Effects of dimethylglycine sodium salt supplementation on growth performance, hepatic antioxidant capacity, and mitochondria-related gene expression in weanling piglets born with low birth weight¹

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ABSTRACT: Dimethylglycine sodium salt (DMG-Na) has exhibited excellent advantages in animal experiments and human health. The present study aimed to investigate the effects of dietary supplementation with 0.1% DMG-Na on the growth performance, hepatic antioxidant capacity, and mRNA expression of mitochondria-related genes in low birth weight (LBW) piglets during weaning period. Sixteen piglets with normal birth weight (NBW) and 16 LBW piglets were fed either a basal diet or a 0.1% DMG-Na supplemented diet from age of 21 to 49 d. Blood and liver samples were collected at the end of the study. The results showed that compared with NBW piglets, LBW piglets exhibited greater ($P < 0.05$) alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase activities in the serum. LBW decreased ($P < 0.05$) the

activity of glutathione peroxidase and increased ($P < 0.05$) the contents of malondialdehyde and H_2O_2 in liver. DMG-Na supplementation increased ($P < 0.05$) body weight gain, feed intake, and feed efficiency, decreased ($P < 0.05$) ALT and AST activities, and reduced the content of H_2O_2 in LBW piglets. LBW piglets had downregulated ($P < 0.05$) mRNA expression of thioredoxin 2, thioredoxin reductases 2, and nuclear respiratory factor-1 (*Nrf1*) in the liver. However, DMG-Na supplementation increased ($P < 0.05$) mRNA expression of *Nrf1* in the liver. In conclusion, DMG-Na supplementation has beneficial effects in alleviating LBW-induced hepatic oxidative damage and changed mitochondrial genes expression levels, which is associated with increased antioxidant enzyme activities and up-regulating mRNA gene abundance.

Key words: low birth weight, mitochondria, oxidative damage

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INTRODUCTION

Growth and development of the fetus are complex biological processes affected by genetics, maternal nutrition, maternal maturity, and environmental and other factors (Redmer et al., 2004; Gootwine, 2005). These factors can influence fetal birth weight either directly or indirectly, and

birth weight in pigs is an important risk factor for preweaning mortality (Roehe and Kalm, 2000). Intrauterine growth retardation (IUGR) is usually defined as impaired growth and development of the mammalian embryo/fetus or its organs during pregnancy (Wu et al., 2006), leading to low birth weight (LBW). This LBW phenomenon occurs naturally in pigs, and it is also a common problem in human medicine (McMillen and Robinson, 2005; Wu et al., 2006). IUGR increases the risk of still born and preterm births, exerts long-term effects on the offspring, and increases the risk of cardiovascular and metabolic diseases in adulthood (Alisi et al., 2011; Devaskar and Chu, 2016;

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Herrera et al., 2017). The fetuses affected by IUGR reportedly have reduced antioxidant capacity and are prone to suffer from oxidative stress (Biri et al., 2007; Liu et al., 2013). Generally, oxidative stress is an imbalance between the production of free radicals, especially reactive oxygen species (ROS) and their scavenging capacity (Lum and Roebuck, 2001). Serious oxidative stress is known to lead to hepatic oxidative damage, including lipid peroxidation and protein peroxidation, which can have adverse effects on overall health.

The liver is one of the most important organs that plays an essential role in the metabolism and transformation of nutrients. Mitochondria are the major sites of ROS and adenosine triphosphate (ATP) production, and are the main regulation centers of energy metabolism (Hock and Kralli, 2009). When oxidative stress occurs, excessive ROS production can lead to an imbalance in redox state of cells, a reduction in the mitochondrial membrane potential, and ultimately disordered mitochondrial functions (Prosperini et al., 2013). Mitochondrial dysfunction occurs in LBW animals as evidenced by reduced ATP synthesis, reduced antioxidant enzyme activity, increased production of ROS, and impaired expression of mitochondria-related genes (Ogata et al., 1990; Park et al., 2003; Lee and Wei, 2005; Liu et al., 2012).

N, N-dimethylglycine (DMG) is a natural intermediate substance associated with the metabolic pathways of animals and plants which also appears in the pathway from choline to glycine metabolism (Prola et al., 2013). In vivo, betaine, a metabolite of choline, transfers a methyl group under the action of betaine homocysteine methyltransferase to produce DMG. In the liver, DMG is then further demethylated to sarcosine and ultimately to glycine (Craig, 2004; Slow et al., 2004). Both sarcosine and glycine play significant roles in physiological and biochemical processes. Notably, glycine is an amino acid involved in the synthesis of glutathione. Glutathione is associated with protection against oxidative stress and the regulation of protein and DNA synthesis (Hogeveen et al., 2013). One important role of DMG is the generation of glycine. Although not considered an essential amino acid, glycine may be conditionally essential for preterm infants. The maternal to fetal transfer of glycine is reportedly limited in humans and other species (Geddie et al., 1996; Paolini et al., 2001; Friesen et al., 2007). Furthermore, DMG could improve immunity and suppress oxidative stress by scavenging excessive free radicals, and thereby prevent unfavorable reactions. (Hariganesh and Prathiba, 2001; Bai et al., 2016). However, to the

best of our knowledge, the effects of DMG-Na on LBW-induced hepatic damage and mitochondrial dysfunction in weaned piglets have not yet been studied. We hypothesized that DMG-Na treatment has protective effects against LBW-induced hepatic oxidative damage and alters the expression of mitochondrial genes. Therefore, the objective of the present study was to investigate whether dietary DMG-Na supplementation could alleviate the negative effects of LBW on the growth performance, hepatic antioxidant capacity, and mitochondrial gene expression in weanling piglets.

