improves the serum redox status in intrauterine growth restricted (IUGR) weaned piglets<sup>1</sup>.

Item	Groups		
	NBWC <sup>2</sup>	IUGRC <sup>3</sup>	IUGRD <sup>4</sup>
SOD, U/mL	143.49 ± 5.68 <sup>a</sup>	118.57 ± 7.10 <sup>b</sup>	133.79 ± 6.22 <sup>a</sup>
GSH-Px, U/mL	356.77 ± 7.96 <sup>a</sup>	286.72 ± 7.11 <sup>b</sup>	330.28 ± 8.08 <sup>a</sup>
GSH, U/mL	49.76 ± 1.77 <sup>a</sup>	35.54 ± 1.14 <sup>b</sup>	46.48 ± 1.18 <sup>a</sup>
GR, U/mL	9.97 ± 0.10 <sup>a</sup>	6.19 ± 0.06 <sup>b</sup>	8.58 ± 0.06 <sup>a</sup>
CAT, U/mL	2.56 ± 0.05 <sup>a</sup>	1.79 ± 0.02 <sup>b</sup>	2.33 ± 0.05 <sup>a</sup>
MDA, nmol/mL	4.96 ± 0.08 <sup>b</sup>	6.10 ± 0.05 <sup>a</sup>	5.23 ± 0.03 <sup>b</sup>

SOD = superoxide dismutase; GSH-Px = glutathione peroxidase; GSH = glutathione; GR = glutathione reductase; CAT = catalase; MDA = methane dicarboxylic aldehyde.

<sup>a, b</sup> Within a row, means with different superscripts were significantly different ( $P < 0.05$ ).

<sup>1</sup> Data are shown as mean ± standard error of mean,  $n = 10$  piglets per group.

<sup>2</sup> NBWC = normal birth weight weaned piglets fed standard basal diets.

<sup>3</sup> IUGRC = one IUGR weaned piglets fed standard basal diets.

<sup>4</sup> IUGRD = another IUGR weaned piglets from the same litter fed standard basal diets plus 0.1% DMG-Na.

**Table 7**

Dietary dimethylglycine sodium salt (DMG-Na) supplementation improves the jejunum redox status in intrauterine growth restricted (IUGR) weaned piglets<sup>1</sup>.

Item	Groups		
	NBWC <sup>2</sup>	IUGRC <sup>3</sup>	IUGRD <sup>4</sup>
SOD, U/g protein	89.72 ± 2.38 <sup>a</sup>	69.76 ± 2.08 <sup>b</sup>	81.55 ± 1.90 <sup>a</sup>
GSH-Px, U/g protein	36.72 ± 1.79 <sup>a</sup>	26.42 ± 1.11 <sup>b</sup>	35.11 ± 1.36 <sup>a</sup>
GSH, μmol/g protein	26.11 ± 1.16 <sup>a</sup>	19.69 ± 0.50 <sup>b</sup>	24.22 ± 0.84 <sup>a</sup>
GR, U/g protein	13.50 ± 0.67 <sup>a</sup>	10.46 ± 0.57 <sup>b</sup>	12.25 ± 0.88 <sup>a</sup>
CAT, U/g protein	1.72 ± 0.04 <sup>a</sup>	1.25 ± 0.02 <sup>b</sup>	1.68 ± 0.04 <sup>a</sup>
MDA, nmol/mg protein	1.34 ± 0.05 <sup>b</sup>	2.02 ± 0.06 <sup>a</sup>	1.43 ± 0.04 <sup>b</sup>

SOD = superoxide dismutase; GSH-Px = glutathione peroxidase; GSH = glutathione; GR = glutathione reductase; CAT = catalase; MDA = methane dicarboxylic aldehyde.

<sup>a, b</sup> Within a row, means with different superscripts were significantly different ( $P < 0.05$ ).

<sup>1</sup> Data are shown as mean ± standard error of mean,  $n = 10$  piglets per group.

<sup>2</sup> NBWC = normal birth weight weaned piglets fed standard basal diets.

<sup>3</sup> IUGRC = one IUGR weaned piglets fed standard basal diets.

<sup>4</sup> IUGRD = another IUGR weaned piglets from the same litter fed standard basal diets plus 0.1% DMG-Na.

#### 4. Discussion

Intrauterine growth restriction has received increasing attention in animal husbandry because of its irreversible oxidative

**Table 8**

Dietary dimethylglycine sodium salt (DMG-Na) supplementation improves the jejunum mitochondrial redox status in intrauterine growth restricted (IUGR) weaned piglets<sup>1</sup>.

