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Dietary dihydroartemisinin supplementation improves growth, intestinal digestive function and nutrient transporters in weaned piglets with intrauterine growth retardation

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ABSTRACT

Two experiments were conducted to (1) investigate the effects of different doses of dihydroartemisinin (DiHA) on growth performance, nutrient digestibility, serum biochemistry, antioxidant and immune function in weaned piglets and determine the optimal dose of DiHA and (2) investigate the effects of DiHA on growth, intestinal digestive function and nutrient transporters in weaned piglets with intrauterine growth retardation (IUGR). In experiment 1, a total of 360 21-day-old weaned piglets were randomly allocated into six dietary treatments, including control group (basal diet), DiHA 20, DiHA 40, DiHA 80, DiHA 160 and DiHA 320 groups (basal diet supplemented with 20, 40, 80, 160, 320 mg/kg DiHA, respectively). At 42 days of age, a digestibility trial was conducted for 7 days. At 49 days of age, the growth performance, serum biochemistry, antioxidant and immune function were measured. The results showed that DiHA at the dose of 80 mg/kg significantly improved ($P < 0.05$) growth performance and decreased the incidence of diarrhea. Diet supplemented with 80 mg/kg DiHA also improved ($P < 0.05$) the nutrient digestibility, ameliorated serum biochemistry and improved serum cytokine levels. Therefore, according to these results, the optimal dose of DiHA in pig diet was considered to be 80 mg/kg. In experiment 2, weaned piglets (21 d) with normal birth weight (NBW) and IUGR fed the basal diet (NBW-CON and IUGR-CON groups) or the 80 mg/kg DiHA diet (IUGR-DiHA group) for 4 wk. Compared with NBW piglets, IUGR piglets decreased ($P < 0.05$) body weight at 0, 7, 14, 21 days of age and average daily gain (ADG) during 0–7 d, 7–14 d, and 0–21 d period as well as ADG and average daily feed intake (ADFI) at 49 days of age. IUGR led to the impaired development of intestine, decreased activities of intestinal digestive enzyme and disaccharidase, reduced concentrations of glucose transporter and mRNA expressions of related nutrient transporters in weaned piglets. After treatment with DiHA in IUGR piglets, ADG and ADFI, the relative weight of jejunum, the activities of ileal amylase and trypsin, the activities of jejunal lactase, maltase and alkaline phosphatase, the concentrations of jejunal glucose transporter 2 and ileal sodium-dependent glucose transporter 1, and mRNA expressions of nutrient transporters were improved ($P < 0.05$). In conclusion, 80 mg/kg DiHA in the diet has beneficial effects in improving the development of small intestine and alleviating the impairment of nutrient digestion and transporters in IUGR weaned piglets.

1. Introduction

Intrauterine growth retardation (IUGR), defined as impaired growth and development of the mammalian embryo/fetus or its organs during pregnancy, is a serious problem in clinical treatment and livestock production (Barker, 1994; Wu, 2006). The naturally occurring IUGR

Abbreviations: ADG, average daily gain; ADFI, average daily feed intake; AKP, alkaline phosphatase; ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CAT, catalase; *Cat1*, cationic amino acid transporter 1; CP, crude protein; Cr, creatinine; DiHA, dihydroartemisinin; DM, dry matter; EA, enzymatically treated *Artemisia annua* L; EE, ether extract; *Fabp2*, fatty acid binding protein 2; *Fasn*, fatty acid synthase; F/G, feed to gain ratio; GPx, glutathione peroxidase; GLUT2, glucose transporter 2; *Glut2*, glucose transporter type 2; HDL-C, high density lipoprotein-cholesterol; IFN-γ, interferon γ; IgG, immunoglobulin G; IL-1β, interleukin 1β; IL-4, interleukin 4; IL-6, interleukin 6; IL-10, interleukin 10; IUGR, intrauterine growth retardation; LDL-C, low density lipoprotein-cholesterol; MDA, malondialdehyde; OM, organic matter; *Pept1*, peptide transporter 1; SGLT1, sodium-dependent glucose transporter 1; *Sglt1*, sodium-dependent glucose transporters; sIgA, secretory immunoglobulin A; *Snat2*, sodium-coupled neutral amino-acid transporter 2; T-AOC, total oxidant capacity; T-Bil, total bilirubin; TC, total cholesterol; TG, triglycerides; TNF-α, tumor necrosis factor α; TP, total protein; T-SOD, total superoxide dismutase

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infants account for approximately 5–10% all over the world, while the incidence of IUGR neonatal animals is about 15–20% (Mcmillen, 2003). IUGR increases morbidity and mortality among the newborns and may result in permanent alterations in metabolism, growth, and development during postpartum period (Alexander, 2003; Barker et al., 2003; Garite et al., 2004).

The small intestine (SI) is the major organ responsible for terminal digestion and absorption of dietary nutrients (Wu, 1998). It is reported that the digestive system of IUGR newborns exhibits some changes at both the tissue and molecular levels. Briefly, the modifications of gut development in IUGR neonates consist of the smaller size and weight, delayed maturation of intestinal mucosa, impaired intestinal integrity, abnormal digestion and absorption of nutrients, decreased activity of brush border enzyme, and altered nutrient transport function (Guilloteau et al., 2009; Mickiewicz et al., 2012; Amdi et al., 2013; Ferenc et al., 2017). Numerous studies have shown that IUGR decreased growth performance, impaired intestinal morphology (Li et al., 2018; Su et al., 2018), reduced the activity of intestinal digestive enzymes (Dong et al., 2015) in weaned piglets. Because the physiological characteristics of pigs are similar to humans, IUGR pigs are good models for human medicine and nutrition research. Therefore, finding an effective way to attenuate the impaired function of SI in IUGR piglets is very meaningful for human health.

Artemisia annua L. (*A. annua*) is an annual weedy herb native to China (Brown, 2010). Our previous research suggested that diet supplemented with 2 g/kg enzymatically treated *Artemisia annua* L. (EA) improved growth performance and nutrient digestibility, decreased the incidence of diarrhea, increased digestive enzyme capacity, reduced the inflammation and enhanced the immunity in the intestine of weaned piglets from 21 to 50 days of age (ongoing manuscript). Artemisinin, extracted from *A. annua*, is well-known for treating malaria worldwide. It is also demonstrated that artemisinin possesses anti-inflammation, anti-malaria, anti-tumor, antioxidation and immunomodulation activities, and has been used in the clinical treatment of malaria, tumor, inflammation and other diseases (Luo and Shen, 2010). So we presumed that artemisinin from *A. annua* could improve the growth and intestinal health by reducing the inflammation and improving the immunity in IUGR piglets. Dihydroartemisinin (DiHA) is a derivative of artemisinin which has higher efficiency, lower toxicity, better absorption and wider distribution than artemisinin (Lu et al., 2009). However, no information is available about the effects of different doses of DiHA on piglets and the effects of DiHA on IUGR piglets are still limited. Therefore, the aim of this study was to investigate the different doses of DiHA on weaned piglets and choose the optimal dose. Then we explored whether dietary DiHA supplementation could improve the growth performance and intestinal development, and increase the nutrients digestion and transport as a nutritional regulator in IUGR weaned piglets.

2. Materials and methods

All experimental design and procedures were approved by Institutional Animal Care and Use Committee of Nanjing Agricultural University following the requirements of the Regulations for the Administration of Affairs Concerning Experimental Animals of China (NJAU-CAST-2018-023 and NJAU-CAST-2018-146). The chemical composition of the diet in the two experiments was presented in Table 1, which was formulated to meet the nutrient requirements of pigs according to the NRC (2012).

2.1. Preparation of DIHA

Dihydroartemisinin (DiHA, $C_{15}H_{24}O_5$, MW, 284.35, chemical structure see Fig. 1) is one of the largest groups of sesquiterpene lactones (Li et al., 2006). DiHA is the derivative of artemisinin and the major active metabolite of all artemisinins. DiHA used in this study was in powder form and purchased from the DASF Biotechnology Co., Ltd (Nanjing, Jiangsu, China). DiHA was prepared fresh every day and then mixed into the basal diet of piglets according to its proportion. The content of DiHA was 99% as determined by high performance liquid chromatography (HPLC) analysis.

