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Dietary Arginine Supplementation Affects Intestinal Function by Enhancing Antioxidant Capacity of a Nitric Oxide–Independent Pathway in Low-Birth-Weight Piglets

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Abstract

Background: Low-birth-weight (LBW) neonates are susceptible to intestinal dysfunction. Furthermore, the antioxidant capacity of LBW neonates is significantly lower compared with that of normal-birth-weight (NBW) neonates both at birth and at weaning. In LBW neonates, dietary supplementation with arginine has shown beneficial effects on intestinal function.

Objective: The present study explored the potential mechanisms of arginine-induced protective effects against intestinal dysfunction in LBW piglets.

Methods: Forty 4-d-old LBW piglets [body weight (BW): 1.05 ± 0.04 kg] (Large White × Landrace) were assigned to 4 treatments and artificially fed a whole-milk powder– and whey protein concentrate–based diet (containing 0.65% arginine) either not supplemented with arginine (LBWC) or supplemented with 0.5%, 1.0%, or 1.5% L-arginine for 21 d. In addition, 10 NBW siblings (BW: 1.96 ± 0.03 kg) were selected and fed the basal diet. Growth performance, intestinal morphology, mRNA expression of tight junction protein, redox-sensitive genes and nitric oxide (NO) synthase, cytokines, and redox indexes were determined. Data were subjected to 1-factor ANOVA.

Results: LBW piglets exhibited poorer growth performance (29.9%), lower Claudin1 mRNA level (63.6%), lower antioxidant capacity (22.9 ~ 24.3%), and higher jejunum interleukin 1 (IL-1) concentration (18.8%) compared with NBW piglets. Dietary supplementation with 0.5% and 1.0% L-arginine significantly enhanced daily BW gain of LBW piglets by 13.6% and 18.2%, respectively. Compared with LBWC, dietary supplementation with 1.0% L-arginine increased the serum insulin concentration (32.2%) and villus height in the jejunum (12.2%) and ileum (20.5%). In the jejunum, the mRNA levels for Claudin1 (105%) and glutathione peroxidase (36%) were higher, and the concentrations of IL-1 (31.7%) and tumor necrosis factor α (TNF-α) (30%) were lower in arginine-treated piglets than in the LBWC group. However, NO synthase activity and NO concentration in the jejunum of LBW piglets were not influenced by L-arginine supplementation.

Conclusion: The results suggested that supplementation with 1.0% L-arginine not only promoted growth performance and improved intestinal functions in LBW piglets but also improved intestinal barrier functions and enhanced antioxidant capacity by an NO-independent pathway. *J Nutr* 2018;148:1751–1759.

Keywords: low birth weight, arginine, intestine, antioxidant capacity, barrier function

Introduction

Low birth weight (LBW) refers to infants born with a weight <2.5 kg regardless of gestational age at birth and piglets weighing <1.0 kg at birth (1–3). LBW in humans and other mammals increases the risk of neonatal mortality and morbidity. It also causes poor postnatal growth (3–6). Many studies have shown that the retarded growth of LBW neonates is partly due to the impairment of the digestive and absorptive functions of the small intestine (2, 7–9). Because of the similarities in gastrointestinal physiology between infants and piglets, LBW piglets have been considered to be an ideal model to study the gastrointestinal dysfunction observed in LBW infants (10, 11).

Current studies have reported that LBW piglets exhibit intestinal dysfunction with an increase in permeability and downregulation of tight junction proteins in the small intestine (12, 13). Tight junction proteins, including occludin, claudins, and zonula occludens, are important in regulating intestinal barrier integrity and preventing the diffusion of macromolecules

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through paracellular pathways (12, 14). Furthermore, many studies have reported a significant decrease in the antioxidant capacity of LBW neonates at birth and weaning (2, 15, 16). The imbalance between reactive oxygen species (ROS) production and antioxidant capacity leads to oxidative stress (17). ROS, such as superoxide anion free radicals and hydroxyl free radicals, are produced mainly in the mitochondria. Excessive ROS elicit oxidative stress, which further induces cell apoptosis and inhibits cell proliferation (18). The intestine is susceptible to oxidative damage and its frequent contact with luminal oxidants from nutrient intake or infection (19). Previous studies have reported that necrotizing enterocolitis, which is inversely correlated with birth weight, is one of the oxidative stress–related diseases in preterm infants (20). Numerous factors induce oxidative stress in necrotizing enterocolitis, such as bacterial LPSs and cytokines, which can trigger ROS generation (21).

Arginine is an essential amino acid for young mammals, especially for those under stress (22–24). Arginine plays an important role in many metabolic pathways, because it serves as a precursor for the synthesis of biologically important molecules, such as NO, creatine, ornithine, and polyamines (25). Earlier studies reported that supplementing arginine at different doses (0.2%, 0.4%, 0.6%, 0.7%, 0.8%, 1.0%, or 1.2%) enhanced growth performance and intestinal development in early-weaned piglets (23, 26–28). Notably, supplementation with 1.5% L-arginine relieved oxidative stress induced by diquat in weaned piglets (29). Mitochondria are considered to be a source of ROS (30). A decrease in ROS-scavenging ability might trigger oxidative stress and impair the function of mitochondria (31). It is well known that arginine can promote the biogenesis and biological function of mitochondria through the NO-signaling pathway (32, 33). However, information on the effects of dietary arginine supplementation on the redox status in the small intestine of LBW neonatal piglets remains elusive.

Thus, we hypothesized that arginine supplementation could enhance growth and improve intestinal development by reducing oxidative stress in LBW piglets. Our objective was to elucidate the benefits of arginine supplementation on intestinal dysfunction in LBW piglets and offer a theoretical basis for developing arginine as a functional component in feeds or formulas of LBW neonates.

Methods

The study was carried out according to the guidelines of the Ethics Committee of Sichuan Agricultural University (China) for the use of animals in research.