Item	Groups		
	NBWC <sup>2</sup>	IUGRC <sup>3</sup>	IUGRD <sup>4</sup>
MnSOD, U/mg protein	72.28 ± 2.54 <sup>a</sup>	54.49 ± 2.09 <sup>b</sup>	69.83 ± 2.11 <sup>a</sup>
GSH-Px, U/mg protein	18.54 ± 0.66 <sup>a</sup>	12.56 ± 0.08 <sup>b</sup>	16.49 ± 0.12 <sup>a</sup>
GSH, μg/g protein	19.63 ± 0.81 <sup>a</sup>	15.41 ± 0.08 <sup>b</sup>	18.02 ± 0.15 <sup>a</sup>
GR, U/mg protein	7.78 ± 0.07 <sup>a</sup>	5.11 ± 0.05 <sup>b</sup>	7.33 ± 0.04 <sup>a</sup>
γ-GCL, U/mg protein	2.28 ± 0.03 <sup>a</sup>	1.63 ± 0.06 <sup>b</sup>	2.08 ± 0.05 <sup>a</sup>

MnSOD = manganese superoxide dismutase; GSH-Px = glutathione peroxidase; GSH = glutathione; GR = glutathione reductase; γ-GCL = γ-glutamylcysteine ligase.

<sup>a, b</sup> Within a row, means with different superscripts were significantly different ( $P < 0.05$ ).

<sup>1</sup> Data are shown as mean ± standard error of mean,  $n = 10$  piglets per group.

<sup>2</sup> NBWC = normal birth weight weaned piglets fed standard basal diets.

<sup>3</sup> IUGRC = one IUGR weaned piglets fed standard basal diets.

<sup>4</sup> IUGRD = another IUGR weaned piglets from the same litter fed standard basal diets plus 0.1% DMG-Na.

damage, delayed postnatal growth, and adverse effects on intestinal health (Wang et al., 2005; Li et al., 2021). Previous studies used IUGR weaned piglets as a model demonstrated their poor growth performance (Zhang et al., 2014), and this outcome agrees with our results, which show that the IUGR group ( $0.76 \pm 0.06$  kg) exhibited lower growth performance than the NBW group ( $1.53 \pm 0.04$  kg). As expected, DMG-Na supplementation could improve the growth performance of weaned piglets with IUGR, which might be explained by its high radical-scavenging capacity and intestinal health benefits (Bai et al., 2016). In addition, the improvement of intestinal function by supplementation of DMG-Na increased the digestion and absorption efficiency of dietary nutrients, which would be beneficial in ameliorating the growth performance of IUGR weaned piglets.

The small intestine is important in nutrient digestion, absorption, and metabolism. Intrauterine growth restriction leads to intestinal diseases in the perinatal period and predisposes individuals to feeding intolerance and digestive diseases in the early postnatal period (Bernstein et al., 2000). The movement of substances across the cell membrane depends on diffusion or active transport, which depend on the intestinal structure (Nusrat et al., 2000). Consistent with our study, it has shown that intrauterine growth restriction leads to intestinal villus atrophy, mucosal oxidative damage, and intestinal dysfunction, thereby causing diarrhea and reduction in feed utilization in piglets (Wang et al., 2005, 2008). Another study also showed that autophagosomes and mitochondrial swelling appeared in the small intestine of the IUGR weaned piglets, which might be related to intrauterine malnutrition and is likely to be alleviated by nutrient replenishment (Minamikawa et al., 1999). It also suggested that DMG-Na could protect the small intestine from oxidative damage and maintaining its normal histomorphology by its antioxidant capacity (Friesen et al., 2007). Another study found that DMG-Na protected cells from oxidative damage (Look et al., 2001), and this might be one possible reason for the results observed in the histomorphological analysis.

Intrauterine growth restriction is a serious complication of the mammalian fetus during pregnancy, limiting fetal development and impairing immune function during the perinatal period. Immunoglobulin A, IgG, and IgM levels are critical because they reflect the immune status and the capacity to fight against various infections. IgA can turn to sIgA in cell gaps and then bind to the corresponding antigen to relieve the oxidative damage in the small intestine (Brandtzaeg, 2002). It has been found that excessive intestinal epithelial cell apoptosis damage intestinal integrity resulting in the release of inflammatory cytokines (Jozawa et al.,

**Table 9**  
Dietary dimethylglycine sodium salt (DMG-Na) supplementation improves the jejunum oxidative damage in intrauterine growth restricted (IUGR) weaned piglets <sup>1</sup>.

Item	Groups		
	NBWC <sup>2</sup>	IUGRC <sup>3</sup>	IUGRD <sup>4</sup>
ROS, % of N	1.00 ± 0.03 <sup>b</sup>	1.59 ± 0.07 <sup>a</sup>	1.18 ± 0.07 <sup>b</sup>
PC, nmol/mg protein	26.54 ± 1.06 <sup>b</sup>	33.20 ± 0.89 <sup>a</sup>	28.96 ± 1.02 <sup>b</sup>
8-OHdG, ng/mg protein	0.31 ± 0.05 <sup>b</sup>	0.53 ± 0.02 <sup>a</sup>	0.39 ± 0.02 <sup>b</sup>
MMP, % of N	1.00 ± 0.05 <sup>a</sup>	0.63 ± 0.04 <sup>b</sup>	0.88 ± 0.08 <sup>a</sup>
Percentage of apoptosis cells counts, %	1.83 ± 0.06 <sup>b</sup>	2.31 ± 0.13 <sup>a</sup>	1.95 ± 0.09 <sup>b</sup>
Percentage of necrotic cells counts, %	1.07 ± 0.08 <sup>b</sup>	1.83 ± 0.08 <sup>a</sup>	1.15 ± 0.12 <sup>b</sup>
ATP, μmol/mg protein	1.35 ± 0.08 <sup>a</sup>	1.09 ± 0.07 <sup>b</sup>	1.32 ± 0.12 <sup>a</sup>
mtDNA, % of N	1.00 ± 0.05 <sup>a</sup>	0.61 ± 0.02 <sup>b</sup>	0.92 ± 0.02 <sup>a</sup>

ROS = reactive oxygen species; PC = protein carbonyls; 8-OHdG = 8-hydroxy-2- deoxyguanosine; MMP = mitochondrial membrane potential; ATP = adenosine triphosphate; mtDNA = mitochondria DNA.