MATERIALS AND METHODS

Ethical Statement

All experimental procedures were conducted in conformity with the Guidelines on Ethical Treatment of Experimental Animals (2006) No. 398 set by the Ministry of Science and Technology in China and the regulation regarding the Management and Treatment of Experimental Animals (2008) No. 45 set by the Jiangsu Provincial People's Government. The experimental protocol was specifically approved by the Animal Care Committee of Nanjing Agricultural University (Nanjing, Jiangsu Province, China).

Animals and Experimental Design

Pregnant sows (Landrace × Yorkshire) with similar parity (second or third) and similar expected dates of confinement were selected. All sows fed the same commercial diet, according to the nutrient requirements of the National Research Council (2012). At birth, the birth weight (BW) and sex of each newborn piglet (Duroc × (Landrace × Yorkshire)) were recorded. A piglet was defined as LBW when its BW was two standard deviations below the mean BW of the total population as described by Wang et al. (2012). Consequently, one male LBW piglet with a mean BW of 0.76 ± 0.06 kg and one normal same-sex littermate with a mean BW of 1.53 ± 0.04 kg were chosen in the present study. In total, 16 pairs of normal birth weight (NBW) and LBW piglets from 16 sows were selected according to their BW. All piglets were allowed to suckle the sow naturally up to 21 d of weaning age. Thereafter, 16 pairs of NBW and LBW piglets were randomly assigned to 1 of the 2 dietary groups. All piglets were fed a basal diet (Control, Diet C) without DMG-Na supplementation or a basal diet plus 0.1% DMG-Na supplementation (DMG-Na,

Diet D) according to 2×2 factorial arrangement. The piglets were divided into 4 experimental groups ($n = 8$): **NC** (NBW piglets fed Diet C), **ND** (NBW piglets fed Diet D), **LC** (LBW piglets fed Diet C), and **LD** (LBW piglets fed Diet D). The chemical composition of diet is present in Table 1, which was formulated according to the NRC (2012) to meet the nutrient requirements of the piglets. DMG-Na (Qilu Sheng Hua Pharmaceutical Co., Ltd., Shandong, People's Republic of China) was used as a dietary supplementation replacing the same amount of corn. Piglets were housed individually in pens with plastic floors (1×0.6 m) at 28 °C and were given ad libitum access to feed and water.

Sample Collection

At 49 d of age, all piglets were sacrificed at 12 h after the last meal. Blood samples were collected by jugular vein puncture, centrifuged at $3,000 \times g$ for 15 min at 4 °C and then stored at -80 °C until analysis. Liver samples were removed immediately after sacrifice, frozen in liquid nitrogen after snipping, and stored at -80 °C for further analysis.

Growth Performance Measurement

Pig BW and feed intake were recorded and measured on a pen basis at weaning and sampling

to calculate total BW gain, feed intake, and feed efficiency (G:F).

Liver Morphology

Liver samples from the left lobe were collected, then fixed in 4% paraformaldehyde, and embedded in paraffin. The samples were sliced into 5- μ m section that was stained with hematoxylin and eosin (H&E) and observed using light microscopy (Nikon Eclipse 80i; Nikon, Melville, NY, USA). The slides were reviewed by 2 technical specialists (Nanjing Microworld Bioengineering), who were “blinded” as to the experimental details.

Assessment of Serum Aminotransferase Activities

The activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (AKP) in the serum were determined with corresponding kits obtained from the Nanjing Jiancheng Institute of Bioengineering (Nanjing, Jiangsu, China).