Experimental animals. Artificial rearing is an effective strategy to assess the effects of nutritional interventions and their impact on the growth and development of piglets (34, 35). Full-term newborn piglets were weighed ≤2 h after parturition to determine birth weight and sex. Piglets were individually tagged with a unique ear tag, and birth weight was used as a criterion to identify LBW and normal-birth-weight (NBW) littermates. Piglets with birth weights <1.0 kg were selected as being LBW according to a previous study (2). An NBW littermate had a birth weight ≤1-SD unit of the mean birth weight of the whole litter. The average ± SEM birth weights for all LBW and NBW piglets in this study were 0.94 ± 0.03 kg and 1.69 ± 0.03 kg, respectively. Bottle-feeding was started at day 4 after birth to allow piglets to ingest sufficient amounts of colostrum. At 4 d of age, a total of 40 LBW piglets (Large White × Landrace; Mianyang, China) from 40 litters were selected and distributed in 4 groups. LBW piglets were fed a whole-milk powder– and whey protein concentrate–based diet (containing 0.65% arginine) either not supplemented with arginine (LBWC) or supplemented with 0.5%, 1.0%, or 1.5% L-arginine in their diet. In addition, 10 NBW siblings derived from 10 of the above-mentioned 40 sows were selected and fed a basal diet without supplemental L-arginine (NBWC). The average ± SEM body weights (BWs) of LBW and NBW piglets on day 4 were 1.05 ± 0.04 kg and 1.96 ± 0.03 kg, respectively. Each treatment group consisted of 10 piglets, and the sex ratio was equal (1:1).

Piglets were housed individually in metabolism cages (0.8 m × 0.7 m × 0.4 m) in an environmentally controlled room. The room temperature was maintained at 30°–32°C during the first week and was gradually reduced to 27°–29°C by the end of the 21-d experimental period. All piglets were bottle-fed with liquid formula milk and had free access to drinking water. The piglets were bottle-fed 7 times/d at 0600, 0900, 1200, 1500, 1800, 2100, and 2400. For each piglet, the weight of the liquid milk was recorded before and after feeding for calculating the dry matter intake based on the ratio of formula powder to water. No medicine or antibiotics were used during the experimental period. The experiment lasted 21 d.

Experimental diets. The basal diet (**Supplemental Table 1**) was formulated according to previous studies (26, 34). To formulate the diets accurately, amino acids in the ingredients (whole-milk powder, whey protein concentrate, and casein) were analyzed according to the AOAC (26, 36). The amino acid composition of the ingredients and milk replacer is summarized in **Supplemental Table 2**. The concentration of arginine in the basal diet was 7.42 g/kg dry matter (6.5 g arginine/kg diet, containing 87.5% dry matter), which is close to 7.69 g arginine/kg dry matter in sow milk (23). The dose of arginine was chosen on the basis of previous studies (26, 27, 29). Appropriate amounts of L-alanine and glucose were added to the milk replacer to formulate isonitrogenous and isoenergetic diets (**Supplemental Table 3**). Alanine was chosen for isonitrogenous control for the following considerations. First, previous studies found that alanine supplementation did not affect food intake (23, 27) or concentration of arginine in plasma (37). Second, in contrast to arginine, alanine is not an antioxidant (38). Third, alanine is a nonessential amino acid and is not toxic, and piglets have a high capacity to catabolize this neutral amino acid (23, 38). Glucose was chosen as an isoenergetic control because most dietary carbohydrates contain glucose. Furthermore, supplemental glucose in arginine-treated groups provides only 0.3–1% (0.08–0.22 MJ/kg digestible energy) of the total digestible energy in the diet. The liquid milk was prepared by mixing 1.0 kg formula powder (dry matter: 87.5%) with 4 L warm water (40°C). L-Arginine was procured from Ajinomoto Company Limited (Shanghai, China). L-Alanine was procured from Yimengsi Company Limited (Shanghai, China).

Sample collection. In this study, our purpose was to elucidate the benefits of appropriate dose L-arginine supplementation on intestine dysfunction in LBW piglets. Therefore, the group with the best growth performance among the 3 arginine-treated groups was chosen to represent the proper arginine treatment to further study the benefits of arginine on intestinal function. On day 22 after the start of the experiment, all of the piglets were weighed. Subsequently, the piglets from the NBWC, LBWC, and the arginine-treated groups with the best

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Abbreviations used: ADG, average daily gain; BW, body weight; CAT, catalase; ENOS, epithelial NO synthase; GPX, glutathione peroxidase; INOS, inducible NO synthase; LBW, low birth weight; LBWC, low-birth-weight piglets fed a diet not supplemented with L-arginine; MDA, malondialdehyde; NBW, normal birth weight; NBWC, normal-birth-weight piglets fed a diet not supplemented with L-arginine; NOS, NO synthase; ROS, reactive oxygen species; SOD, superoxide dismutase.

growth performance (1.0% arginine) were sampled ($n = 10$). Blood samples were collected from the jugular vein into polyethylene tubes (Axygen Biotechnology Co., Ltd.) after overnight feed deprivation (34) and stored for 30 min before centrifugation at 3000 × *g* for 15 min (4°C). The supernatant was collected and stored immediately at −20°C for further analyses.

After blood sampling, the piglets were killed by intravenous injection of pentobarbital sodium (50 mg/kg BW) followed by a subsequent exsanguination protocol approved by the Sichuan Agricultural University Animal Care Advisory Committee. The entire small intestine was removed and cut into 3 segments—duodenum, jejunum, and ileum—according to the description presented in our previous study (39). A 10-cm segment of the jejunum was emptied, carefully flushed with saline, and placed on an ice-cold surface. The mucosa of the jejunum was gently scraped with a glass slide, snap-frozen in liquid nitrogen, and then stored at −80°C for further analyses. Furthermore, a 1-cm segment of jejunum was collected, flushed with saline, snap-frozen in liquid nitrogen, and stored at −80°C for further analyses. Subsequently, 2-cm segments of the mid-duodenum, jejunum, and ileum were collected. The segments were flushed gently with ice-cold PBS (pH 7.4) and then fixed in 10% fresh, chilled formaldehyde solution for histomorphologic measurements.