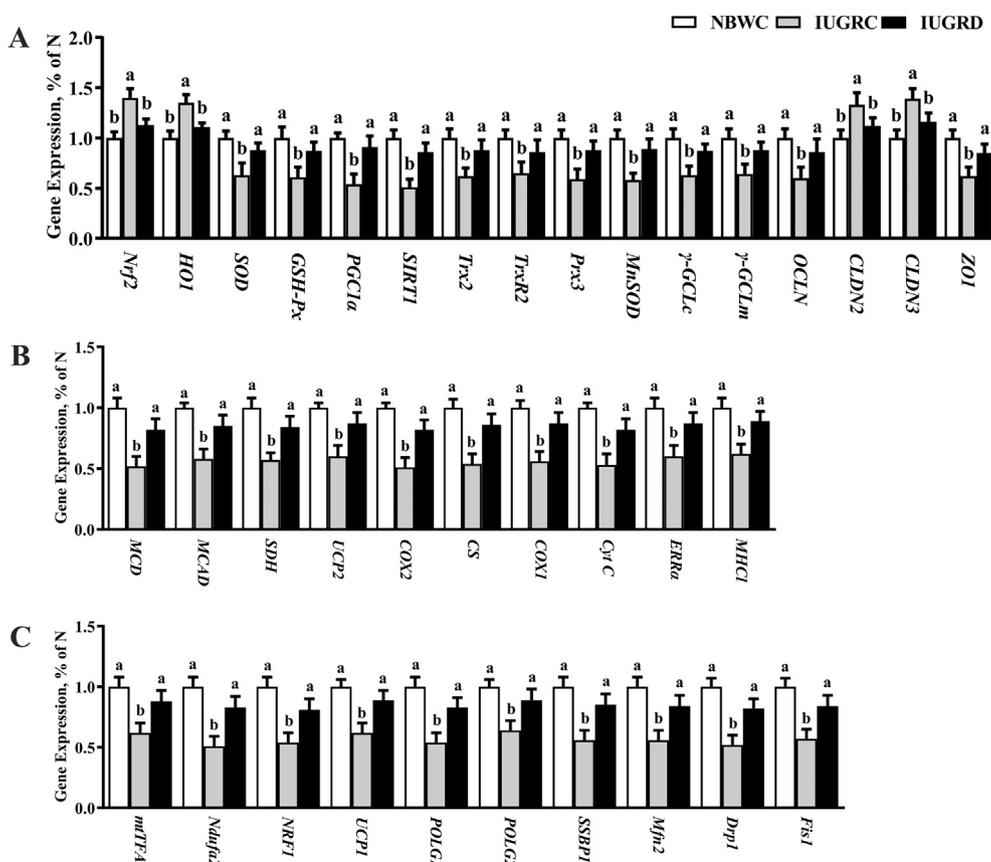
<sup>a, b</sup> Within a row, means with different superscripts were significantly different (*P* < 0.05).

<sup>1</sup> Data are shown as mean ± standard error of mean, *n* = 10 piglets per group.

<sup>2</sup> NBWC = normal birth weight weaned piglets fed standard basal diets.

<sup>3</sup> IUGRC = one IUGR weaned piglets fed standard basal diets.