Table 1
Composition and nutrient level of the basal diet (air-dry basis).

Ingredients	Ratio (%)	Calculated nutrient levels	Content
Corn	57.70	Digestible energy (MJ/kg)	14.04
Soybean meal (46%)	12.50	Crude protein (%)	18.31
Expanded corn	8.00	Lysine (%)	1.31
Full-fat soybean	8.00	Methionine (%)	0.40
Fermented soybean meal	4.00	Methionine + Cystine (%)	0.70
Whey powder	3.00	Threonine (%)	0.80
Fish meal (crude protein 67%)	3.00	Calcium (%)	0.85
Dicalcium phosphate	1.80	Total phosphorus (%)	0.72
Limestone	0.50		
L-lysine (78%)	0.30		
L-threonine	0.10		
DL-methionine	0.08		
Wheat middling	0.02		
Premix[1]	1		
Total	100		

[a]The premix supplied the following per kg complete diet: vitamin A, 12,000 IU; vitamin D_3, 3000 IU; α-tocopherol, 50 mg; vitamin K_3, 4 mg; vitamin B_1, 4 mg; vitamin B_2, 10 mg; vitamin B_6, 7 mg; vitamin B_{12}, 0.05 mg; niacin, 30 mg; pantothenic acid, 15 mg; folic acid, 0.3 mg; biotin, 0.08 mg; choline chloride, 500 mg; Fe ($FeSO4•H_2O$), 110 mg; Cu ($CuSO_4•5H_2O$), 7 mg; Zn (ZnO), 110 mg; I (KIO_3), 0.3 mg; Mn ($MnSO4•H_2O$), 5 mg; Se (Na_2SeO_3), 0.3 mg.

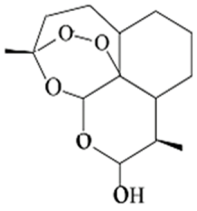

Fig. 1. The chemical structure of dihydroartemisinin.

2.2. Experiment 1

2.2.1. Animals and experimental design

In experiment 1, a total of 360 21-day-old [Duroc × (Yorkshire × Landrace)] weaned piglets with an initial body weight (BW) of 6.30 ± 0.09 kg were randomly assigned to 1 of 6 replicates (pens) of 10 piglets (five barrows and five gilts) per pen in a 28-d experiment. The dietary treatment consisted of six groups: CON group (control group, basal diet), DiHA 20 group (basal diet + 20 mg/kg DiHA), DiHA 40 group (basal diet + 40 mg/kg DiHA), DiHA 80 group (basal diet + 80 mg/kg DiHA), DiHA 160 group (basal diet + 160 mg/kg DiHA), DiHA 320 group (basal diet + 320 mg/kg DiHA). The piglets were housed in an environmentally controlled pen (ten pigs/pen) with pen dimensions of 3.40 m × 3.60 m, with plastic floors, and one nipple waterer and one four-space self-feeder. The house temperature was thermostatically maintained at 25 to 28 °C. All piglets were provided *ad libitum* access to feed and water during the whole trial period.

2.2.2. Sampling and measurements

The study design timeline of Experiment 1 was presented in Fig. 2. The piglets were individually weighed at 21 d and 49 day of age. Feed consumptions were measured daily per pen throughout the experiment. Growth performance was evaluated by average daily feed intake (ADFI), average daily gain (ADG) and feed to gain ratio (F/G). On the last day of the experiment (49 d of age), two piglets were randomly selected from each pen (one gilt and one barrow) and approximately

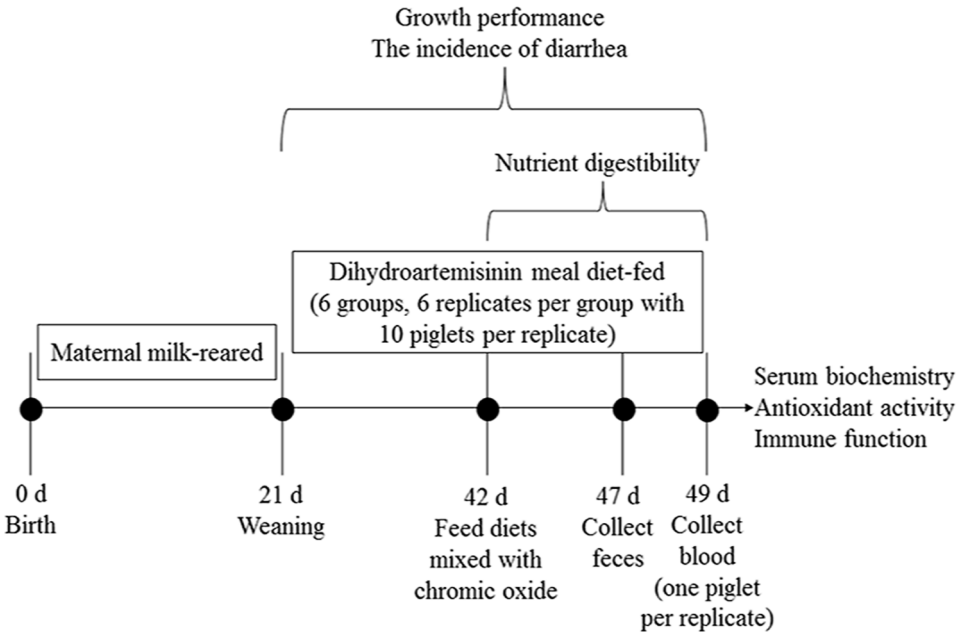

Fig. 2. The study design timeline of Experiment 1.

10 mL of blood samples were collected by jugular venipuncture. Serum was separated by centrifugation at 3000 × g for 10 min at 4 °C and stored at −20 °C for further analysis.

2.2.3. The incidence of diarrhea

Fecal scores were monitored each morning and quantified using a scale ranking from 0 to 3 according to Marquardt et al. (1999), where 0 = normally shaped feces, 1 = soft feces, 2 = mild diarrhea, and 3 = severe diarrhea. Piglets were counted as having diarrhea when the fecal score was greater than 1. The incidence of diarrhea was calculated according to the following formula: [total number of diarrhea pigs on each day during the experiment / (total number of pigs × days of experiment)] × 100%. Piglets were not given veterinary treatments for diarrhea during the experiment.

2.2.4. Nutrient digestibility

At 42 days of age, piglets in all pens were fed diets mixed with chromic oxide at a level of 2.0 g/kg as an indigestible marker for the calculation of nutrient digestibility (Fenton and Fenton, 1979). On the last 3 d of the experiment (d 47 to 49), the fresh feces were collected, pooled and mixed from each pen, and then frozen immediately. Before chemical analysis, the fecal samples were lyophilized at 60 °C for 72 h, after which they were ground to pass through a 1 mm screen. All feed and fecal samples were determined for dry matter (DM), organic matter (OM), crude protein (CP) and ether extract (EE) according to the methods of AOAC (2007). Chromium was analyzed using UV absorption spectrophotometry (UV-1201; Shimadzu, Kyoto, Japan) following the procedure described by Fenton and Fenton (1979). The apparent total tract digestibility (ATTD) of nutrients was calculated by the following formula: Digestibility (%) = [1 − (Nf × Cd)/(Nd × Cf)] × 100, where Nf = Nutrient concentration in feces (% DM), Nd = Nutrient concentration in diet (% DM), Cf = Chromium concentration in feces (% DM), and Cd = Chromium concentration in diet (% DM).