Analysis of amino acids and insulin. Free amino acids in the serum were detected by using an automatic amino acid analyzer L-8800 (Hitachi). Serum insulin concentration was determined by immunoassays using a reagent kit (Shanghai Xinle Bioengineering Co. Ltd.) according to the manufacturer's instructions.

Assay of enzyme activity. Jejunal mucosa samples were homogenized in a 4°C saline solution (1:9 wt:vol) and centrifuged at 2500 × *g* for 15 min at 4°C. The supernatant was then transferred into Eppendorf tubes and stored at −80°C to analyze enzyme activity. Superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT) activities, and malondialdehyde (MDA) and protein carbonyl concentrations were determined through enzymatic colorimetric methods according to commercial SOD, GPX, CAT, MDA, and protein carbonyl assay kits (Nanjing Jiancheng Bioengineering Institute). The protein concentration in the mucosa was measured using the Pierce BCA Protein Assay kit (Thermo Fisher Scientific). Analyses were conducted according to the manufacturer's instructions. Results are expressed per mg of jejunal protein.

Cytokine analysis. Concentrations of jejunal cytokines IL-1, IL-6, and TNF-α were determined by using commercial IL-1, IL-6, and TNF-α ELISA kits (Shanghai Xinle Bioengineering Co. Ltd.) according to the instructions provided by the manufacturer. Results are expressed as per nanogram of protein.

Small intestinal morphology analysis. Duodenum, jejunum, and ileum samples were fixed in 10% formaldehyde solution, embedded in paraffin, cut into 5-μm-thick sections, and stained with hematoxylin and eosin. Villus height and crypt depth were measured using an Olympus CK40 microscope (Olympus Optical Company). A minimum of 10 well-orientated villi and associated crypts of each section were measured.

RNA isolation and RT-qPCR. Total RNA of jejunal samples was extracted using TRIzol reagent (TaKaRa) according to the manufacturer's instructions. The concentration of RNA was detected by using a spectrophotometry method. The integrity of RNA was determined by spectrophotometry and agarose gel electrophoresis. Reverse transcription with the use of the PrimeScript RT reagent kit (TaKaRa) was carried out according to the manufacturer's instructions.

Real-time PCR was performed using the CFX96 real-time PCR detection system (Bio-Rad). A SYBR Green PCR reagent kit (TaKaRa) was used to measure the expression of the target genes. The PCR reaction mixture (20 μL) contained 10 μL SYBR Premix Ex Taq II, 2 μL cDNA template, 1 μL forward primer, 1 μL reverse primer, and 6 μL ultrapure water. The PCR procedure was as follows: pre-denaturating at 95°C for 30 s, 40 cycles at 95°C for 5 s, annealing at optimal temperature for 30 s, and 1 cycle at 95°C for 15 s. The temperature was increased from 65°C to 95°C at a rate of 0.5°C/s to obtain the melting curve. The target genes and primers are presented in **Supplemental Table 4**. The primers were synthesized by Invitrogen Biotechnology Company Limited. The housekeeping gene *GAPDH* was used as the reference gene to normalize mRNA expression of target genes (40). Gene expression data of replicate samples were analyzed by using the $2^{-\Delta\Delta CT}$ method (41).

Statistical analyses. Data were analyzed using SPSS 17.0 software (IBM Corporation). Normality of data and homogeneity of variance were tested with the use of Levene's test. Data were subjected to 1-factor ANOVA, and differences between treatment means were determined by using Duncan's post hoc test. The sample size of each treatment was $n = 10$. Data are expressed as means ± SEMs. A P value <0.05 was used to indicate a significant difference, and a P value between 0.05 and 0.10 indicated a trend.

Results

Food intake, BWs, and general health. No diarrhea occurred in either NBW or LBW piglets throughout the trial. Average daily food intake per kilogram BW among groups did not differ in the study (between days 4 and 25 of age) (**Table 1**). However, absolute daily food intake in all LBW piglets was less than that of NBWC piglets ($P < 0.05$), whereas absolute daily food intake in piglets supplemented with 1.0% L-arginine was greater ($P < 0.05$) than that of LBWC piglets. Dietary supplementation with 0.5% and 1.5% L-arginine did not improve absolute daily food intake of LBW piglets ($P > 0.05$). Arginine intakes in arginine-treated piglets were greater ($P < 0.05$) than those of NBWC and LBWC piglets. Compared with NBW piglets, a significantly lower BW, average daily gain (ADG), and higher feed efficiency ratio was observed in all LBW piglets (**Table 2**). Dietary supplementation with 0.5% and 1.0%

TABLE 1 Effects of L-arginine supplementation on relative and absolute food intakes by LBW piglets[1]

Variable	NBWC	LBWC	LBWC+0.5% Arg	LBWC+1.0% Arg	LBWC+1.5% Arg	*P*
RADMI, g · (kg BW^{-1} · d^{-1})	42.2 ± 2.9	43.2 ± 2.4	42.5 ± 0.9	43.0 ± 2.1	42.2 ± 1.5	0.82
AADMI, g/d	213 ± 9^{a}	145 ± 4^{c}	156 ± 6^{b,c}	163 ± 5^{b}	152 ± 5^{b,c}	<0.01
RAI, g · (kg BW^{-1} · d^{-1})	0.27 ± 0.01^{d}	0.28 ± 0.01^{d}	0.49 ± 0.00^{c}	0.71 ± 0.01^{b}	0.91 ± 0.01^{a}	<0.01
AAI, g/d	1.38 ± 0.05^{d}	0.94 ± 0.02^{e}	1.79 ± 0.02^{c}	2.68 ± 0.08^{b}	3.27 ± 0.10^{a}	<0.01

[1]Values are means ± SEMs, $n = 10$/group. Labeled means in a row without a common superscript letter differ, $P < 0.05$. AADMI, absolute average daily dry matter intake; AAI, absolute arginine intake; BW, body weight; LBW, low-birth-weight; LBWC, low-birth-weight piglets fed a diet not supplemented with L-arginine; LBWC+0.5% Arg, low-birth-weight piglets fed a diet supplemented with 0.5% L-arginine; LBWC+1.0% Arg, low-birth-weight piglets fed a diet supplemented with 1.0% L-arginine; LBWC+1.5% Arg, low-birth-weight piglets fed a diet supplemented with 1.5% L-arginine; NBWC, normal-birth-weight piglets fed a diet not supplemented with L-arginine; RADMI, relative average daily dry matter intake; RAI, relative arginine intake.