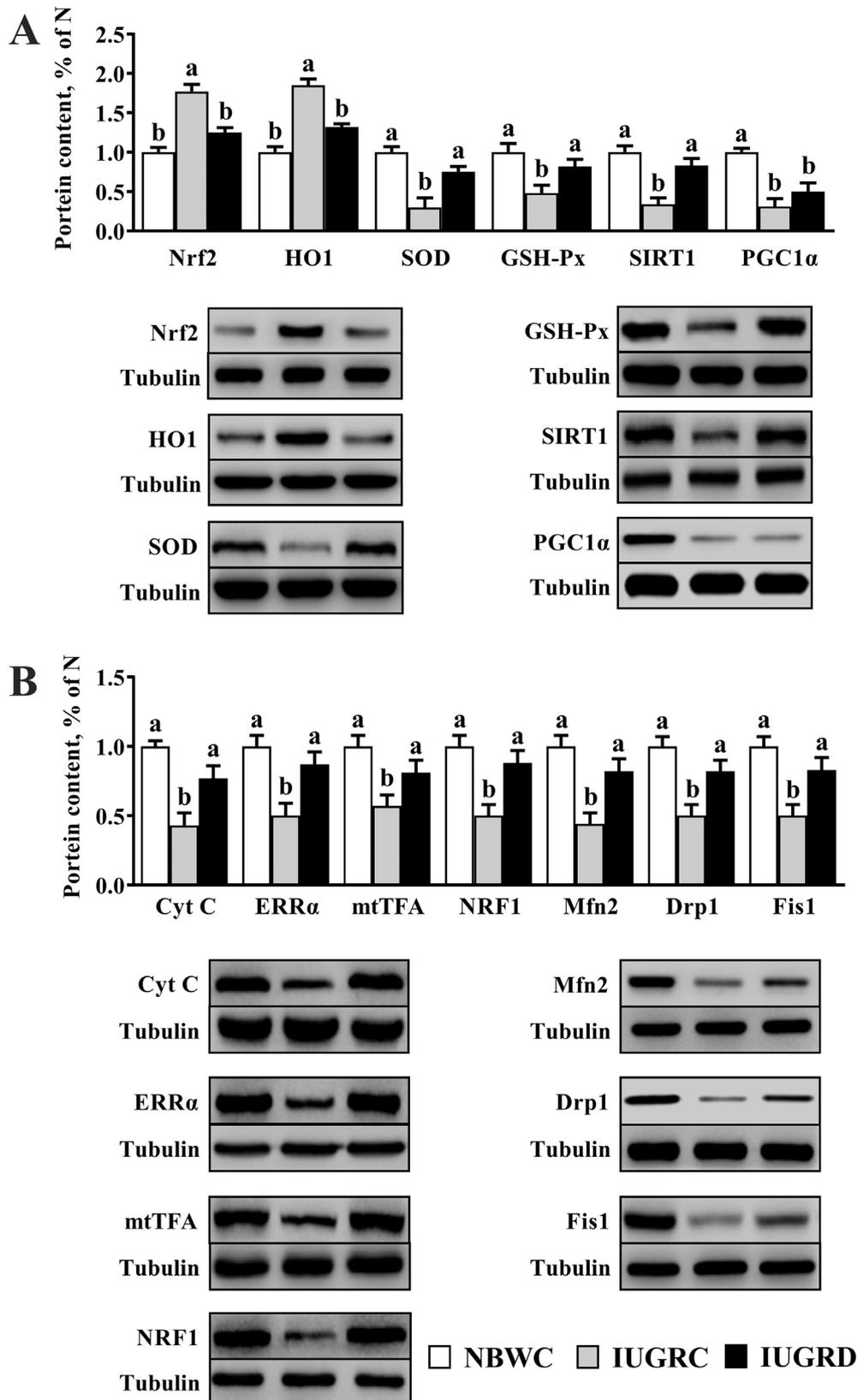
<sup>4</sup> IUGRD = another IUGR weaned piglets from the same litter fed standard basal diets plus 0.1% DMG-Na.



**Fig. 2.** Dietary dimethylglycine sodium salt (DMG-Na) supplementation improves the jejunum redox status-related and mitochondrial function-related gene expression in intrauterine growth restricted (IUGR) weaned piglets. Data are shown as mean ± standard error of mean, *n* = 10 piglets per group. Data on bars with different letters (a, b) were significantly different (*P* < 0.05). All the experiment was repeated 3 times. NBWC, normal birth weight weaned piglets fed standard basal diets; IUGRC, one IUGR weaned piglets fed standard basal diets; IUGRD, another IUGR weaned piglets from the same litter fed standard basal diets plus 0.1% DMG-Na. *Nrf2* = nuclear factor erythroid 2-related factor 2; *HO1* = heme oxygenase 1; *SOD* = copper and zinc superoxide dismutase; *GSH-Px* = glutathione peroxidase; *MnSOD* = manganese superoxide dismutase; *γ-GCLc* =  $\gamma$ -glutamylcysteine ligase c; *γ-GCLm* =  $\gamma$ -glutamylcysteine ligase m; *Trx2* = thioredoxin 2; *TrxR2* = thioredoxin reductase 2; *Prx3* = peroxiredoxin 3; *Sirt1* = sirtuin 1; *PGC1 $\alpha$*  = peroxisome proliferator-activated receptor $\gamma$ coactivator-1 $\alpha$ ; *OCLN* = occluding; *CLDN2* = claudin2; *CLDN3* = claudin3; *ZO1* = zonula occludens-1; *MCD* = lipid oxidation enzymes malonyl-CoA decarboxylase; *MCAD* = medium-chain acyl-CoA dehydrogenase; *SDH* = mitochondrial proteins succinate dehydrogenase; *UCP2* = uncoupling protein 2; *COX2* = cyclooxygenase 2; *CS* = citrate synthase; *COX1* = cyclooxygenase 1; *Cyt C* = Cytochrome C; *ERR $\alpha$*  = estrogen-related receptor  $\alpha$ ; *MHC1* = major histocompatibility complex I; *mtTFA* = mitochondrial transcription factor A; *Ndufa2* = NADH dehydrogenase (ubiquinone) iron-sulfur protein 2; *NRF1* = nuclear respiratory factor 1; *UCP1* = uncoupling protein 1; *POLG1* =  $\gamma$  DNA polymerases catalytic subunit; *POLG2* =  $\gamma$  DNA polymerases accessory subunit; *SSBP1* = single-strand DNA binding protein 1; *Drp1* = dynamin-related protein 1; *Fis1* = mitochondrial fission 1; *Mfn2* = mitochondrial mitofusin 2.