Assay of Hepatic Antioxidant System

One gram of liver was homogenized at 8,000 rpm for 10 s in 4 mL of 0.9% sterile saline solution on ice and centrifuged at $3,500 \times g$ for

Table 1. Ingredients and nutrient contents of the basal diet (dry matter basis)

Ingredients	Contents, %	Nutrient composition	Contents, %
Corn	40.00	Digestible energy (MJ/kg)	14.34
Rice, broken	15.00	Crude protein	20.20
Soybean meal, fermented	10.00	Leucine	1.45
Soybean meal, de-hulled	6.00	Methionine + cystine	0.79
Spray dried animal plasma	5.00	Lysine	1.34
Whey powder	7.00	Threonine	0.81
Fish meal	4.00	Tryptophan	0.23
Sugar	4.50	Isoleucine	0.74
Glucose	3.00	Valine	0.89
Soybean oil	1.50	Total calcium	0.85
L-lysine-HCl (98%)	0.30	Total phosphorus	0.70
L-methionine	0.15		
L-threonine	0.20		
L-tryptophan	0.05		
L-isoleucine	0.05		
L-valine	0.05		
Salt	0.30		
Limestone	1.10		
CaHPO ₄	0.80		
Premix ^a	1.00		
Total	100.00		

^aIn premix, provided per kg of diet: Vitamin A, 15,000 IU; Vitamin D₃, 3,000 IU; Vitamin E, 150 mg; Vitamin K₃, 3 mg; Vitamin B₁, 3 mg; Vitamin B₂, 6 mg; Vitamin B₆, 5 mg; Vitamin B₁₂, 0.03 mg; Niacin, 45 mg; Vitamin C, 250 mg; Calcium pantothenate, 9 mg; Folic acid, 1 mg; Biotin, 0.3 mg; Choline chloride, 500 mg; Fe, 170 mg; Cu, 150 mg; Zn, 150 mg; Mn, 80 mg; Mg, 68 mg; I, 0.90 mg; Co, 0.30 mg; Se, 0.2 mg.

15 min at 4 °C, and then this hepatic supernatant was carried out to measure the activities of total antioxidative capability (**T-AOC**), superoxide dismutase (**SOD**), glutathione peroxidase (**GSH-Px**), and the contents of malondialdehyde (**MDA**), H_2O_2 , and nitric oxide using colorimetric kits with a spectrophotometer according to the instructions provided with the kits obtained from the Nanjing Jiancheng Institute of Bioengineering (Nanjing, Jiangsu, China). The protein concentrations were determined by using the bicinchoninic acid assay (Bainor et al., 2011).

Isolation of Hepatic Mitochondria

Hepatic mitochondria were isolated at 4 °C by using a standard procedure described by Weinbach (1961). Namely, approximately 1 g of liver samples was immediately immersed in isolation buffer (0.1 μ mol/L EDTA-2Na, 0.01 M Tris-HCl, 0.8% sodium chloride solution, 0.01 M sucrose, pH = 7.4). Tissue samples were then homogenized with additional 9 volumes (wt/vol) of homogenization media in a homogenizer and centrifuged at $600 \times g$ for 10 min. The collected supernatants were then centrifuged again at $11,000 \times g$ for 15 min. The supernatants were removed and resuspended in isolation buffer and centrifuged again at $11,000 \times g$ for 15 min to acquire mitochondria. The concentrations of protein in extracts were measured by using the bicinchoninic acid protein assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China).

Measurement of Hepatic Mitochondrial Metabolite Concentration

Protein oxidation in hepatic mitochondria was determined by the concentration of protein carbonyls (**PC**). The PC concentration was calculated in triplicate by using the method described by Weber et al. (2015), and the results were presented in nmol/mg protein. The content of 8-hydroxy-2-deoxyguanosine (**8-OHdG**) in hepatic mitochondria was measured using an enzyme linked immunosorbent assay (**ELISA**) kit (Nanjing Jiancheng Bioengineering Institute) according to the manufacturers' instructions, and the results were presented in ng/mg protein.

Detection of ROS Generation From Primary Hepatocytes

The ROS assay kit (S0033; Beyotime Biotech) was used to detect the intracellular ROS

concentrations via a sensitive fluorescent dichloro-fluorescein-diacetate (**DCFH-DA**) probe, as previously described in detail (Sang et al., 2012). Briefly, the freshly isolated hepatocytes were incubated with 2, 7-DCFH-DA (10 μ M) and DNA stain Hoechst 33342 (10 mmol/L) at 37 °C for 30 min. Then the DCFH fluorescence of the mitochondrial was measured at an emission wavelength of 530 nm and an excitation wavelength of 485 nm with an FLX 800 microplate fluorescence reader (Biotech Instruments, Inc., USA). ROS production was presented as the mean DCFH-DA fluorescence intensity over that of the control.

Measurement of Mitochondrial Membrane Potential

The mitochondrial membrane potential assay kit (C2006; Beyotime Biotech) was used to monitor the change in mitochondrial membrane potential, as previously described in detail (Zhang et al., 2011). The mitochondrial membrane potential of hepatocytes was calculated as the fluorescence ratio of aggregates (red) to monomers (green).

RNA Extraction and Quantitative Real-Time Polymerase Chain Reaction

RNA was isolated using Trizol Reagent (TaKaRa Biotechnology, Dalian, Liaoning, China) from snap-frozen liver sample using the manufacturer's protocol. RNA integrity was checked on 1% agarose gel with ethidium bromide staining. The RNA concentration and purity were determined from OD 260/280 readings (ratio > 1.8) using a NanoDrop ND-1000 UV spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). After determining the RNA concentration, 1 μ g of total RNA was reverse-transcribed into cDNA using PrimeScript RT Reagent Kit (TaKaRa Biotechnology, Dalian, Liaoning, China) according to the manufacturer's guidelines.