TABLE 2 Effects of L-arginine supplementation on the growth of LBW piglets[1]

Variable	NBWC	LBWC	LBWC+0.5% Arg	LBWC+1.0% Arg	LBWC+1.5% Arg	*P*
Initial BW on day 4, kg	1.96 ± 0.03[a]	1.05 ± 0.04[b]	1.04 ± 0.04[b]	1.05 ± 0.04[b]	1.04 ± 0.04[b]	<0.01
Final BW on day 25, kg	8.09 ± 0.17[a]	5.67 ± 0.16[c]	6.29 ± 0.30[b]	6.50 ± 0.12[b]	6.18 ± 0.18[b,c]	<0.01
ADG, g/d	292 ± 7[a]	220 ± 6[c]	250 ± 13[b]	260 ± 5[b]	245 ± 8[b,c]	<0.01
FE, g gain/g feed	1.38 ± 0.03[c]	1.52 ± 0.03[b]	1.60 ± 0.02[a]	1.60 ± 0.03[a]	1.61 ± 0.02[a]	<0.01

[1]Values are means ± SEMs, $n = 10$/group. Labeled means in a row without a common superscript letter differ, $P < 0.05$. ADG, average daily gain; BW, body weight; FE, feed efficiency; LBW, low-birth-weight; LBWC, low-birth-weight piglets fed a diet not supplemented with L-arginine; LBWC+0.5% Arg, low-birth-weight piglets fed a diet supplemented with 0.5% L-arginine; LBWC+1.0% Arg, low-birth-weight piglets fed a diet supplemented with 1.0% L-arginine; LBWC+1.5% Arg, low-birth-weight piglets fed a diet supplemented with 1.5% L-arginine; NBWC, normal-birth-weight piglets fed a diet not supplemented with L-arginine.

L-arginine-enhanced ($P < 0.05$) ADG of piglets by 14% and 18%, respectively, compared with LBWC piglets. Consequently, the BWs of the 25-d-old piglets supplemented with 0.5% and 1.0% L-arginine were 11% and 15% greater, respectively ($P < 0.05$), than those of LBWC piglets. Furthermore, there was a higher feed efficiency ratio in all of the 3 arginine-treated groups as compared with the LBWC group ($P < 0.05$).

Concentration of serum amino acids. The concentrations of ornithine, lysine, leucine, isoleucine, valine, and phenylalanine in the serum of LBWC piglets were significantly lower than those of NBWC piglets (Table 3). Dietary supplementation with 1.0% L-arginine resulted in a higher serum concentration of ornithine compared with that in the LBWC group ($P < 0.05$). Compared with LBWC piglets, dietary supplementation with 1.0% L-arginine tended to increase the serum concentration of arginine by 20% ($P = 0.09$).

Concentration of serum insulin. The serum insulin concentration of LBWC piglets was significantly lower than that of NBW piglets (Figure 1). The serum insulin concentration in the group supplemented with 1.0% L-arginine was significantly higher than that of LBWC piglets.

Intestinal morphology. The histomorphologic changes in the intestine, including duodenum, jejunum, and ileum, were evaluated (Table 4). Crypt depth in the duodenum was significantly higher in LBW piglets than in NBWC piglets. Compared with LBWC piglets, villus height in the jejunum and ileum of piglets supplemented with 1.0% L-arginine was significantly higher, and compared with NBWC piglets, villus height in the jejunum of piglets supplemented with 1.0% L-arginine was significantly higher.

TABLE 3 Effect of L-arginine supplementation on serum amino acid concentrations in LBW piglets[1]

Variable	NBWC	LBWC	LBWC+1.0% Arg	*P*
Arginine	108 ± 8	99 ± 7	119 ± 5	0.09
Citrulline	88 ± 17	88 ± 6	98 ± 4	0.75
Ornithine	90 ± 8[a]	53 ± 5[b]	77 ± 5[a]	<0.01
Glutamic acid	279 ± 27	237 ± 14	272 ± 13	0.27
Proline	226 ± 19	181 ± 9	205 ± 8	0.07
Lysine	198 ± 18[a]	136 ± 9[b]	135 ± 12[b]	<0.01
Methionine	69 ± 5	55 ± 4	55 ± 3	0.06
Threonine	297 ± 35	269 ± 24	249 ± 28	0.51
Leucine	181 ± 14[a]	139 ± 6[b]	132 ± 8[b]	<0.01
Isoleucine	127 ± 11[a]	91 ± 6[b]	104 ± 10[a,b]	0.04
Valine	387 ± 14[a]	269 ± 15[b]	260 ± 16[b]	< 0.01
Phenylalanine	128 ± 6[a]	101 ± 5[b]	114 ± 6[ab]	0.01

[1]Values are means ± SEMs, $n = 10$/group. Labeled means in a row without a common superscript letter differ, $P < 0.05$. LBW, low-birth-weight; LBWC, low-birth-weight piglets fed a diet not supplemented with L-arginine; LBWC+1.0% Arg, low-birth-weight piglets fed a diet supplemented with 1.0% L-arginine; NBWC, normal-birth-weight piglets fed a diet not supplemented with L-arginine.