2019). Based on the current results, IUGR weaned piglets showed increased concentrations of jejunum IL-1 $\beta$ , IL-6, and TNF- $\alpha$  and decreased concentration of jejunum IL-8. In agreement with this

work, it has been demonstrated that IUGR newborn piglets have increased TNF- $\alpha$  and IL-6 concentrations, which suggested that they were prone to suffer from the inflammatory injury (Huang



**Fig. 3.** Dietary dimethylglycine sodium salt (DMG-Na) supplementation improves the jejunum redox status-related and mitochondrial function-related protein expression in intrauterine growth restricted (IUGR) weaned piglets. Data are shown as mean ± standard error of mean  $n = 3$  piglets per group. Data on bars with different letters (a, b) were significantly different ( $P < 0.05$ ). All the experiment was repeated 3 times. NBWC, normal birth weight weaned piglets fed standard basal diets; IUGRC, one IUGR weaned piglets fed standard basal diets; IUGRD, another IUGR weaned piglets from the same litter fed standard basal diets plus 0.1% DMG-Na. Nrf2 = nuclear factor erythroid 2-related factor 2; HO1 = heme oxygenase 1; SOD = superoxide dismutase; GSH-Px = glutathione peroxidase; Sirt1 = sirtuin 1; PGC1α = peroxisome proliferator-activated receptor-coactivator-1α; Cyt C = Cytochrome C; ERRα = estrogen-related receptor α; mtTFA = mitochondrial transcription factor A; NRF1 = nuclear respiratory factor 1; Mfn2 = mitochondrial mitofusin2; Drp1 = dynamin-related protein 1; Fis1 = mitochondrial fission 1.

et al., 2019). It was found that DMG-Na could enhance the body's immune status and relieve oxidative damage by scavenging the excessive generated free radicals (Hariganesh and Prathiba, 2000). It has been suggested that intrauterine growth restriction could damage the small intestine in piglets and lead to abnormalities in intestinal morphology and immune function (Wang et al., 2012). DMG-Na increases serum immunoglobulin levels and attenuates the intestinal inflammatory response of IUGR weaned piglets through its immunomodulatory function (Bai et al., 2019), which might represent a possible explanation for our observations.

Small intestine plays a crucial role in preventing bacterial invasion, and its digestive enzymes, cytokines, and immunoglobulins are related to growth and immune function. Consistent with the current results, it has been revealed that the intestinal structure and function of IUGR weaned piglets was impaired usually resulting in the abnormalities secretion of digestive enzyme. This may closely relate to intestinal epithelial cell apoptosis and proliferation imbalance, and finally affects the digestion and utilization of feed seriously (Baserga et al., 2004; D'Inca et al., 2011). Studies have also demonstrated that DMG-Na may increase digestive enzyme activity and enhance the intestinal brush border ability to promote the digestion and absorption of nutrients (Bai et al., 2019). In this work, the addition of 0.1% DMG-Na to the diet improved the digestion and absorption of nutrients in the IUGR group, mainly due to the benefits of DMG-Na in protecting the intestinal structure and function.

Oxidative damage can enhance ROS levels, decrease antioxidant capacity, and destroy the mitochondrial structure. The intestinal mitochondrial swelling in the IUGR group in the current study suggests they suffer oxidative damage and accompanied by a disrupted redox status (Mizushima, 2007). Oxidative damage can be ameliorated by the SOD enzyme, which catalyzes the conversion of endogenous superoxide anions to hydrogen peroxide through disproportionation, and ultimately neutralizes the same using intracellular enzyme GSH-Px (Bayrak et al., 2008). Meanwhile, MnSOD enzyme, GSH-related metabolic enzymes, and  $\gamma$ -GCL enzymes are crucial mitochondria antioxidant enzymes that involved in suppressing of mitochondrial oxidative damage (Langston et al., 2011; Tang et al., 2012). It was revealed that DMG-Na could be used as an antioxidant additive to improve the individuals' antioxidant ability, thus improving their performance (Friesen et al., 2007; Bai et al., 2016). Taken together with our findings, it is suggested that DMG-Na could improve the antioxidant capacity through scavenging the excess generated ROS to maintain the balance of the intracellular redox status.

There is a dynamic balance between ROS levels and the antioxidant system. However, this balance could be disturbed if subjected to environmentally induced conditions resulting in oxidative damage (Sun and Zemel, 2009). Excessive ROS can induce mitochondrial and DNA damage, ultimately affecting antioxidant capacity (Bandyopadhyay, 1999; Sastre et al., 2000). Intrauterine growth restriction is closely related to oxidative damage, mitochondrial dysfunction, high ROS levels, and metabolic syndrome occurrence (Biri et al., 2007). It has been suggested that excessive ROS induces mitochondrial DNA (mtDNA) damage, impairs mitochondrial function, and produces more endogenous ROS (Simmons, 2012). The MMP levels, negatively correlated with ROS level, act as an indicator of the starting of mitochondria-dependent apoptosis (Kowaltowski and Vercesi, 1999). One study indicated that IUGR weaned piglets showed reduced antioxidant enzyme activity and mtDNA levels (Park et al., 2004), consistent with our findings. Another study also found that IUGR piglets were susceptible to oxidative damage because of their decreased antioxidant capacity (Biri et al., 2007; Hracsko et al., 2007). Therefore, it can be concluded from the current results that the reduction in

antioxidant capacity of the IUGR group leads to impaired intestinal function, and DMG-Na could relieve oxidative damage by scavenging for excessive ROS, which have verified that natural antioxidants could suppress the oxidative damage in cells (Friesen et al., 2007; Zhang et al., 2014).