Quantitative real-time polymerase chain reaction (**qRT-PCR**) was performed on an ABI StepOnePlus Real-Time PCR System (Applied Biosystems, Grand Island, NY, USA) according to the manufacturer's instructions. The sequences of primers used in this experiment are shown in Table 2. The cDNA samples were amplified by qRT-PCR with SYBR Premix Ex Taq reagents (Takara Biotechnology, Dalian, Liaoning, China). Briefly, the reaction mixture of 20 μ L was prepared using 2 μ L of cDNA, 0.4 μ L each of forward and reverse primers, 0.4 μ L of ROX reference dye

(50 ×; Life Technologies, Grand Island, New York), 10 µL of SYBR Premix Ex Taq (2×), and 6.8 µL of double-distilled H₂O. Each sample was tested in duplicate. qRT-PCR consisted of a prerun at 95 °C for 30 s and 40 cycles of denaturation at 95 °C for 5 s, followed by a 60 °C annealing step for 30 s. The conditions of the melting curve analysis were as follows: one cycle of denaturation at 95 °C for 10 s, followed by an increase in temperature from 65 to 95 °C at a rate of 0.5 °C/s. The relative levels of mRNA expression were calculated using the 2^{-ΔΔC_t} method (Livak and Schmittgen, 2001), in which the β-actin gene was amplified as an internal standard, and the values of pigs in NC group were used as a calibrator.

Statistical Analysis

Two-way ANOVA was used to determine the main effects (BW and diet) and their interaction using the general linear model procedure of SPSS

software (version 21; SPSS, Inc., Chicago, IL, USA). Data are presented as mean ± SEM. When significant interactions were observed, means separation was conducted by one-way ANOVA using Duncan's multiple range tests, which were considered significant at $P < 0.05$ and P values between 0.05 and 0.10 were considered a trend.

RESULTS

Growth Performance

The growth performance is shown in Table 3. LBW piglets exhibited lower ($P < 0.05$) total BW gain and feed intake during experimental period. However, dietary supplementation with DMG-Na increased ($P < 0.05$) total BW gain, feed intake, and feed conversion ratio (G:F) in LBW piglets. The interactions between BW and Diet were not significant.

Table 2. Sequences for real-time PCR primers

Gene ¹	Accession number	Forward and reverse primers (5'-3')	Length, bp
SOD1	NM_001190422.1	F: CATTCCATCATTTGGCCGCAC R: TTACACCACAGGCCAAACGA	118
GPX1	NM_214201.1	F: CCTCAAGTACGTCCGACCAG R: TGAGCATTTGCGCCATTCA	85
Trx2	NM_214313.2	F: CCAAGATGGTGAAGCAG R: TGGCTGAGAAATCGACCA	98
Trx-R2	NM_001243705.1	F: GACAGAAAGTGCCCTTGA R: AGCCATCTCCCAGCAAC	78
Nrf1	XM_005657993.1	F: CCTTGTGGTGGGAGGAATGT R: TATGCTGGCTGACCTTGTGG	75
Sirt1	NM_001145750.1	F: TTGCAACAGCATCTTGCCTG R: GGACATCGAGGAACCACCTG	91
PGC-1α	NM_213963.2	F: GCAGTTCTCACAGAGACGCT R: TAGAGACGGCTCTTCTGCCT	162
TFAM	NM_001130211.1	F: AGCGAGGTCTGAAGAGTTGC R: GAGGTCCGCTCCTGACTTTC	176
β-actin	DQ845171.1	F: TCATGGACTCTGGGGATGGG R: GCAGCTCGTAGCTCTTCTCC	273

¹SOD1 = superoxide dismutase 1; GPX1 = glutathione peroxidase 1; Trx2 = thioredoxin 2; Trx-R2 = thioredoxin reductase 2; Nrf1 = nuclear respiratory factor 1; SIRT1 = sirtuin 1; PGC-1α = peroxisome proliferator-activated receptor-γ coactivator 1α; TFAM = mitochondrial transcription factor A.

Table 3. Effects of dietary dimethylglycine sodium salt (DMG-Na) supplementation on growth performance of normal birth weight and low birth weight weanling piglets

Items ¹	NBW		LBW		SEM	P-value		
	CON	DMG-Na	CON	DMG-Na		BW	Diet	BW × Diet
Total body weight gain, kg	9.33	11.15	7.94	9.85	1.35	0.012	0.001	0.927
Total feed intake, kg	17.00	16.70	14.90	15.30	0.11	0.033	0.045	0.838
G:F, kg/kg	0.55	0.67	0.53	0.64	0.06	0.075	0.011	0.859

¹NBW-CON = normal birth weight group given a control diet; NBW-(DMG-Na) = normal birth weight group given a DMG-Na supplemented diet; LBW-CON = low birth weight group given a control diet; LBW-(DMG-Na) = low birth weight group given a DMG-Na supplemented diet; SEM = standard error of the mean; BW = birth weight; G:F = weight gain/feed intake.