2.2.5. Serum biochemistry

The concentrations of serum total protein (TP, A045-1-1), albumin (ALB, A028-1-1), blood urea nitrogen (BUN, C013-2-1), total bilirubin (T-Bil, C019-1-1), creatinine (Cr, C011-2-1), total cholesterol (TC, A111-2-1), triglycerides (TG, A110-2-1), high density lipoprotein-cholesterol (HDL-C, A112-2-1), and low density lipoprotein-cholesterol (LDL-C, A113-2-1) were measured by an automatic biochemistry blood analyzer (HITACHI 7020, Hitachi, Tokyo, Japan). The activities of aspartate aminotransferase (AST, C010-3-1), alanine aminotransferase (ALT, C009-3-1) and alkaline phosphatase (AKP, A059-2-2) were determined using corresponding diagnostic kits (Nanjing Jiancheng Bioengineering Institute, Jiangsu Province, China) according to the instructions of the manufacturer. The detailed instructions of these testing kits were clearly described in our supplemental files (http://doi.org/10.5281/zenodo.4022066).

2.2.6. Antioxidant activity

The level of total antioxidant capacity (T-AOC, A015-1-2), the activities of total superoxide dismutase (T-SOD, A001-1-2), glutathione peroxidase (GPx, A005-1-2) and catalase (CAT, A007-1-1), and the concentration of malondialdehyde (MDA, A003-1-2) in serum were determined by assay kits of Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China) according to the instructions of the manufacturer. The detailed instructions of these testing kits were clearly described in our supplemental files (http://doi.org/10.5281/zenodo.4022066).

2.2.7. Immune function

The concentrations of interleukin 1β (IL-1β, GR-E71042), interleukin 6 (IL-6, GR-E71048), tumor necrosis factor α (TNF-α, GR-E71237), interferon γ (IFN-γ, GR-E71030), interleukin 4 (IL-4, GR-E71047), interleukin 10 (IL-10, GR-E71034), secretory immunoglobulin A (sIgA, GR-E71085) and immunoglobulin G (IgG, GR-E71136) in serum were determined by ELISA methods using each antibody and biotinylated secondary antibody according to the instruction of manufacturer (YILI Biological Technology Co., Ltd, Shanghai, China). The detailed instructions of these testing kits were clearly described in our supplemental files (http://doi.org/10.5281/zenodo.4022066).

2.3. Experiment 2

2.3.1. Animals and experimental design

In experiment 2, twelve healthy sows with the similar parity (second or third) were fed the same gestating diets during the whole pregnancy period. At day 114 (within standard deviation (SD) 1 of gestation), one normal birth weight (NBW) piglets [Duroc × (Landrace × Yorkshire)]

(1.56 ± 0.02 kg) and two IUGR piglets (0.99 ± 0.03 kg) were selected from each litter according to the selection criteria for IUGR. Previous study suggested that newborn piglets with a birth weight close to the average BW of the herd (within 0.5 SD) were defined as NBW, while those with a lower BW (within 2 SD) were defined as IUGR (D'Inca et al., 2010). All piglets were marked and reared with maternal milk until weaning at 21 days of age. The piglets were individually weighed at 0, 7, 14, 21 days of age to observe the growth of piglets during suckling period. After weaning, NBW piglets were randomly assigned to the NBW-CON group and two IUGR piglets from one litter were allocated to the IUGR-CON group and IUGR-DiHA group (n = 12, six barrows and six gilts). All piglets were housed individually in plastic floored pens at an ambient temperature of 25 to 28 °C and allowed feed and water *ad libitum* throughout the whole experimental period. At 49 days of age, the body weights of piglets were measured individually and feed consumptions were recorded daily to evaluate the growth performance in terms of ADG, ADFI and F/G.

2.3.2. Sampling and measurements

At 49 days of age, eight piglets of each group with nearly similar body weight (n = 8, four barrows and four gilts) were sacrificed by intramuscular injection of sodium pentobarbital (50 mg/kg BW). The SI was immediately dissected without mesentery and divided into duodenum, jejunum and ileum according to previous study described by Wang et al. (2008). The digesta samples of jejunum and ileum were collected by gently massaging the intestinal tract. Then the weights and lengths of each segment from emptied SI were measured. Subsequently, the jejunal and ileal mucosa were scraped from the luminal surface by using a sterile glass microscope. All the digesta and mucosa samples were rapidly frozen in liquid nitrogen and stored at −80 °C until analysis.

2.3.3. Analysis of digestive enzyme activity in small intestine

The samples of jejunal and ileal digesta from −80 °C were homogenized in an ice-cold phosphate-buffered saline-EDTA (2.0 M NaCl, 0.05 M Na_3PO_4, 2×10^{-3} M EDTA, pH7.4) with a ratio of 1:4 (wt/vol). Then the homogenate was centrifuged at 2500 × g at 4 °C for 15 min and the supernatant was stored at −20 °C until further analysis. The activities of amylase (C016-1-1), trypsin (A080-2-2) and lipase (A054-1-1) of intestinal digesta were determined using corresponding diagnostic kits of Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China) according to the instructions of the manufacturer. The detailed instructions of these testing kits were clearly described in our supplemental files (http://doi.org/10.5281/zenodo.4022066).

2.3.4. Analysis of absorption-related enzyme activity and nutrient transporter in small intestine

The samples of jejunal and ileal mucosa from −80 °C were homogenized in an ice-cold phosphate-buffered saline-EDTA (2.0 M NaCl, 0.05 M Na_3PO_4, 2×10^{-3} M EDTA, pH 7.4) with a ratio of 1:4 (wt/vol). Then the homogenate was centrifuged at 4000 × g at 4 °C for 15 min and the supernatant was stored at −20 °C until further analysis. The activities of lactase (A082-1-1), sucrose (A082-2-1), maltase (A082-3-1), Na^+-K^+-ATPase (A016-2-1) and alkaline phosphatase (AKP, A059-2-2) of intestinal mucosa were determined using corresponding diagnostic kits of Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China) according to the instructions of the manufacturer. The concentrations of glucose transporter 2 (GLUT2, GR-E71160) and sodium-dependent glucose transporter 1 (SGLT1, GR-E71998) were determined by ELISA methods using each antibody and biotinylated secondary antibody according to the instructions of the manufacturer (YILI Biological Technology Co., Ltd, Shanghai, China). The detailed instructions of these testing kits were clearly described in our supplemental files (http://doi.org/10.5281/zenodo.4022066).

2.3.5. Analysis of gene expression

Total RNA from jejunal and ileal mucosa was extracted using Trizol reagent (TaKaRa, Dalian, China). The concentration of RNA was quantified by a spectrophotometer (NanoDrop 2000c, Thermo Scientific, USA) and the integrity of RNA was evaluated by agarose gel electrophoresis. Then 1 μg of total RNA for each sample was reverse-transcribed to the complementary DNA (cDNA) using the PrimeScript RT Master Mix (Perfect Real Time) kit (TaKaRa, Dalian, Liaoning, China) according to the manufacturer's instructions. Quantitative real-time polymerase chain reaction (qRT-PCR) was carried out in duplicate on the StepOnePlus ABI Prism Sequence Detection System (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions of the TB GreenPremix Ex Taq (Tli RNaseH Plus) kit (TaKaRa, Dalian, Liaoning, China). The thermal cycling parameters were 95 °C for 30 s for one cycle, followed by 95 °C for 5 s and 60 °C for 30 s for forty cycles. Primer sequences of glucose transporter type 2 (*Glut2*), sodium-dependent glucose transporters 2 (*Sglt1*), sodium-coupled neutral amino-acid transporter 2 (*Snat2*), cationic amino acid transporter 1 (*Cat1*), peptide transporter 1 (*Pept1*), fatty acid binding protein 2 (*Fabp2*), fatty acid synthase (*Fasn*) and *β-actin* genes used for qRT-PCR were presented in Table 2. The relative levels of mRNA expression were calculated using the $2^{-\Delta\Delta Ct}$ method after normalization with those of β-actin as a housekeeping gene (Schmittgen and Livak, 2008).