Activation of Nrf2 and HO1 is vital in relieving oxidative damage by regulating antioxidant gene expression (SDO, GSH-Px, and  $\gamma$ -GCL) (Bartel, 2004; Lim et al., 2005). Mitochondria contain a large content of Trx2, Trx-R2, and Prx3 proteins, which act together to prevent the individual from oxidative damage through scavenging excessive generated free radicals and regulating its mitochondria-dependent apoptotic pathways (Mohamed et al., 2014). PGC1 $\alpha$  is a coactivator with pleiotropic functions, which can regulate mitochondrial function gene expression (COX1, Cyt C, ERR $\alpha$ , MHC1, mtTFA, Ndufa2, NRF1, NRF2, UCP1, mtDNA replication and repair [POLG1, POLG2, SSBP1], and mitochondrial fission [Drp1, Fis1] and fusion [Mfn2]), as it induces mitochondrial genes both at the level of the nuclear and mitochondrial genomes (Chiu et al., 2002; Hwang et al., 2013). SIRT1, originally described as a factor regulating apoptosis and DNA repair, is overly sensitive to cellular redox and nutritional status and is known to control genomic stability and cellular metabolism (Michelet et al., 2006). Studies found that SIRT1 physically interacts with and deacetylates PGC1 $\alpha$  at multiple lysine sites, consequently enhances PGC1 $\alpha$  level and regulates antioxidant ability, lipid oxidation enzymes (MCD, MCAD), and mitochondrial gene expression (SDH, UCP2, COX2, and CS) (Lin et al., 2005; St-Pierre et al., 2006). ZO1 accompanied with OCLN and CLDN gene families is the key regulator of intestinal permeability (Sinclair, 2005). To our knowledge, this is the first work to show the dairy effects of DMG-Na on the jejunum's redox status and mitochondrial function in IUGR weaned piglets via the SIRT1/PGC1 $\alpha$  pathway.

## 5. Conclusions and perspectives

The present study demonstrated that dietary DMG-Na supplementation could effectively alleviate intestinal structure damage, redox status imbalance, and intestinal dysfunction in IUGR weaned piglets via the activating of the SIRT1/PGC1 $\alpha$  network. This study indicates DMG-Na can serve as a health-promoting substance to inhibit oxidative damage and provides a new perspective for nutritional regulation of intestinal function in IUGR weaned piglets.

### Author contributions

**Kaiwen Bai:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Luyi Jiang:** Investigation. **Qiming Li:** Investigation. **Jingfei Zhang:** Investigation. **Lili Zhang:** Project administration. **Tian Wang:** Resources, Writing – review & editing, Supervision, Funding acquisition.

### 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.

### Acknowledgements

This research was supported by National Key Research and Development Program of China (No. 2018YFD0501101) and the National Natural Science Foundation of China (No. 31802094).

## Appendix

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.aninu.2021.12.007>.