Liver Morphology

Histology of the liver sections in the NBW groups presented normal hepatocyte architecture with well-preserved cytoplasm (Figure 1A and B). However, LBW piglets had moderate alteration in hepatic examination, which clearly revealed the dissolution of the cell structure (Figure 1C). The vacuolization of hepatocyte (arrowhead) and the disorganization of parenchyma were also more obvious in LBW piglets. These morphological changes, especially cytoplasmic vacuolization, were partially alleviated after DMG-Na supplementation (Figure 1D).

Serum ALT, AST, and ALP Activities

As shown in Table 4, LBW piglets had greater ($P < 0.05$) ALT, AST, and AKP activities in the serum than NBW piglets, whereas DMG-Na supplementation decreased ($P < 0.05$) ALT and AST activities in LBW piglets. However, no difference was found for AKP activity with DMG-Na supplementation ($P > 0.05$). In addition, both BW and diet had no interaction effects on these 3 parameters ($P > 0.05$).

Hepatic Antioxidant Capacity

LBW decreased ($P < 0.05$) the activity of GSH-Px and increased ($P < 0.05$) the contents of

MDA and H_2O_2 in liver, compared with NBW piglets (Table 5). The production of nitric oxide had a trend to be greater ($P = 0.086$) in LBW piglets. Additionally, without DMG-Na supplementation, hepatic T-AOC and SOD activities were not affected by LBW. Piglets with DMG-Na treatment had a trend to increase the activities of hepatic GSH-Px ($P = 0.055$) and SOD ($P = 0.087$) and decrease hepatic MDA content ($P = 0.094$) in comparison with those given control diets. However, the content of H_2O_2 was reduced ($P < 0.05$) in DMG-Na-treated groups. Additionally, BW and diet had an interaction effect ($P < 0.05$) on hepatic H_2O_2 content, in which dietary DMG-Na supplementation decreased hepatic H_2O_2 content in LBW piglets rather than NBW piglets.

Contents of PC, 8-OHdG, ROS, and Membrane Potential in Hepatic Mitochondria

As shown in Figure 2, LBW piglets had greater ($P < 0.05$) concentrations of PC, 8-OHdG, and ROS in hepatic mitochondria compared with NBW piglets. In addition, mitochondrial membrane potential tended to decrease ($P = 0.080$) in LBW piglets. However, DMG-Na supplementation did not eliminate the differences in contents of PCs, 8-OHdG, ROS, and membrane potential in hepatic mitochondria between NBW and LBW piglets. There was no interaction effects ($P > 0.05$) between BW and diet on these parameters.

Figure 1. Effects of dimethylglycine sodium salt (DMG-Na) on hepatic histology of normal birth weight and low birth weight weanling piglets. Liver sections from the different groups were stained with hematoxylin and eosin (original magnification: 400×). Arrowheads indicated the vacuolization of hepatocyte. (A) Normal birth weight piglets with control diets. (B) Normal birth weight piglets with diets supplemented with 0.1% DMG-Na. (C) Low birth weight piglets with control diets. (D) Low birth weight piglets with diets supplemented with 0.1% DMG-Na.

Table 4. Effects of dietary dimethylglycine sodium salt (DMG-Na) supplementation on activities of ALT, AST, AKP in the serum of normal birth weight and low birth weight weanling piglets

Items ¹	NBW		LBW		SEM	P-value		
	CON	DMG-Na	CON	DMG-Na		BW Diet	BW × Diet	
ALT, U/L	21.77	21.10	26.67	24.08	0.56	0.002	0.009	0.102
AST, U/L	30.59	30.17	35.88	32.88	0.65	0.005	0.017	0.063
AKP, Unit ²	20.010	21.46	23.70	23.22	0.89	0.007	0.602	0.301

¹NBW-CON = normal birth weight group given a control diet; NBW-(DMG-Na) = normal birth weight group given a DMG-Na supplemented diet; LBW-CON = low birth weight group given a control diet; LBW-(DMG-Na) = low birth weight group given a DMG-Na supplemented diet; SEM = standard error of the mean; BW = birth weight; ALT = alanine aminotransferase; AST = aspartate aminotransferase; AKP = alkaline phosphatase.

²Unit: one unit alkaline phosphatase activity is defined as the amount of alkaline phosphatase in 100 mL serum that liberates 1 mg of hydroxy-benzene from disodium phenyl phosphate per quarter at 37 °C.