2.4. Statistical analysis

All data were analyzed by using SPSS statistical software (Ver. 20.0 for windows, SPSS, Inc., Chicago, IL, USA). In experiment 1, the statistical differences between treatment groups were determined via one-way analysis of variance (ANOVA) and Tukey's post hoc test for multiple comparisons. The orthogonal ploynomial contrast test was also performed to determine linear and quadratic effects of increasing inclusion level of DiHA in the diet. In experiment 2, the body weights of the piglets at 0, 7, 14, and 21 days of age and the average daily gain of the piglets from 0 to 7, 7 to 14, 14 to 21, and 0 to 21 days of age were analyzed by unpaired independent t-tests. Other parameters were analyzed by ANOVA and Tukey's post hoc test for multiple comparisons. Results were presented as means ± SEM. A value of $P < 0.05$ was considered as statistically significant.

Table 2
Primer sequences used for quantitative real-time PCR assays.

Gene	Accession no.	Primer, 5′-3′
β-actin	XM_003124280.4	CACGCCATCCTGCGTCTGGA
		AGCACCGTGTTGGCGTAGAG
Glut2	AF054835.1	CCAGGCCCCATCCCCTGGTT
		GCGGGTCCAGTTGCTGAATGC
Sglt1	NM_001164021.1	GGCTGGACGAAGTATGGTGT
		ACAACCACCCAAATCAGAGC
Snat2	NM_001317081.1	GATTGTGGGCAGTGGAATCC
		AAGACCCTCCTTCATTGGCA
Cat1	NM_001012613	CATCAAAAACTGGCAGCTCA
		TGGTAGCGATGCAGTCAAAG
Pept1	NM_214347.1	GGATAGCCTGTACCCCAAGCT
		CATCCTCCACGTGCTTCTTGA
Fabp2	NM_001031780.1	TCGGGATGAAATGGTCCAGACT
		TGTGTTCTGGGCTGTGCTCCA
Fasn	NM_001099930.1	TGGAGGTGCGCCAGATAC
		GGTTCAGCGTTGCCTGCT

Glut2, glucose transporter type 2; *Sglt1*, sodium-dependent glucose transporters 2; *Snat2*, sodium-coupled neutral amino-acid transporter 2; *Cat1*, cationic amino acid transporter 1; *Pept1*, peptide transporter 1; *Fabp2*, fatty acid binding protein 2; *Fasn*, fatty acid synthase.

Table 3
Effect of different doses of dihydroartemisinin on growth performance and incidence of diarrhea in weaned piglets.

Item	Treatment[1]						SEM[2]	*P* value	
	CON	DiHA 20	DiHA 40	DiHA 80	DiHA 160	DiHA 320		Linear[3]	Quadratic[3]
ADFI (g/d/pig)	477.12^{c}	528.90^{ab}	540.54^{a}	542.43^{a}	510.41^{b}	461.37^{c}	6.068	0.059	<0.001
ADG (g/d/pig)	264.97^{b}	285.32^{b}	279.71^{b}	342.86^{a}	282.78^{b}	273.16^{b}	5.663	0.248	<0.001
F/G	1.80^{ab}	1.86^{ab}	1.94^{a}	1.60^{c}	1.81^{ab}	1.71^{bc}	0.026	0.026	0.574
The incidence of diarrhea (%)	5.25^{a}	3.50^{b}	1.61^{d}	1.43^{e}	1.66^{d}	1.87^{c}	0.236	<0.001	<0.001

[1] CON group, basal diet; DiHA 20, DiHA 40, DiHA 80, DiHA 160 and DiHA 320 groups, basal diet supplemented with 20, 40, 80, 160, 320 mg/kg dihydroartemisinin (DiHA), respectively.
[2] Standard error of the mean based on pooled estimate of variation.
[3] Orthogonal polynomials were used to evaluate linear and quadratic responses of different levels of dihydroartemisinin treatment. [a-e]Means within the same row with different superscripts differ significantly ($P < 0.05$). ADFI, average daily feed intake; ADG, average daily gain; F/G, feed to gain ratio.

3. Results

3.1. Experiment 1

3.1.1. Growth performance and incidence of diarrhea

As shown in Table 3, ADFI in DiHA 20, DiHA 40, DiHA 80 and DiHA 160 groups were higher ($P < 0.05$) than those of the CON group. Diet supplemented with 80 mg/kg DiHA increased ($P < 0.05$) ADG and decreased F/G compared to the CON group. Piglets fed different doses of DiHA diet significantly decreased ($P < 0.05$) the incidence of diarrhea compared with those fed the basal diet. Meanwhile, DiHA supplementation quadratically increased ADFI ($P < 0.001$) and ADG ($P < 0.001$) and linearly decreased F/G ($P = 0.026$). There were linear ($P < 0.001$) and quadratic ($P < 0.001$) influences on the incidence of diarrhea when the DiHA levels increased.

3.1.2. Nutrient digestibility

Compared to the CON group, the ATTD of OM and EE in DiHA 40, DiHA 80, DiHA 160 and DiHA 320 groups were increased ($P < 0.05$) compared to those of the CON group (Table 4). Dietary DiHA supplementation at different levels significantly increased ($P < 0.05$) the ATTD of DM and CP compared to those of CON group. DiHA inclusion affected the ATTD of OM ($P < 0.001$ and $P < 0.001$), DM ($P < 0.001$ and $P < 0.001$), CP ($P < 0.001$ and $P < 0.001$) and EE ($P < 0.001$ and $P < 0.001$) linearly and quadratically.

3.1.3. Serum biochemistry

Piglets fed 80 mg/kg DiHA diet increased ($P < 0.05$) TP concentration and decreased ($P < 0.05$) TG concentration compared with those fed the basal diet (Table 5). Piglets fed 160 and 320 mg/kg DiHA diet significantly increased ($P < 0.05$) the T-Bil concentrations and AKP activities compared with those fed the basal diet. Diet supplemented with 320 mg/kg DiHA increased ($P< 0.05$) the activity of AST compared to the CON group. In addition, the concentrations of TP ($P = 0.023$), T-Bil ($P = 0.006$), AST ($P = 0.007$) and AKP ($P = 0.007$) were linearly increased.

3.1.4. Antioxidant activity

The levels of T-AOC in DiHA 40, DiHA 80, DiHA 160 and DiHA 320 groups were higher ($P < 0.05$) than those of the CON group (Table 6). The activities of CAT in DiHA 80, DiHA 160 and DiHA 320 groups were increased ($P < 0.05$) compared to those of the CON group. Dietary DiHA supplementation linearly increased T-AOC level ($P < 0.001$) and CAT activity ($P = 0.004$).

3.1.5. Immune function

Compared to the CON group, DiHA 40, DiHA 80, DiHA 160 and DiHA 320 groups significantly decreased ($P < 0.05$) the concentration of IL-1β and increased ($P < 0.05$) the concentrations of IL-10, sIgA and IgG (Table 7). The concentrations of IL-4 in DiHA 80 and DiHA 160 groups were higher ($P < 0.05$) than those of the CON group. In addition, DiHA supplementation linearly decreased TNF-α concentration ($P = 0.011$) and increased sIgA concentration ($P < 0.001$). There were linear and quadratic influences on the concentrations of IL-1β ($P = 0.004$ and $P = 0.002$), IL-4 ($P = 0.001$ and $P = 0.010$), IL-10 ($P < 0.001$ and $P = 0.004$), and IgG ($P < 0.001$ and $P = 0.035$) as supplemental DiHA level increased.

3.2. Experiment 2

3.2.1. Growth performance

During the suckling period, the body weights of IUGR piglets at 0, 7, 14, 21 days of age were lower ($P < 0.05$) than those of the NBW piglets (Fig. 3(a)). The average daily gain of IUGR piglets during 0–7 d, 7–14 d and 0–21 d period were decreased ($P < 0.05$) compared to those of the NBW piglets (Fig. 3(b)).