## References

- Bai K, Jiang L, Zhu S, Feng C, Zhao Y, Zhang L, et al. Dimethylglycine sodium salt protects against oxidative damage and mitochondrial dysfunction in the small intestines of mice. *Int J Mol Med* 2019;43:2199–211.
- Bai K, Xu W, Zhang J, Kou T, Niu Y, Wan X, et al. Assessment of free radical scavenging activity of dimethylglycine sodium salt and its role in protective protection against lipopolysaccharide-induced oxidative stress in mice. *PLoS One* 2016;12(11):e0155393.
- Bai P, Cantó C, Oudart H, Brunyánszki A, Cen Y, Thomas C, et al. Houtkooper. PARP-1 inhibition increases mitochondrial metabolism through SIRT1 activation. *Cell Metabol* 2011;6(13):461–8.
- Bandyopadhyay U. Reactive oxygen species: oxidative damage pathogenesis. *Curr Sci* 1999;5:658–66.
- Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 2004;23(116):281–97.
- Baserga M, Bertolotto C, MacLennan NK, Hsu JL, Pham T, Laksana GS, et al. Uteroplacental insufficiency decreases small intestine growth and alters apoptotic homeostasis in term intrauterine growth retarded rats. *Early Hum Dev* 2004;79:93–105.
- Bayrak O, Uz E, Bayrak R, Turgut F, Atmaca AF, Sahin S, et al. Curcumin protects against ischemia/reperfusion injury in rat kidneys. *World J Urol* 2008;26:285–91.
- Berlett BS, Stadtman ER. Protein oxidation in aging, disease, and oxidative stress. *J Biol Chem* 1997;15(272):20313–6.
- Bernstein IM, Horbar JD, Badger GJ, Ohlsson A, Golan A. Morbidity and mortality among very-low-birth-weight neonates with intrauterine growth restriction. *Am J Obstet Gynecol* 2000;182(1 Pt 1):198–206.
- Biri A, Bozkurt N, Turp A, Kavutcu M, Himmetoglu O, Durak I. Role of oxidative stress in intrauterine growth restriction. *Gynecol Obstet Invest* 2007;64:187–92.
- Brandtzaeg PE. Current understanding of gastrointestinal immunoregulation and its relation to food allergy. *Ann N Y Acad Sci* 2002;964:13–45.
- Cheng K, Wang T, Li S, Song Z, Zhang H, Zhang L, et al. Effects of early resveratrol intervention on skeletal muscle mitochondrial function and redox status in neonatal piglets with or without intrauterine growth retardation. *Oxid Med Cell Longev* 2020;22(2020):4858975.
- Chiu H, Brittingham JA, Laskin DL. Differential induction of heme oxygenase-1 in macrophages and hepatocytes during acetaminophen-induced hepatotoxicity in the rat: effects of hemin and biliverdin. *Toxicol Appl Pharmacol* 2002;1(181):106–15.
- D'Inca R, Gras-Le Guen C, Che L, Sangild PT, Le Huérou-Luron I. Intrauterine growth restriction delays feeding-induced gut adaptation in term newborn pigs. *Neonatology* 2011;99:208–16.
- Dillin A, Hsu AL, Arantes-Oliveira N, Lehrer-Graiwier J, Hsin H, Fraser AG, et al. Rates of behavior and aging specified by mitochondrial function during development. *Science* 2002;20(298):2398–401.
- Dong L, Zhong X, Ahmad H, Li W, Wang Y, Zhang L, et al. Intrauterine growth restriction impairs small intestinal mucosal immunity in neonatal piglets. *J Histochem Cytochem* 2014;62:510–8.
- Feng C, Bai K, Wang A, Ge X, Zhao Y, Zhang L, et al. Effects of dimethylglycine sodium salt supplementation on growth performance, hepatic antioxidant capacity, and mitochondria-related gene expression in weanling piglets born with low birth weight. *J Anim Sci* 2018;7(96):3791–803.
- Friesen RW, Novak EM, Hasman D, Innis SM. Relationship of dimethylglycine, choline, and betaine with oxoprolin in plasma of pregnant women and their newborn infants. *J Nutr* 2007;137:2641–6.
- Hariganesh K, Prathiba J. Effect of dimethylglycine on gastric ulcers in rats. *J Pharm Pharmacol* 2000;52:1519–22.
- He Q, Ren P, Kong X, Xu W, Tang H, Yin Y, et al. Intrauterine growth restriction alters the metabolome of the serum and jejunum in piglets. *Mol Biosyst* 2011;7:2147–55.
- Hracsko Z, Safar Z, Orvos H, Novak Z, Pal A, Varga IS. Evaluation of oxidative stress markers after vaginal delivery or caesarean section. *Vivo* 2007;21:703–6.
- Huang S, Li N, Liu C, Li T, Wang W, Jiang L, et al. Characteristics of the gut microbiota colonization, inflammatory profile, and plasma metabolome in intrauterine growth restricted piglets during the first 12 hours after birth. *J Microbiol* 2019;57:748–58.
- Hwang JW, Yao H, Caito S, Sundar IK, Rahman I. Redox regulation of SIRT1 in inflammation and cellular senescence. *Free Radic Biol Med* 2013;61:95–110.
- Jozawa H, Inoue-Yamauchi A, Arimura S, Yamanashi Y. Loss of C/EBP $\delta$  enhances apoptosis of intestinal epithelial cells and exacerbates experimental colitis in mice. *Gene Cell* 2019;24:619–26.
- Kowaltowski AJ, Vercesi AE. Mitochondrial damage induced by conditions of oxidative stress. *Free Radic Biol Med* 1999;26:463–71.
- Langston JW, Li W, Harrison L, Aw TY. Activation of promoter activity of the catalytic subunit of  $\gamma$ -glutamylcysteine ligase (GCL) in brain endothelial cells by insulin requires antioxidant response element 4 and altered glycemic status: implication for GCL expression and GSH synthesis. *Free Radic Biol Med* 2011;1(51):1749–57.