Gene expressions of tight junction proteins. Figure 2 shows the gene expressions of tight junction proteins in the jejunum. Claudin1 mRNA level in the jejunum of LBWC piglets was significantly lower than that in NBWC piglets. Furthermore, 1.0% L-arginine supplementation significantly upregulated Claudin1 mRNA levels in the jejunum of LBW piglets compared with those in LBWC piglets.

Redox status in the jejunum. The antioxidant gene expression in the jejunum is presented in Table 5. Supplementation with 1.0% L-arginine significantly upregulated *GPX* mRNA level in the jejunum of LBW piglets when compared with that in LBWC piglets.

The activities of CAT and GPX were significantly lower in the jejunum of LBWC piglets when compared with NBWC piglets (Table 6). The activities of CAT and GPX in the jejunum of piglets supplemented with 1.0% ($P = 0.06$) L-arginine ($P = 0.09$) tended to be higher than in LBWC piglets. Conversely,

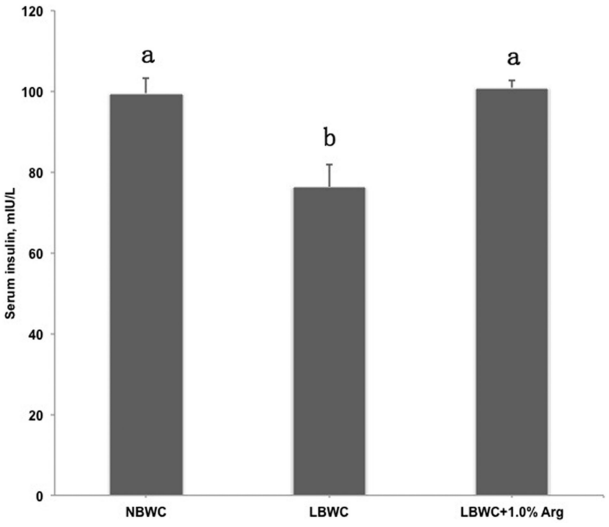

FIGURE 1 Effects of L-arginine supplementation on serum insulin concentration in LBW piglets. Values are means ± SEMs; $n = 10$/group. Means without a common letter differ, $P < 0.05$. LBW, low-birth-weight; LBWC, low-birth-weight piglets fed a diet not supplemented with L-arginine; LBWC+1.0% Arg, low-birth-weight piglets fed a diet supplemented with 1.0% L-arginine; NBWC, normal-birth-weight piglets fed a diet not supplemented with L-arginine.

TABLE 4 Effect of L-arginine supplementation on intestinal morphology in LBW piglets[1]

Variable	NBWC	LBWC	LBWC+1.0% Arg	*P*
Duodenum, μm				
Villus height	513 ± 22	494 ± 10	503 ± 19	0.76
Crypt depth	125 ± 10[b]	161 ± 7[a]	180 ± 4[a]	0.01
Jejunum, μm				
Villus height	426 ± 17[b]	433 ± 10[b]	486 ± 19[a]	0.04
Crypt depth	126 ± 5	135 ± 8	134 ± 9	0.61
Ileum, μm				
Villus height	384 ± 22[a]	331 ± 14[b]	399 ± 15[a]	0.03
Crypt depth	103 ± 7	118 ± 7	114 ± 7	0.33

[1]Values are means ± SEMs, $n = 10$/group. Labeled means in a row without a common superscript letter differ, $P < 0.05$. LBW, low-birth-weight; LBWC, low-birth-weight piglets fed a diet not supplemented with L-arginine; LBWC+1.0% Arg, low-birth-weight piglets fed a diet supplemented with 1.0% L-arginine; NBWC, normal-birth-weight piglets fed a diet not supplemented with L-arginine.

TABLE 5 Effect of L-arginine supplementation on antioxidant gene expression in the jejunum of LBW piglets[1]

	Fold of LBWC			
Variable	NBWC	LBWC	LBWC+1.0% Arg	*P*
SOD	0.85 ± 0.11	1.00 ± 0.11	1.20 ± 0.13	0.14
CAT	1.49 ± 0.20	1.00 ± 0.07	1.22 ± 0.05	0.06
GPX	1.04 ± 0.08[b]	1.00 ± 0.09[b]	1.36 ± 0.07[a]	0.01
NFE2L2	0.81 ± 0.04	1.00 ± 0.08	1.10 ± 0.10	0.15
HO1	0.89 ± 0.07	1.00 ± 0.11	0.69 ± 0.13	0.12
NQO1	1.00 ± 0.11	1.00 ± 0.14	1.28 ± 0.12	0.21

[1]Values are means ± SEMs, $n = 10$/group. Labeled means in a row without a common superscript letter differ, $P < 0.05$. *CAT*, catalase; *GPX*, glutathione peroxidase; *HO1*, heme oxygenase 1; LBW, low-birth-weight; LBWC, low-birth-weight piglets fed a diet not supplemented with L-arginine; LBWC+1.0% Arg, low-birth-weight piglets fed a diet supplemented with 1.0% L-arginine; NBWC, normal-birth-weight piglets fed a diet not supplemented with L-arginine; *NQO1*, NAD(P)H quinone oxidoreductase 1; *NFE2L2*, nuclear factor erythroid 2–like 2; *SOD*, superoxide dismutase.

the concentration of MDA in the jejunum of 1.0% arginine-supplemented piglets tended to be lower than in LBWC piglets ($P < 0.07$).

Concentrations of cytokines. The concentration of IL-1 in the jejunum of LBWC piglets was significantly higher than that in NBWC piglets (Table 7). The concentrations of IL-1 and TNF-α in the jejunum of piglets supplemented with 1.0% L-arginine were significantly lower than those in LBWC piglets.

NO content and NO synthase activity. There was no significant change in the mRNA levels of epithelial NO synthase (*ENOS*) ($P = 0.47$) and inducible NO synthase ($P = 0.38$) in the jejunum of LBW piglets supplemented with 1.0% L-arginine when compared with LBWC piglets (Figure 3A, B). Supplementation with 1.0% L-arginine had no effect on NO content ($P = 0.91$) or NO synthase (NOS) activity ($P = 0.59$) in the jejunum of LBW piglets when compared with LBWC piglets (Figure 3C, D).