During the experimental period, IUGR-CON piglets showed a decreased ($P < 0.05$) average body weight (ABW) at 21 and 49 days of

Table 4
Effect of different doses of dihydroartemisinin on nutrient digestibility in weaned piglets.

Item	Treatment[1]						SEM[2]	*P* value	
	CON	DiHA 20	DiHA 40	DiHA 80	DiHA 160	DiHA 320		Linear[3]	Quadratic[3]
ATTD of DM (%)	76.01^{d}	79.66^{c}	85.55^{b}	89.20^{a}	85.52^{b}	81.20^{c}	0.970	<0.001	<0.001
ATTD of OM (%)	78.95^{c}	80.74^{c}	87.14^{b}	89.45^{a}	86.11^{b}	85.91^{b}	0.967	<0.001	<0.001
ATTD of CP (%)	59.24^{d}	71.04^{c}	76.92^{b}	82.14^{a}	76.21^{b}	74.28^{bc}	1.547	<0.001	<0.001
ATTD of EE (%)	56.83^{c}	58.22^{c}	65.41^{b}	71.35^{a}	66.19^{b}	63.80^{b}	1.141	<0.001	<0.001

[1] CON group, basal diet; DiHA 20, DiHA 40, DiHA 80, DiHA 160 and DiHA 320 groups, basal diet supplemented with 20, 40, 80, 160, 320 mg/kg dihydroartemisinin (DiHA), respectively.
[2] Standard error of the mean based on pooled estimate of variation.
[3] Orthogonal polynomials were used to evaluate linear and quadratic responses of different levels of dihydroartemisinin treatment. [a-d]Means within the same row with different superscripts differ significantly ($P < 0.05$). ATTD, apparent total tract digestibility; DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract.

Table 5
Effect of different doses of dihydroartemisinin on serum biochemistry in weaned piglets.

Item	Treatment[1]						SEM[2]	*P* value	
	CON	DiHA 20	DiHA 40	DiHA 80	DiHA 160	DiHA 320		Linear[3]	Quadratic[3]
TP (g/L)	39.46^{b}	41.03^{ab}	44.18^{ab}	47.67^{a}	44.92^{ab}	44.76^{ab}	0.906	0.023	0.100
ALB (g/L)	20.96	20.97	19.65	20.53	20.50	20.58	0.309	0.715	0.454
BUN (mmol/L)	11.54	11.79	11.48	11.80	11.52	11.57	0.044	0.714	0.349
T-Bil (μmol/L)	0.32^{b}	0.40^{ab}	0.42^{ab}	0.32^{b}	0.52^{a}	0.50^{a}	0.023	0.006	0.658
Cr (μmol/L)	150.55	144.02	144.05	142.71	144.66	147.37	2.339	0.765	0.341
AST (U/L)	8.00^{b}	8.16^{b}	9.96^{ab}	9.19^{ab}	9.07^{ab}	11.61^{a}	0.372	0.007	0.584
ALT (U/L)	7.30	7.22	7.42	7.29	7.88	7.67	0.200	0.400	0.840
AKP (U/L)	34.88^{b}	35.70^{ab}	36.63^{ab}	34.07^{b}	42.62^{a}	42.66^{a}	1.076	0.007	0.229
TC (mmol/L)	2.05	2.06	1.99	1.97	2.19	1.99	0.043	0.939	0.909
TG (mmol/L)	0.37^{a}	0.39^{a}	0.35^{ab}	0.29^{b}	0.34^{ab}	0.36^{ab}	0.010	0.153	0.104
LDL-C (mmol/L)	0.72	0.70	0.70	0.68	0.68	0.71	0.016	0.761	0.605
HDL-C (mmol/L)	1.59	1.59	1.63	1.60	1.59	1.63	0.039	0.862	0.991

[1] CON group, basal diet; DiHA 20, DiHA 40, DiHA 80, DiHA 160 and DiHA 320 groups, basal diet supplemented with 20, 40, 80, 160, 320 mg/kg dihydroartemisinin (DiHA), respectively.
[2] Standard error of the mean based on pooled estimate of variation.
[3] Orthogonal polynomials were used to evaluate linear and quadratic responses of different levels of dihydroartemisinin treatment. ^{a,b}Means within the same row with different superscripts differ significantly ($P < 0.05$). TP, total protein; ALB, albumin; BUN, blood urea nitrogen; T-Bil, total bilirubin; Cr, creatinine; AST, aspartate aminotransferase; ALT, alanine aminotransferase; AKP, alkaline phosphatase. TC, total cholesterol; TG, triglyceride; LDL-C, low density lipoprotein-cholesterol; HDL-C, high density lipoprotein-cholesterol.

Table 6
Effect of different doses of dihydroartemisinin on antioxidant activity in weaned piglets.

Item	Treatment[1]						SEM[2]	*P* value	
	CON	DiHA 20	DiHA 40	DiHA 80	DiHA 160	DiHA 320		Linear[3]	Quadratic[3]
T-AOC (U/mL)	1.11^{b}	1.10^{b}	1.93^{a}	2.13^{a}	2.06^{a}	2.29^{a}	0.108	<0.001	0.174
T-SOD (U/mL)	237.63	228.59	238.56	234.49	240.79	245.55	2.331	0.141	0.307
GPx (U/mL)	351.03	353.97	383.13	360.88	355.78	360.76	5.351	0.777	0.308
CAT (U/mL)	30.30^{b}	35.92^{ab}	37.52^{ab}	42.75^{a}	40.87^{a}	41.32^{a}	1.30	0.004	0.145
MDA (U/mL)	3.62	3.66	3.21	3.28	3.39	3.83	0.12	0.899	0.138

[1] CON group, basal diet; DiHA 20, DiHA 40, DiHA 80, DiHA 160 and DiHA 320 groups, basal diet supplemented with 20, 40, 80, 160, 320 mg/kg dihydroartemisinin (DiHA), respectively.
[2] Standard error of the mean based on pooled estimate of variation.
[3] Orthogonal polynomials were used to evaluate linear and quadratic responses of different levels of dihydroartemisinin treatment. ^{a,b}Means within the same row with different superscripts differ significantly ($P < 0.05$). T-AOC, total antioxidant capacity; T-SOD, total superoxide dismutase; GPx, glutathione peroxidase; CAT, catalase; MDA, malondiadehyde.

age, ADG and ADFI compared with the NBW-CON piglets (Table 8). DiHA treatment effectively improved ($P < 0.05$) ABW at 49 d of age, ADG and ADFI of IUGR piglets.

3.2.2. Development of small intestine

The body weight before slaughter in IUGR-CON group was lower ($P < 0.05$) than that of the NBW-CON group (Table 9). IUGR piglets increased ($P < 0.05$) the relative weight of jejunum and ileum, and

Table 7
Effect of different doses of dihydroartemisinin on immune function in weaned piglets.

Item	Treatment[1]						SEM[2]	*P* value	
	CON	DiHA 20	DiHA 40	DiHA 80	DiHA 160	DiHA 320		Linear[3]	Quadratic[3]
IL-1β (pg/mL)	259.67^{a}	287.67^{ab}	226.16^{bc}	202.41^{c}	211.42^{c}	241.69^{bc}	7.466	0.004	0.002
IL-6 (pg/mL)	652.38	675.57	610.95	566.68	661.32	648.87	15.352	0.739	0.188
TNF-α (pg/mL)	164.21	154.41	158.31	146.30	143.05	141.92	2.971	0.011	0.818
IFN-γ (pg/mL)	30.56	28.36	28.50	29.92	28.92	28.22	0.771	0.616	0.874
IL-4 (pg/mL)	42.71^{c}	43.10^{c}	44.24^{c}	58.53^{a}	53.33^{ab}	47.64^{bc}	1.356	0.001	0.010
IL-10 (pg/mL)	80.76^{b}	81.92^{b}	113.31^{a}	109.49^{a}	102.37^{a}	107.91^{a}	2.970	<0.001	0.004
sIgA (μg/mL)	31.93^{b}	31.41^{b}	41.32^{a}	43.68^{a}	43.66^{a}	45.19^{a}	1.188	<0.001	0.093
IgG (mg/mL)	17.39^{b}	17.66^{b}	22.27^{a}	22.33^{a}	23.46^{a}	22.87^{a}	0.541	<0.001	0.035

[1] CON group, basal diet; DiHA 20, DiHA 40, DiHA 80, DiHA 160 and DiHA 320 groups, basal diet supplemented with 20, 40, 80, 160, 320 mg/kg dihydroartemisinin (DiHA), respectively.
[2] Standard error of the mean based on pooled estimate of variation.
[3] Orthogonal polynomials were used to evaluate linear and quadratic responses of different levels of dihydroartemisinin treatment. $^{a-c}$Means within the same row with different superscripts differ significantly ($P < 0.05$). IL-1β, interleukin 1β; IL-6, interleukin 6; TNF-α, tumor necrosis factor α; IFN-γ, interferon γ; IL-4, interleukin 4; IL-10, interleukin 10; sIgA, secretory immunoglobulin A; IgG, immunoglobulin G.