Table 5. Effects of dietary dimethylglycine sodium salt (DMG-Na) supplementation on hepatic redox status of normal birth weight and low birth weight weanling piglets

Items ¹	NBW		LBW		SEM	P-value		
	CON	DMG-Na	CON	DMG-Na		BW Diet	BW × Diet	
T-AOC, U/mg protein	1.04	1.05	0.912	1.028	0.06	0.241	0.335	0.419
SOD, U/mg protein	323.70	345.59	327.00	346.60	11.52	0.853	0.087	0.922
GSH-Px, U/mg protein	84.99	86.85	75.85	83.94	2.44	0.023	0.055	0.215
MDA, nmol/mg protein	2.83	2.78	3.49	2.94	0.17	0.025	0.094	0.168
H ₂ O ₂ , mmol/g protein	6.15 ^b	6.32 ^b	7.97 ^a	6.35 ^b	0.24	0.001	0.006	0.001
nitric oxide, µmol/g protein	1.61	1.67	2.09	1.68	0.14	0.086	0.229	0.099

^{a,b}Means not sharing the same superscript differ at $P < 0.05$.

¹NBW-CON = normal birth weight group given a control diet; NBW-(DMG-Na) = normal birth weight group given a DMG-Na supplemented diet; LBW-CON = low birth weight group given a control diet; LBW-(DMG-Na) = low birth weight group given a DMG-Na supplemented diet; SEM = standard error of the mean; BW = birth weight; T-AOC = total antioxidative capability; SOD = superoxide dismutase; GSH-Px = glutathione peroxidase; MDA = malondialdehyde.

Gene Expression

The expression of genes related to hepatic antioxidant capacity and mitochondria is presented in Figure 3. Compared with NBW piglets, LBW piglets had downregulated ($P < 0.05$) mRNA expression levels of *Trx2*, *Trx-R2*, and nuclear respiratory factor-1 (*Nrf1*) in the liver. The expression levels of *SOD1* ($P = 0.095$), *GPx1* ($P = 0.065$), and *TFAM* ($P = 0.095$) had a trend to decrease in LBW piglets. After dietary supplementation with DMG-Na, mRNA expression of *Nrf1* in the liver of piglets was increased ($P < 0.05$). A tendency for increased ($P = 0.065$) mRNA gene abundance of *GPx1* was seen in the piglets fed the DMG-Na diet. Similarly, DMG-Na supplementation tended to alleviate the lower expression levels of *TFAM* ($P = 0.065$) and *PGC-1α* ($P = 0.082$) induced by LBW. In addition, BW and diet had no interaction effects ($P > 0.05$) on above expression levels of genes.

DISCUSSION

Births affected by prenatal intrauterine growth restriction may have long-term negative effects on

the individual physiological function. This applies for human babies as well as piglets. Some studies have shown that IUGR delayed the postnatal growth and gut development in pigs (Wang et al., 2005; Hu et al., 2015). In addition, IUGR pigs have reduced antioxidant capacity and are prone to oxidative damage (Biri et al., 2007). As a dietary additive, DMG-Na could improve nutrient digestibility in sows and suppress oxidative stress (Cools et al., 2010; Bai et al., 2016). In this study, we investigated the growth performance, hepatic antioxidant capacity, and the mitochondrial function of piglets with LBW caused by IUGR. In addition, we assessed the effects of DMG-Na supplementation on the above indexes of LBW piglets during the post-weaning period.

The data presented herein revealed that LBW significantly reduced total BW gain and feed intake in piglets after weaning. These findings indicate that LBW negatively affects BW and post-weaning growth of piglets, which were consistent with the results of previous studies that showed LBW piglets exhibited poor growth performance compared with normal piglets (Michiels et al., 2013; Zhang et al., 2014; Zhang et al., 2016). As expected, dietary

Figure 2. Effects of dimethylglycine sodium salt (DMG-Na) on hepatic reactive oxygen species (ROS) concentrations (A), protein carbonyls contents (B), 8-hydroxy-2-deoxyguanosine contents (C), and mitochondrial membrane potential (D) of normal birth weight and low birth weight weanling piglets. (A)–(D) The value of NC group was set to 100%. NBW/CON = normal birth weight group given a control diet; NBW/DMG-Na = normal birth weight group given a DMG-Na supplemented diet; LBW/CON = low birth weight group given a control diet; LBW/DMG-Na = low birth weight group given a DMG-Na supplemented diet; BW = birth weight; data are presented as mean $\pm$ SEM ($n = 8$).

supplementation with DMG-Na was able to ameliorate LBW-induced inferior growth performance by significantly increasing total BW gain and feed intake, and improving feed efficiency. Dietary supplementation with 167-mg DMG-Na per kg has been demonstrated to improve apparent total tract digestibility in broiler chickens (Kalmar et al., 2010). Similarly, short-term supplementation of DMG leads to an improved nutrient digestibility in sows (Cools, et al., 2010). These beneficial effects of DMG have been attributed to its emulsifying action at the gut level, through which nutrients become more available for digestion and absorption (Kalmar et al., 2011). This might be the reason for the significant effects of dietary treatment with 0.1% DMG-Na on growth performance in LBW piglets.