Discussion

In humans, LBW is common in newborn infants and has been found to impair gut development and cause growth retardation (42, 43). The pig (*Sus scrofa*) is an excellent model organism used in biomedical studies because it is closely related to humans in terms of anatomy, genetics, and physiology. The present study investigated how dietary L-arginine supplementation improved growth performance and affected intestinal function in a pig model. Arginine deficiency might limit the maximal growth of neonatal piglets, especially in LBW neonates (23, 44). The present study showed that LBW reduced the growth performance of piglets, and L-arginine supplementation promoted the growth performance of LBW piglets. This agrees with the findings of previous studies (26–28) that showed that dietary arginine supplementation improved the growth of LBW piglets. The possible mechanisms for the enhanced growth of LBW piglets in response to dietary arginine supplementation might be due to the following reasons.

First, dietary arginine supplementation increased arginine availability (23). The results of the present study showed that 1.0% L-arginine supplementation tended to increase serum arginine concentration without affecting the concentration of lysine. In the present study, the average arginine intakes by LBW piglets supplemented with 0%, 0.5%, 1.0%, and 1.5%

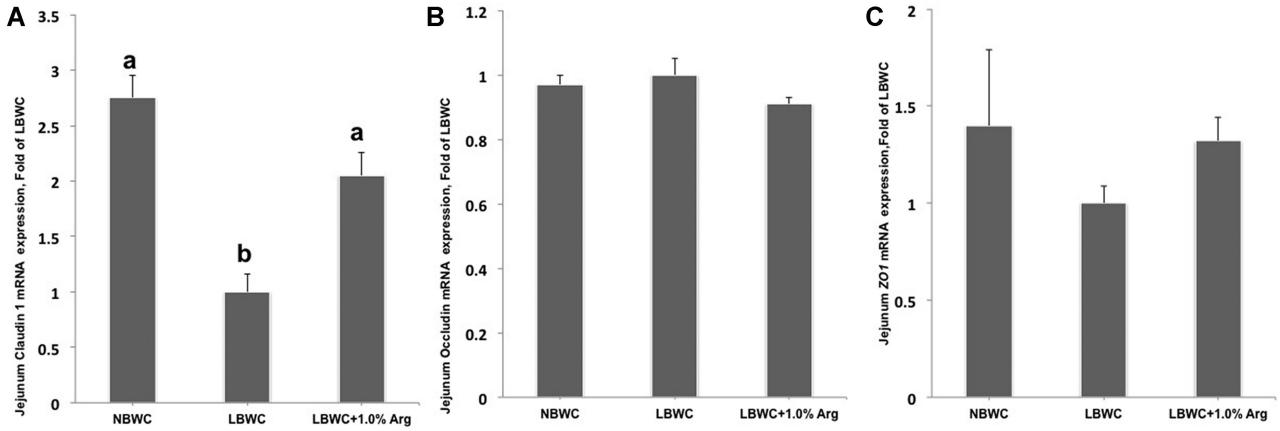

FIGURE 2 Effects of L-arginine supplementation on relative mRNA expression of Claudin1 (A), Occludin (B), and *ZO1* (C) in the jejunum of LBW piglets. Values are means ± SEMs; $n = 10$/group. Means without a common letter differ, $P < 0.05$. LBW, low-birth-weight; LBWC, low-birth-weight piglets fed a diet not supplemented with L-arginine; LBWC+1.0% Arg, low-birth-weight piglets fed a diet supplemented with 1.0% L-arginine; NBWC, normal-birth-weight piglets fed a diet not supplemented with L-arginine; *ZO1*, zonula occludens 1.

TABLE 6 Effect of L-arginine supplementation on antioxidant activity in the jejunum of LBW piglets[1]

Variable	NBWC	LBWC	LBWC+1.0% Arg	*P*
PC, nmol/mg protein	14.8 ± 0.7	15.9 ± 1.1	15.1 ± 0.5	0.59
MDA, nmol/mg protein	0.67 ± 0.06	0.82 ± 0.04	0.65 ± 0.04	0.07
SOD, U/mg protein	63.8 ± 0.9	65.2 ± 2.4	63.2 ± 0.9	0.66
CAT, U/mg protein	20.6 ± 1.3[a]	15.9 ± 0.4[b]	17.9 ± 0.8[a,b]	0.01
GPX, U/mg protein	90.4 ± 3.6[a]	68.5 ± 3.7[b]	79.2 ± 4.3[a,b]	<0.01

[1] Values are means ± SEMs, $n = 10$/group. Labeled means in a row without a common superscript letter differ, $P < 0.05$. CAT, catalase; GPX, glutathione peroxidase; LBW, low-birth-weight; LBWC, low-birth-weight piglets fed a diet not supplemented with L-arginine; LBWC+1.0% Arg, low-birth-weight piglets fed a diet supplemented with 1.0% L-arginine; MDA, malondialdehyde; NBWC, normal-birth-weight piglets fed a diet not supplemented with L-arginine; PC, protein carbonyl; SOD, superoxide dismutase.