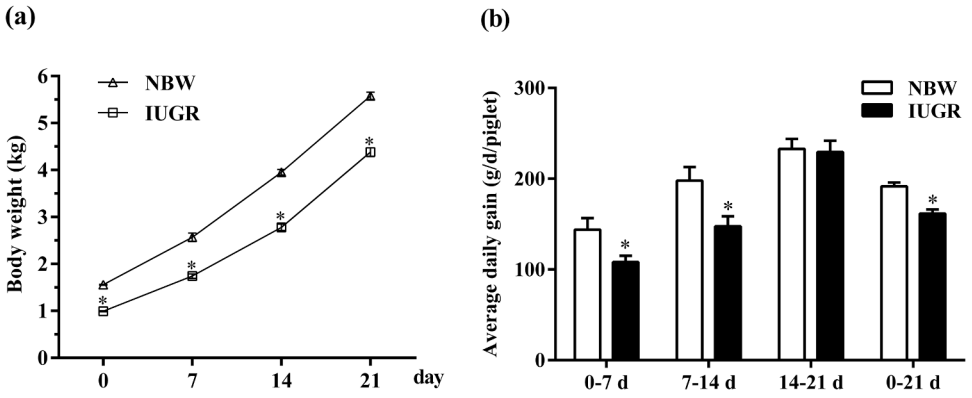

Fig. 3. Analysis of body weight (a) and average body weight gain (b) in normal birth weight (NBW) and intrauterine growth retardation (IUGR) piglets during suckling period from 0 to 21 days of age. Data were analyzed using unpaired independent t-tests. Values were presented as mean ± SEM (n = 12). *A significant difference (P < 0.05) was observed when compared with NBW group. NBW, normal birth weight piglets; IUGR, intrauterine growth retardation piglets.

Table 8
Effect of diet supplemented with dihydroartemisinin on growth performance in weaned piglets with intrauterine growth retardation.

Items	Treatment[1]			*P* value[2]	
	NBW-CON (NC)	IUGR-CON (IC)	IUGR-DiHA (ID)	NC *vs.* IC	IC *vs.* ID
21 d ABW (kg)	5.58 ± 0.08	4.35 ± 0.14*	4.42 ± 0.13	<0.001	0.903
49 d ABW (kg)	14.28 ± 0.30	9.66 ± 0.22*	10.94 ± 0.21#	<0.001	0.006
ADG (g/d/piglet)	258.70 ± 3.52	187.27 ± 3.07*	204.92 ± 4.53#	<0.001	0.012
ADFI (g/d/piglet)	396.00 ± 6.49	282.67 ± 5.83*	304.36 ± 4.77#	<0.001	0.043
F/G	1.53 ± 0.02	1.51 ± 0.03	1.49 ± 0.03	0.880	0.865

[1] NBW-CON (NC) group, normal body weight weaned piglets fed with basal diet; IUGR-CON (IC) group, intrauterine growth retardation weaned piglets fed with basal diet. IUGR-DiHA (ID) group, intrauterine growth retardation weaned piglets fed with 80 mg/kg dihydroartemisinin. Data were presented as mean ± SEM (n = 8).

[2] A value of *P < 0.05 was considered as statistically significant difference when compared with NBW-CON group.

# P < 0.05 was considered as statistically significant difference when compared with IUGR-CON group. ABW, average body weight; ADG, average daily gain; ADFI, average daily feed intake; F/G, feed to gain ratio.

Table 9
Effect of diet supplemented with dihydroartemisinin on the development of small intestine in weaned piglets with intrauterine growth retardation.

Items	Treatment[1]			*P* value[2]	
	NBW-CON (NC)	IUGR-CON (IC)	IUGR-DiHA (ID)	NC *vs.* IC	IC *vs.* ID
BW (kg)	15.14 ± 0.50	10.41 ± 0.50*	13.14 ± 0.38#	<0.001	0.001
Duodenum weight/BW (g/kg)	0.99 ± 0.06	0.83 ± 0.06	1.03 ± 0.05	0.137	0.067
Jejunum weight/BW (g/kg)	17.42 ± 0.69	21.28 ± 0.77*	18.74 ± 0.50#	0.001	0.033
Ileum weight/BW (g/kg)	27.53 ± 1.37	32.25 ± 1.04*	29.06 ± 1.22	0.032	0.178
SI length (m)	13.25 ± 0.30	11.72 ± 0.46	12.03 ± 0.54	0.061	0.881
Duodenum length (cm)	24.25 ± 0.59	20.38 ± 0.53*	25.00 ± 0.60#	<0.001	<0.001
Jejunum length (m)	5.20 ± 0.12	4.39 ± 0.10*	4.54 ± 0.14	<0.001	0.659
Ileum length (m)	7.80 ± 0.18	6.91 ± 0.28	7.07 ± 0.32	0.070	0.914
Duodenum length/SI (cm/m)	1.84 ± 0.07	1.76 ± 0.09	2.06 ± 0.08#	0.773	0.045
Jejunum length/SI (cm/m)	39.27 ± 0.03	37.69 ± 1.02	38.04 ± 1.10	0.419	0.957
Ileum length/SI (cm/m)	58.90 ± 0.04	58.94 ± 0.05	58.74 ± 0.06#	0.794	0.026

[1] NBW-CON (NC) group, normal body weight weaned piglets fed with basal diet; IUGR-CON (IC) group, intrauterine growth retardation weaned piglets fed with basal diet. IUGR-DiHA (ID) group, intrauterine growth retardation weaned piglets fed with 80 mg/kg dihydroartemisinin. Data were presented as mean ± SEM (n = 8).

[2] A value of *P < 0.05 was considered as statistically significant difference when compared with NBW-CON group.

# P < 0.05 was considered as statistically significant difference when compared with IUGR-CON group. BW, body weight; SI, small intestine.

reduced (P < 0.05) the length of duodenum and jejunum when compared to the NBW-CON piglets. IUGR piglets fed the DiHA diet increased (P < 0.05) the body weight, duodenum length, the relative length of duodenum, and decreased the relative jejunum weight and relative ileum length compared to those of IUGR piglets fed the basal diet.

3.2.3. Activity of digestive enzyme in small intestine

IUGR-CON piglets exhibited lower (P < 0.05) activities of amylase and trypsin in the ileum compared to the NBW-CON piglets (Table 10). Dietary DiHA supplementation significantly enhanced (P < 0.05) the amylase activity in the jejunum and the activities of amylase and trypsin in the ileum of IUGR piglets.

3.2.4. Activities of disaccharidase, Na^+-K^+-ATPase and AKP in small intestine

IUGR decreased (P < 0.05) the activities of lactase, sucrase, maltase and AKP in the jejunum and activities of lactase, sucrase and Na^+-K^+-ATPase in the ileum of weaned piglets (Table 11). After DiHA intervention, IUGR piglets displayed increased (P < 0.05) activities of lactase, maltase and AKP in the jejunum and Na^+-K^+-ATPase in the ileum.