The assessment of serum aminotransferase activities and histological changes suggested that liver damage induced by LBW was alleviated after DMG-Na administration. The activities of serum AST, ALT, and AKP are important indicators of liver function. Hepatic AST is distributed in the cytoplasm and mitochondria, and hepatic ALT is only present in the cytoplasm. Once the liver has been damaged, the hepatic cell structure is destroyed, and ALT and AST are released into the

blood circulation. Elevated serum AKP has been associated with impaired liver function caused by hepatic cholestasis and some destruction of hepatic cell membranes. In the current study, piglets were food-deprived for 12 h prior to blood samples collection to avoid the influence of certain components of the feed on serum biochemical indicators. We observed that LBW piglets had greater ALT, AST, and AKP activities in the serum than NBW piglets. Similarly, Zhang et al. (2018) confirmed that LBW piglets showed a greater rise in plasma aminotransferase activities. The abnormal increase of serum aminotransferase activities indicates severe damage of liver tissue. Boehm et al. (1990) have also demonstrated that IUGR could lead to overall hepatocellular dysfunction in infants with LBW. Moreover, our study found that DMG-Na intervention could attenuate hepatic damage in LBW piglets. Reduced AST and ALT activities in serum suggested that LBW-induced hepatic injury was ameliorated after DMG-Na administration, and this was directly supported by morphological observation where the hepatocyte vacuolation was partially attenuated by DMG-Na treatment.

Oxidative stress is a systematic response associated with the pathophysiology of many

Figure 3. Effects of dietary dimethylglycine sodium salt (DMG-Na) supplementation on the hepatic antioxidant and mitochondria related mRNA expressions in normal birth weight and low birth weight weanling piglets. (A) Relative mRNA expressions of *SOD1*, *GPx1*, *Trx2*, and *Trx-R2*. (B) Relative mRNA expressions of *Nrf1*, *PGC-1α*, *Sirt1*, and *TFAM*. NBW/CON = normal birth weight group given a control diet; NBW/DMG-Na = normal birth weight group given a DMG-Na supplemented diet; LBW/CON = low birth weight group given a control diet; LBW/DMG-Na = low birth weight group given a DMG-Na supplemented diet; BW = birth weight; data are presented as mean $\pm$ SEM ($n = 8$).

different disorders, including hepatic redox status and function in piglets as shown in the present study. Oxidative damage is an important factor that induces liver injury of IUGR piglets. As small and highly reactive molecules, H_2O_2 and nitric oxide play a dual role in normal cell progression, as both toxins and signaling molecules (Luo et al., 2016). In this trial, LBW piglets showed a significant increase in hepatic H_2O_2 concentration. The overproduction of free radicals could exceed the capability of the antioxidant defense system, which could in turn initiate many oxidative reactions, resulting in oxidative damage. However, DMG-Na supplementation could prevent these alterations and particularly reduce the concentration of H_2O_2 considerably. MDA is the most common product of lipid peroxidation (Pirincicoglu et al., 2010) and its contents

can directly reflect the extent of lipid oxidative injury. In the present study, a considerable increase in MDA concentration was observed in the liver of LBW piglets, when compared with normal piglets. Similarly, Liu et al. (2012b) and Zhang et al. (2018) also reported greater contents of MDA in the liver of LBW piglets. The increase in lipid peroxidation could be attributed to increased generation of free radicals and a reduction in antioxidant capacity. The classical antioxidant enzymes, such as SOD and GSH-Px, comprise the first line of defense against ROS. Furthermore, T-AOC reflects the activity of nonenzymatic antioxidant defense system and the levels of antioxidant enzymes. In the present study, we found that hepatic GSH-Px activity was reduced in LBW piglets but the T-AOC and SOD activities showed no difference. These findings are not entirely

consistent with those of [Zhang et al. \(2016\)](#). We speculated that unreduced SOD activity might be a compensatory response to reduce oxidative stress caused by LBW. In addition, [Yin et al. \(2014\)](#) found that plasma SOD activity was reduced in piglets at 1-d post-weaning and was then recovered at 3, 5, and 7 d, which is also suggestive of the body's compensatory response to stress. Supplementation with DMG-Na had a trend to increase the activities of SOD and GSH-Px in the liver. [Bai et al. \(2016\)](#) reported that gastric intubation with DMG-Na significantly improves the activities of hepatic SOD and GSH-Px in mice. This disparity between our studies could be explained by the differences in species and models of administration additives.

It has been reported that the excess generation of free radicals in the body could inhibit antioxidant capacity and disrupt the functions of mitochondrial lipids and protein ([Lee and Wei, 2005](#); [Prosperini et al., 2013](#)). Mitochondrial membranes are rich in unsaturated fatty acids that make them the most vulnerable targets of free radicals. Under oxidative stress, excessive free radicals interact with hepatic mitochondrial membranes. This causes an elevation in MDA contents and subsequent disintegration of the mitochondrial membrane ([Chen and Yu, 1994](#)). Increased concentrations of free radicals are also accompanied by a reduction in the mitochondrial membrane potential level, which usually marks the initial stages of mitochondrial damage. Our current study found that LBW piglets showed greater concentrations of ROS, 8-OHdG, and PC in hepatic mitochondria when compared with normal piglets. These results indicated that LBW was closely linked to oxidative stress and mitochondrial damage. Similarly, [Liu et al. \(2012a\)](#) demonstrated that the membrane potential was decreased in isolated mitochondria from the liver of LBW piglets. However, our analysis of ROS, 8-OHdG, PC levels, and membrane potential did not support the notion that mitochondrial damage in LBW piglets could be prevented by our nutritional strategy. No significant effects of dietary DMG-Na supplementation on the above indexes of mitochondrial damage were observed.