L-arginine from days 4 to 25 were 0.94, 1.79, 2.68, and 3.27 g/d, respectively, based on their dietary arginine concentration and average daily dry matter intake. On the basis of the growth performance analysis, the best daily arginine intake from days 4 to 25 (1–7 kg BW) by LBW piglets was 2.68 g/d. The daily arginine intake by LBW piglets (2.63 g/d) in the study by Wang et al. (26) was close to that in our study. Meanwhile, in the present study, daily food intake per kilogram BW among groups did not differ (Table 1). The results indicated that dietary intake of all nutrients (including protein, fat, carbohydrates, vitamins, and minerals), except for arginine, did not differ between LBWC piglets and arginine-supplemented LBW piglets; the enhanced growth of LBW piglets in response to L-arginine supplementation was partly attributed to the increase in arginine availability. In addition, this study suggests that the best ratio of lysine to arginine (100:71) for LBW piglets (1–7 kg BW) is higher than that for young piglets (100:45 for 5- to 7-kg piglets) recommended by the NRC (45). Kim et al. (23) reported that the ideal dietary ratio of lysine to arginine for young piglets should be ≥100:55 to foster maximal weight gain in piglets <21 d old based on the results that elevating the ratio of lysine to arginine from 100:35 to 100:45 and 100:55, respectively, dose dependently increased the BW gain of piglets. However, it is possible that the ideal dietary ratio of lysine to arginine for young piglets is >100:55 because the study by Kim et al. did not involve a higher-arginine-supplemented group. In addition, preweaning supplementation with 0.8% arginine in piglets showed a 10-d carry-over effect of arginine in increasing ADG (46). Therefore, the requirements of arginine were influenced by birth weight, BW, and age and varied with condition, which necessitates further studies.

Second, dietary L-arginine supplementation partly promoted the secretion of insulin. Arginine is a potent stimulator of insulin secretion (47). Increases in the serum concentration of anabolic hormones improve the efficiency of nutrient utilization and enhance tissue protein synthesis (23). The results of the present study showed that dietary supplementation with 1.0% L-arginine increased serum insulin concentration in artificially reared LBW piglets. Wang et al. (26) showed that 0.6% arginine supplementation increased the concentration of insulin and phosphorylated Akt, thus improving the development and morphology of the small intestine in intrauterine growth–restricted piglets. However, Kim et al. (23) observed that dietary supplementation with 0.2% arginine did not affect plasma insulin concentration but improved the BW of NBW piglets. As described earlier, these results indicated that the interaction between dietary arginine supplementation and insulin secretion is complicated. Consequently, further studies need to be done to elucidate how dietary arginine supplementation influences insulin secretion.

TABLE 7 Effect of L-arginine supplementation on cytokine concentrations in the jejunum of LBW piglets[1]

Variable	NBWC	LBWC	LBWC+1% Arg	*P*
IL-1, ng/g protein	18.6 ± 0.80[b]	22.1 ± 1.42[a]	15.1 ± 0.91[c]	<0.01
IL-6, ng/g protein	12.8 ± 0.70	13.9 ± 0.96	10.8 ± 0.94	0.07
TNF-α, ng/g protein	10.8 ± 0.85[a,b]	12.8 ± 0.61[a]	8.96 ± 1.05[b]	0.02

[1] Values are means ± SEMs, $n = 10$/group. Labeled means in a row without a common superscript letter differ, $P < 0.05$. LBW, low-birth-weight; LBWC, low-birth-weight piglets fed a diet not supplemented with L-arginine; LBWC+1.0% Arg, low-birth-weight piglets fed a diet supplemented with 1.0% L-arginine; NBWC, normal-birth-weight piglets fed a diet not supplemented with L-arginine.

Third, dietary arginine supplementation improved intestinal health and function. Studies showed that the retarded growth of LBW piglets accompanied intestinal dysfunction (2, 8, 9). The small intestine is a critical organ in humans and in other animals for digestion and absorption of food, thus regulating the growth and development of infants and young piglets. The present study corroborated the findings of previous studies, which reported that intestinal morphology was impaired (8, 34, 48) and the expression of tight junction proteins was downregulated in LBW piglets (12). Previous research has found that LBW piglets are vulnerable to oxidative stress (2, 15, 16, 49), which is induced by many factors, such as birth and weaning (17, 50). To evaluate the effect of oxidative stress on intestinal function in LBW piglets, the redox status of the jejunum in LBW piglets was determined in the present study. SOD, CAT, and GPX are antioxidant enzymes that constitute important parts of antioxidant system in the body to scavenge ROS (51, 52). Protein carbonyl and MDA are the most important biomarkers of protein oxidation and lipid peroxidation, respectively (53, 54). In the present study, there was a significant decrease in the activities of GPX and CAT, and the concentration of MDA tended to increase in the jejunum of LBW piglets, which indicated oxidative damage in LBW piglets. When the production of ROS overwhelms the endogenous antioxidant capacity, excess ROS can lead to oxidative damage of DNA, proteins, and lipids and increase membrane permeability, which might be linked to gut-related diseases (55, 56). Oxidative stress is often associated with elevated concentrations of proinflammatory cytokines, especially TNF-α (57). In the present study, there was an increase in the concentrations of IL-1 and TNF-α in the jejunum of LBWC piglets when compared with NBWC piglets. Our previous study reported that oxidative stress induced by diquat significantly increased the *Tnfa* mRNA level in the jejunum of piglets (39). TNF-α is a proinflammatory cytokine involved in systemic inflammation and can increase intestinal epithelial barrier permeability by activating the extracellular signal–regulated protein kinase 1 and 2 signaling pathway (58). Therefore, oxidative stress in the jejunum could be a vital factor leading to intestinal dysfunction in LBW piglets. Thus, enhancing the antioxidant capacity of the intestine might ameliorate intestinal dysfunction.

Studies have shown that arginine could alleviate oxidative stress and increase antioxidant capacity in pigs (29, 59). In the present study, supplementation with 1.0% L-arginine increased the mRNA expression of *GPX* and tended to increase the mRNA of *CAT* in the jejunum of LBW piglets when compared with LBWC piglets. Consequently, supplementation with 1.0% L-arginine tended to increase the activity of CAT and GPX and