Table 10
Effect of diet supplemented with dihydroartemisinin on digestive enzyme activity of intestinal digesta in weaned piglets with intrauterine growth retardation.

Items	Treatment[1]			*P* value[2]	
	NBW-CON (NC)	IUGR-CON (IC)	IUGR-DiHA (ID)	NC *vs.* IC	IC *vs.* ID
Jejunum					
Amylase (U/mg protein)	111.57 ± 8.41	103.13 ± 6.44	135.91 ± 9.12#	0.744	0.030
Trypsin (U/μg protein)	24.87 ± 4.39	24.08 ± 2.46	23.59 ± 3.21	0.986	0.994
Lipase (U/g protein)	52.96 ± 5.51	44.20 ± 6.09	46.12 ± 3.29	0.463	0.962
Ileum					
Amylase (U/mg protein)	140.50 ± 8.12	94.85 ± 2.45*	131.36 ± 9.27#	0.001	0.008
Trypsin (U/μg protein)	40.55 ± 5.09	14.57 ± 1.09*	29.88 ± 3.24#	<0.001	0.021
Lipase (U/g protein)	31.80 ± 5.03	28.21 ± 5.13	27.45 ± 5.76	0.883	0.994

[1] NBW-CON (NC) group, normal body weight weaned piglets fed with basal diet; IUGR-CON (IC) group, intrauterine growth retardation weaned piglets fed with basal diet. IUGR-DiHA (ID) group, intrauterine growth retardation weaned piglets fed with 80 mg/kg dihydroartemisinin. Data were presented as mean ± SEM (n = 8).
[2] A value of *P < 0.05 was considered as statistically significant difference when compared with NBW-CON group.
# P < 0.05 was considered as statistically significant difference when compared with IUGR-CON group.

3.2.5. Nutrient transporters

The concentrations of GLUT2 in both jejunum and ileum, and SGLT1 concentration in the ileum of IUGR-CON group were decreased (P < 0.05) compared to those of the NBW-CON group (Table 12). DiHA inclusion significantly increased (P < 0.05) the GLUT2 in the jejunum and SGLT1 in the ileum of IUGR piglets. In the jejunum, IUGR piglets showed lower (P < 0.05) mRNA expressions of *Glut2, Snat2, Fabp* and *Fasn* compared to those of the NBW piglets (Fig. 4(a)). The expressions of these genes in IUGR piglets were all up-regulated (P < 0.05) after DiHA treatment. In the ileum, IUGR piglets down-regulated (P < 0.05) the mRNA expressions of *Glut2, Sglt1, Snat2, Pept1, Fabp* and *Fasn*, when compared with the NBW piglets (Fig. 4(b)). Diet supplemented with DiHA promoted (P < 0.05) the mRNA expressions of *Glut2, Sglt1, Snat2, Pept1, Fabp* and *Fasn* of the IUGR piglets.

4. Discussion

IUGR is characterized by fetal growth less than normal for the population, which results in the restriction of growth and development (Rosenberg, 2008). Intestinal dysfunction and abnormal development also occur in IUGR neonates, which may exert a long-term effect on gut health during the postpartum period (Thornbury et al., 1992; Trahair et al., 1997). Therefore, it is important for early nutritional intervention in IUGR fetus or animals. The aim of experiment 1 was to determine the optimal dose of DiHA through investigating the different doses of DiHA on the growth performance, nutrient digestibility, serum biochemistry, antioxidant activity and immune function of weaned piglets. Then we used this dose in the experiment 2. We presumed that supplementation with DiHA could improve growth performance, enhance the intestinal digestion and absorption, and increase the levels of nutrient transporters of IUGR piglets.

In experiment 1, piglets in DiHA3 group significantly increased ADFI and ADG, decreased F/G compared to CON group. Previous study demonstrated that broilers fed diet supplemented with 1 g/kg EA increased ADG in the starter phase (1–21 d) and the whole trial (1–42 d) (Wan et al., 2016). Research also found that 2 g/kg EA inclusion increased ADFI and ADG of weaned piglets from 21 to 50 days of age (Niu et al., 2020). The incidence of diarrhea was considered as the reflection of the resistance to pathogens as well as the nutrient absorption of animals (Zhao et al., 2015). *Artemisia* species have been used in traditional Chinese medicine for centuries to treat diarrhea (Shahbazfar et al., 2011). In this experiment, different levels of DiHA in the diet could reduce the incidence of diarrhea. Moreover, the lowest incidence of diarrhea was observed in the DiHA3 group, as compared with other groups. These results showed that diet supplemented with 80 mg/kg improved the growth performance and decreased the

Table 11
Effect of diet supplemented with dihydroartemisinin on the activity of absorption-related enzyme of intestinal mucosa in weaned piglets with intrauterine growth retardation.

Items	Treatment[1]			*P* value[2]	
	NBW-CON (NC)	IUGR-CON (IC)	IUGR-DiHA (ID)	NC *vs.* IC	IC *vs.* ID
Jejunum					
Lactase (U/mg protein)	32.56 ± 1.11	20.95 ± 1.58*	30.71 ± 1.83#	<0.001	<0.001
Sucrase (U/mg protein)	39.66 ± 1.09	31.07 ± 2.16*	29.24 ± 1.32	0.003	0.699
Maltase (U/mg protein)	113.40 ± 2.84	56.81 ± 2.80*	79.75 ± 4.03#	<0.001	<0.001
Na^+-K^+-ATPase (U/mg protein)	3.69 ± 0.23	3.23 ± 0.27	3.81 ± 0.30	0.466	0.308
AKP (U/g protein)	524.40 ± 24.52	343.65 ± 28.34*	458.98 ± 35.19#	0.002	0.038
Ileum					
Lactase (U/mg protein)	9.00 ± 0.52	4.29 ± 0.29*	5.62 ± 0.24	<0.001	0.554
Sucrase (U/mg protein)	34.75 ± 1.77	22.15 ± 1.30*	26.94 ± 2.68	0.001	0.232
Maltase (U/mg protein)	62.87 ± 4.79	50.18 ± 4.52	51.95 ± 3.40	0.123	0.954
Na^+-K^+-ATPase (U/mg protein)	1.16 ± 0.12	0.73 ± 0.08*	1.22 ± 0.11#	0.025	0.012
AKP (U/g protein)	325.24 ± 22.32	282.14 ± 23.16	298.87 ± 28.50	0.456	0.833

[1] NBW-CON (NC) group, normal body weight weaned piglets fed with basal diet; IUGR-CON (IC) group, intrauterine growth retardation weaned piglets fed with basal diet. IUGR-DiHA (ID) group, intrauterine growth retardation weaned piglets fed with 80 mg/kg dihydroartemisinin. Data were presented as mean ± SEM (n = 8).
[2] A value of *P < 0.05 was considered as statistically significant difference when compared with NBW-CON group.
# P < 0.05 was considered as statistically significant difference when compared with IUGR-CON group. AKP, alkaline phosphatase.

Table 12
Effect of diet supplemented with dihydroartemisinin on nutrient transporter in weaned piglets with intrauterine growth retardation.

Items	Treatment[1]			*P* value[2]	
	NBW-CON (NC)	IUGR-CON (IC)	IUGR-DiHA (ID)	NC *vs.* IC	IC *vs.* ID
Jejunum					
GLUT2 (ng/mg protein)	2.49 ± 0.08	1.65 ± 0.05*	2.04 ± 0.09#	<0.001	0.001
SGLT1 (ng/μg protein)	1.71 ± 0.07	1.43 ± 0.09	1.56 ± 0.08	0.054	0.518
Ileum					
GLUT2 (ng/mg protein)	3.02 ± 0.18	2.22 ± 0.08*	2.57 ± 0.10	0.001	0.151
SGLT1 (ng/μg protein)	1.99 ± 0.06	1.59 ± 0.04*	1.83 ± 0.03#	<0.001	0.006

[1] NBW-CON (NC) group, normal body weight weaned piglets fed with basal diet; IUGR-CON (IC) group, intrauterine growth retardation weaned piglets fed with basal diet. IUGR-DiHA (ID) group, intrauterine growth retardation weaned piglets fed with 80 mg/kg dihydroartemisinin. Data were presented as mean ± SEM (n = 8).