Moreover, we examined the expression levels of genes associated with the hepatic redox status and mitochondria. The mRNA abundance of *SOD1* exhibited no notable differences between LBW and normal piglets. These results are consistent with unreduced activity of hepatic SOD in LBW piglets, indicating that this enzyme could mediate the restoration of homeostasis. The mRNA abundance of *GPx1* showed a tendency to be downregulated by

LBW in the liver of piglets; however, this change was not significant. Similarly, [Zhang et al. \(2016\)](#) reported no alterations in the mRNA abundance of *GPx1* in LBW piglets, compared with NBW piglets. As part of the antioxidant defense system, the *Trx2* system can maintain free radicals at a non-toxic level, which is crucial for maintaining redox balance and cell viability. Furthermore, changes in mRNA levels of *Trx-R2* are closely related to mitochondrial lipid peroxidation and mitochondrial DNA integrity. Increased mitochondrial ROS production in *Trx2*-deficient mice was observed by [Pérez et al. \(2008\)](#). Moreover, [Li et al. \(2017\)](#) demonstrated that high glucose-induced mitochondrial oxidative damage could be prevented by elevating *Trx2* levels. Our findings showed that the mRNA abundances of *Trx2* and *Trx-R2* were obviously lower in LBW piglets than in normal piglets. This further suggested that LBW caused mitochondrial damage in liver. Our dietary intervention exerted expectable outcomes. There was significant enhancement of *Trx2* expression after treatment with DMG-Na, which might also explain the improvements observed in the hepatic antioxidant system of LBW piglets. Mitochondrial damage was observed in present study, and several transcriptional factors such as *Nrf1*, *PGC-1 α* , *Sirt1*, and *TFAM* were closely linked to mitochondrial regulation. Hepatic mRNA expression of *Nrf1* was found to be downregulated in LBW piglets, which is in agreement with previous study ([Liu et al., 2012b](#)). As a nuclear-encoded regulator of mtDNA replication and transcription that could be regulated by *PGC-1 α* and *Nrf1* to initiate mitochondrial biogenesis ([Ekstrand et al., 2004](#)), *TFAM* tended to be reduced in LBW piglets, compared with NBW piglets. One member of the deacetylase family, *Sirt1*, was shown to regulate oxidative stress in cells and was capable of activating mitochondrial biogenesis by transcriptional activation of the signaling pathway involved in *PGC-1* ([Cheng et al., 2003](#)). [Wolfe et al. \(2012\)](#) demonstrated that LBW newborns underwent postnatal suppression of hepatic *Sirt1* activity. Studies in rodents have also provided evidence that LBW results in low expression of *Sirt1* in skeletal muscle ([Chen et al., 2009](#)). However, we did not find any notable differences in *Sirt1* expression between LBW and normal piglets. We speculated that this discrepancy could be attributed to differences in breed, age, and selection criteria associated with experimental animals. The present study found that mRNA expression of *Nrf1*, *TFAM*, and *PGC-1 α* in LBW piglets was reversed to levels similar to those of normal piglets after dietary

supplemented with DMG-Na. These data indicated that DMG-Na might improve the antioxidant defense system and ameliorate mitochondrial damage in LBW piglets, by increasing the expression of some hepatic antioxidant genes and mitochondria-related genes. Based on the above results, we were unable to explicitly explain why supplementation with DMG-Na could increase the expression of some antioxidant genes and mitochondrial genes, but has no significant effect on ROS concentration, PC, and 8-OHdG levels or the membrane potential. The experimental period of the present study was only 28 d. Thus, hepatic mitochondrial damage induced by LBW possibly requires a longer duration before amelioration could be observed. Further research is needed to explore the possible reasons behind these observations, particularly to examine the long-term effects of DMG-Na.

In conclusion, the present results indicate that dietary supplementation of DMG-Na has no mitigating effect on mitochondrial damage. However, DMG-Na supplementation is beneficial in improving the growth performance of LBW piglets. Moreover, LBW piglets fed DMG-Na supplemented diets exhibit reduced serum ALT, AST activities and hepatic H_2O_2 content, and increase mRNA abundance of *Nrf1* in the liver, which may account for the upregulated expression of hepatic antioxidant genes and improved liver morphology. Altogether, this study can help expand our understanding of the potential protective roles of DMG-Na in LBW piglets and provide a new concept for nutrition interventions in LBW offspring.

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