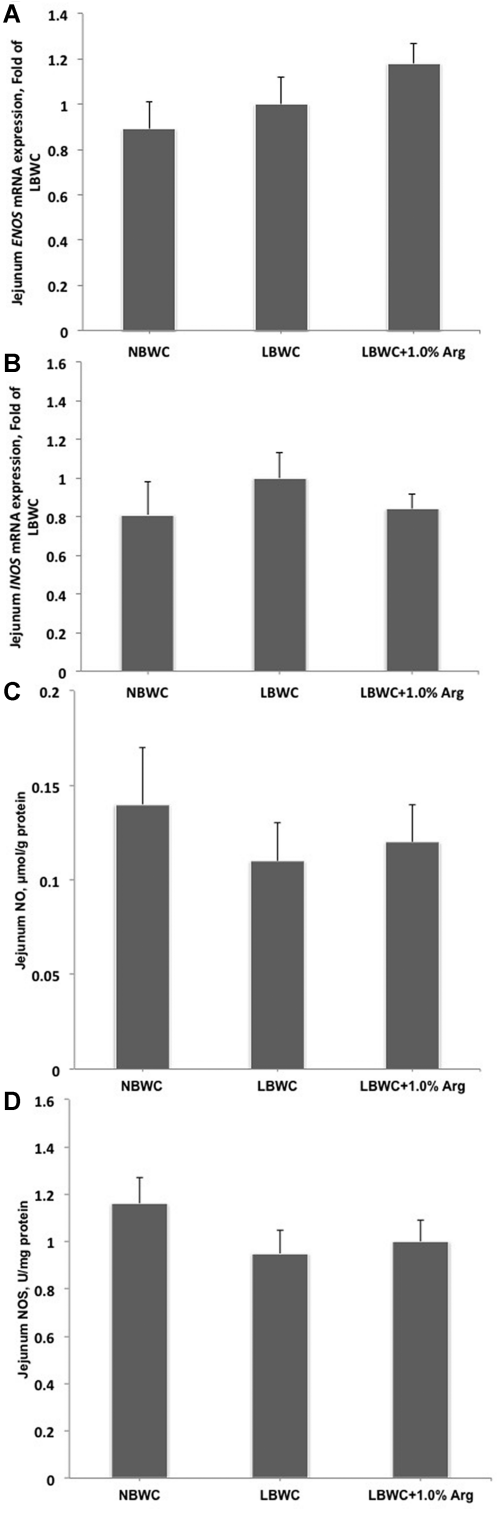

FIGURE 3 Effects of L-arginine supplementation on relative mRNA expressions of *ENOS* (A) and *INOS* (B), content of NO (C), and activity of NOS (D) in the jejunum of LBW piglets. Values are means ± SEMs; $n = 10$/group. *ENOS*, epithelial NO synthase; *INOS*, inducible NO synthase; LBW, low-birth-weight; LBWC, low-birth-weight piglets fed a diet not supplemented with L-arginine; LBWC+1.0% Arg, low-birth-weight piglets fed a diet supplemented with 1.0% L-arginine; NBWC, normal-birth-weight piglets fed a diet not supplemented with L-arginine; NOS, NO synthase.

decrease MDA concentration in the jejunum of LBW piglets when compared with LBWC piglets. MDA is an important biomarker of lipid peroxidation; decreasing the content of MDA helps maintain the integrity of the membrane and permeability of the cell. Intestinal epithelial barriers consist of many junctional complexes, which can tightly bind the epithelial cells and maintain the epithelial barrier (14). Supplementation with 1.0% L-arginine significantly increased the expression of Claudin1, indicating that arginine might improve the tight junction function in LBW piglets. Supplementation with 1.0% L-arginine significantly increased the villus height in the jejunum and ileum of LBW piglets. Similar effects of arginine supplementation on intestinal morphology have been reported in both weaned piglets and intrauterine growth–restricted suckling piglets (26, 60). Therefore, the possible mechanism by which arginine improves the intestinal morphology and barrier is through alleviation of oxidative stress. Our previous study reported that arginine alleviated oxidative stress partly through the inhibition of the expression of the proinflammatory cytokine *TNF-α* (39). The results of the present study showed that 1.0% L-arginine supplementation significantly decreased the concentrations of IL-1 and TNF-α. This suggests that supplementation with 1.0% L-arginine could effectively ameliorate intestinal dysfunction in LBW piglets by enhancing the antioxidant capacity of the jejunum.

Arginine is an essential amino acid for young animals and takes part in many metabolic pathways (61). NO is an important messenger and has diverse roles, such as the regulation of cell survival and proliferation, immune function, and cellular redox state (32, 62). Studies have found that NO could act as a free radical scavenger (63). In this study, dietary supplementation with 1.0% L-arginine exhibited a positive effect by enhancing the antioxidant capacity of the jejunum in LBW piglets. This might be because arginine acted as an NO precursor (64). To show whether the NO pathway is responsible for the arginine-enhanced antioxidant capacity, the mRNA expression of *ENOS* and inducible NO synthase, NOS activity, and NO content were determined. However, in the present study, L-arginine supplementation had no effect on mRNA expression of *NOS*, NOS activity, or NO content in the jejunum of LBW piglets. Dhar et al. (65) reported that arginine supplementation reduced high glucose-induced oxidative stress through an ENOS-independent pathway. The beneficial effects of arginine supplementation on the intestinal health of LBW piglets might be partially attributed to improved antioxidant capacity of the jejunum. However, such a beneficial effect is independent of the NO pathway.

In conclusion, the results of the present study showed that LBW piglets exhibited poor growth performance and lower serum insulin concentrations and antioxidant capacity in the jejunum when compared with NBW piglets. Furthermore, dietary supplementation with 1.0% L-arginine ameliorated growth depression, promoted intestinal development, and improved intestinal function in LBW piglets. L-Arginine supplementation could also improve intestinal barrier function via enhanced antioxidant capacity through an NO-independent pathway in the jejunum of LBW piglets. The present study elucidated the benefits of L-arginine supplementation on intestinal dysfunction in LBW piglets and provided insights on the mechanisms responsible for small intestine dysfunction in LBW neonates.

Acknowledgments

The authors' responsibilities were as follows—PZ, BY, and DC: contributed to the experimental design; YS, YT, and HZ:

conducted the study; XM, JY, YL, JL, ZH, and GT: assisted with all of the data analyses and helped in drafting the manuscript; PZ and YS: wrote the manuscript; JH, HC, and PZ: revised the manuscript; and all authors: read and approved the final manuscript.

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