- Lebacqz-Verheyden AM, Vaerman JP, Heremans JF. A possible homologue of mammalian IgA in chicken serum and secretions. *Immunology* 1972;22:165–75.
- Li T, Huang S, Lei L, Tao S, Xiong Y, Wu G, et al. Intrauterine growth restriction alters nutrient metabolism in the intestine of porcine offspring. *J Anim Sci Biotechnol* 2021;8(12):15.
- Lim LP, Lau NC, Garrett-Engle P, Grimson A, Schelter JM, Castle J, et al. Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. *Nature* 2005;17(433):769–73.
- Lin J, Handschin C, Spiegelman BM. Metabolic control through the PGC-1 family of transcription coactivators. *Cell Metabol* 2005;1:361–70.
- Look MP, Riezler R, Berthold HK, Stabler SP, Schliefer K, Allen RH, et al. Decrease of elevated N,N-dimethylglycine and N-methylglycine in human immunodeficiency virus infection during short-term highly active antiretroviral therapy. *Metabolism* 2001;50:1275–81.
- Michelet L, Zaffagnini M, Massot V, Keryer E, Vanacker H, Miginiac-Maslow M, et al. Thioredoxins, glutaredoxins, and glutathionylation: new crosstalks to explore. *Photosynth Res* 2006;89:225–45.
- Minamikawa T, Williams DA, Bowser DN, Nagley P. Mitochondrial permeability transition and swelling can occur reversibly without inducing cell death in intact human cells. *Exp Cell Res* 1999;10(246):26–37.
- Mizushima N. Autophagy: process and function. *Genes Dev* 2007;21:2861–73.
- Mohamed JS, Lopez MA, Boriek AM. Mechanical stretch up-regulates microRNA-26a and induces human airway smooth muscle hypertrophy by suppressing glycogen synthase kinase-3 $\beta$ . *J Biol Chem* 2010;17(285):29336–47.
- Mohamed JS, Hajira A, Pardo PS, Boriek AM. MicroRNA-149 inhibits PARP-2 and promotes mitochondrial biogenesis via SIRT-1/PGC-1 $\alpha$  network in skeletal muscle. *Diabetes* 2014;63:1546–59.
- National Research Council. Nutrient requirements of swine. 11th ed. Washington, DC: National Academy Press; 2012.
- Nusrat A, Parkos CA, Verkade P, Foley CS, Liang TW, Innis-Whitehouse W, et al. Tight junctions are membrane microdomains. *J Cell Sci* 2000;113:1771–81.
- Park HK, Jin CJ, Cho YM, Park DJ, Shin CS, Park KS, et al. Changes of mitochondrial DNA content in the male offspring of protein-malnourished rats. *Ann N Y Acad Sci* 2004;1011:205–16.
- Sastre J, Pallardó FV, Viña J. Mitochondrial oxidative stress plays a key role in aging and apoptosis. *IUBMB Life* 2000;49:427–35.
- Simmons RA. Developmental origins of diabetes: the role of oxidative stress. *Best Pract Res Clin Endocrinol Metabol* 2012;26:701–8.
- Sinclair DA. Toward a unified theory of caloric restriction and longevity regulation. *Mech Ageing Dev* 2005;126:987–1002.
- St-Pierre J, Drori S, Uldry M, Silvaggi JM, Rhee J, Jäger S, et al. Suppression of reactive oxygen species and neurodegeneration by the PGC-1 transcriptional coactivators. *Cell* 2006;20(127):397–408.
- Sun X, Zemel MB. Leucine modulation of mitochondrial mass and oxygen consumption in skeletal muscle cells and adipocytes. *Nutr Metab (Lond)* 2009;5(6):26.
- Tang Y, Gao C, Xing M, Li Y, Zhu L, Wang D, et al. Quercetin prevents ethanol-induced dyslipidemia and mitochondrial oxidative damage. *Food Chem Toxicol* 2012;50:1194–200.
- Valavanidis A, Vlachogianni T, Fiotakis C. 8-hydroxy-2'-deoxyguanosine (8-OHdG): a critical biomarker of oxidative stress and carcinogenesis. *J Environ Sci Health C Environ Carcinog Ecotoxicol Rev* 2009;27:120–39.
- Wang J, Chen L, Li D, Yin Y, Wang X, Li P, et al. Intrauterine growth restriction affects the proteomes of the small intestine, liver, and skeletal muscle in newborn pigs. *J Nutr* 2008;138:60–6.
- Wang T, Huo YJ, Shi F, Xu RJ, Hutz RJ. Effects of intrauterine growth retardation on development of the gastrointestinal tract in neonatal pigs. *Biol Neonate* 2005;88:66–72.
- Wang Y, Zhang L, Zhou G, Liao Z, Ahmad H, Liu W, et al. Dietary L-arginine supplementation improves the intestinal development through increasing mucosal Akt and mammalian target of rapamycin signals in intra-uterine growth retarded piglets. *Br J Nutr* 2012;28(108):1371–81.
- Xu W, Bai K, He J, Su W, Dong L, Zhang L, Wang T. Leucine improves growth performance of intrauterine growth retardation piglets by modifying gene and protein expression related to protein synthesis. *Nutrition* 2016;32:114–121.
- Zhang H, Chen Y, Li Y, Yang L, Wang J, Wang T. Medium-chain TAG attenuate hepatic oxidative damage in intra-uterine growth-retarded weanling piglets by improving the metabolic efficiency of the glutathione redox cycle. *Br J Nutr* 2014;28(112):876–85.
- Zhang H, Li Y, Chen Y, Zhang L, Wang T. N-Acetylcysteine protects against intra-uterine growth retardation-induced intestinal injury via restoring redox status and mitochondrial function in neonatal piglets. *Eur J Nutr* 2019;58:3335–47.
- Zhang J, Xu L, Zhang L, Ying Z, Su W, Wang T. Curcumin attenuates D-galactosamine/lipopolysaccharide-induced liver injury and mitochondrial dysfunction in mice. *J Nutr* 2014;144:1211–8.