[2] A value of *P < 0.05 was considered as statistically significant difference when compared with NBW-CON group.

# P < 0.05 was considered as statistically significant difference when compared with IUGR-CON group. GLUT2, glucose transporter 2; SGLT1, sodium-dependent glucose transporter 1.

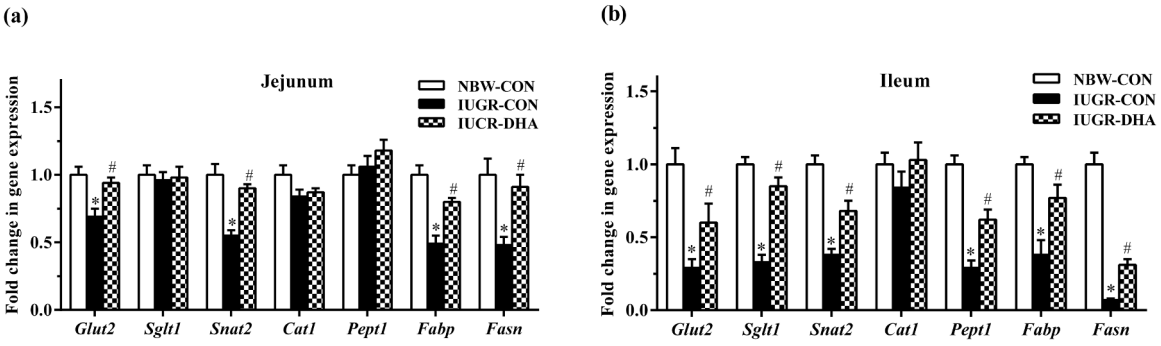

Fig. 4. Effect of diet supplemented with dihydroartemisinin on mRNA expressions of nutrient transporters in the jejunum (a) and ileum (b) of weaned piglets with intrauterine growth retardation. NBW-CON group, normal body weight weaned piglets fed with basal diet; IUGR-CON group, intrauterine growth retardation weaned piglets fed with basal diet. IUGR-DiHA group, intrauterine growth retardation weaned piglets fed with 80 mg/kg dihydroartemisinin. Values were presented as mean ± SEM (n = 8). *A significant difference (P < 0.05) was observed when compared with NBW-CON group; #A significant difference (P < 0.05) was observed when compared with IUGR-CON group. *Glut2*, glucose transporter type 2; *Sglt1*, sodium-dependent glucose transporters 2; *Snat2*, sodium-coupled neutral amino-acid transporter 2; *Cat1*, cationic amino acid transporter 1; *Pept1*, peptide transporter 1; *Fabp2*, fatty acid binding protein 2; *Fasn*, fatty acid synthase.

incidence of diarrhea. DiHA at different levels increased the ATTD of DM, OM, CP and EE and 80 mg/kg DiHA diet showed the highest nutrient digestibility numerically. The increased growth may be attributed to the improvement of nutrient digestibility. Serum biochemistry can reflect the condition of nutrients metabolism and the health status of organ and body (Hu et al., 2015; Liu et al., 2015). The concentrations of serum TP, ALB and BUN are usually regarded as hallmarks of protein synthesis and metabolism (Zhou et al., 2015). The increased content of serum TP in DiHA 80 group suggested that protein anabolism of the body was improved. The increased activities of serum ALT, AST and AKP and concentration of T-Bil are thought to be the biomarkers for the hepatic dysfunction (Tang et al., 2012; Wang et al., 1996). The higher concentration of T-Bil and activity of AKT in DiHA 160 and DiHA 320, and the higher activity of AST in DiHA 320 group were observed, suggesting that liver function may be impaired when piglets fed the diet supplemented with 160 and 320 mg/kg DiHA. TG is the most common nontoxic form of fatty acids. The high plasma concentration of TG indicates the abnormal lipid metabolism of the body, which may lead to atherosclerosis and coronary heart disease (He et al., 2015). Piglets fed diet supplemented with 80 mg/kg DiHA significantly decreased the TG concentration in this experiment. The result showed that 80 mg/kg DiHA diet could regulate lipid metabolism and improve the health of weaned piglets. Oxidative stress is the imbalance of oxidation and antioxidation, which causes the overproduction of free oxygen radicals and results in membrane damage, protein modification (including enzymes) and DNA damage (Pietta, 2000). So it is important to improve the antioxidant activity to protect body from lipid peroxidation and protein oxidation. The enzymatic antioxidant system includes SOD, GPx and CAT. In this study, the activities of CAT in DiHA 80, DiHA 160 and DiHA 320 groups were increased as well as the T-AOC levels, indicating that DiHA supplementation could improve the antioxidant capacity of weaned piglets. In terms of immune function, the present study demonstrated that the content of pro-inflammation cytokine IL-1β was reduced and the concentrations of anti-inflammation cytokines IL-4 and IL-10 were enhanced in the serum of DiHA 80 and DiHA 160 groups. The elevated immunoglobulins sIgA and IgG were observed in treatment groups except the DiHA 20 group. These data illustrated that DiHA supplementation improved the immunity of weaned piglets. According to the results, we concluded that diet supplemented with 80 mg/kg DiHA improved the growth and nutrient digestibility, decreased the incidence of diarrhea, ameliorated protein and lipid metabolism, and increased antioxidant and immune function of weaned piglets. In addition, excessive dose of DiHA had negative effects on serum biochemistry. Therefore, the optimal dose of DiHA in piglet diet might be 80 mg/kg in the present study. In practical pig production, it is difficult to select IUGR piglets from healthy sows with similar parity (second or third) over the same period. In addition, the effect of different doses of DiHA on NBW piglets can reflect the effectiveness of DiHA *in vivo*. So the optimal dose for NBW piglets can be used for IUGR piglets.

In experiment 2, 80 mg/kg DiHA was used to determine whether dietary DiHA supplementation could improve the growth performance, intestinal digestive and absorptive function and nutrient transporter levels of IUGR weaned piglets. In this study, IUGR piglets showed lower body weight during the suckling period, and decreased ADG, ADFI, initial BW and final BW during the weaning period. The results of the

present study were in agreement with previous observations that IUGR decreased the growth in rats (He et al., 2018), neonatal piglets (Boubred et al., 2017), suckling piglets (Zhang et al., 2017) and weaned piglets (Feng et al., 2018; Niu et al., 2019). As expected, diet supplemented with DiHA was able to ameliorate the growth performance by increasing the BW, ADG and ADFI in IUGR piglets.

The intestine is responsible for the first physiological stage to bring nutrients to the body's cells and plays an important role in modulating the development of young mammals (Guilloteau et al., 2010). Whether the positive effect of DiHA on growth of IUGR piglets was attributed to its treatment for intestinal health need further investigation. The length of SI represent the levels of intestinal development. In the current experiment, the length of duodenum and jejunum were decreased in IUGR piglets, indicating that IUGR impaired the development of SI. The result was similar to previous report that there was a decrease in the relative length of duodenum and jejunum in IUGR suckling piglets (Dong et al., 2015). Wang et al. (2012) also found that the relative length of the entire SI was reduced in newborn piglets. Interestingly, DiHA treatment altered the mal-development of the SI in IUGR piglets. These results elucidated that DiHA feeding to piglets during the weaning period could ameliorate the development of SI.

Intestinal digestive enzymes (such as amylase, trypsin and lipase) hydrolyze large molecules into small molecules that can be absorbed in gut (Schneeman and Daniel, 1980). The activities of amylase and trypsin in the ileum were decreased in IUGR piglets, which were consistent with the results reported by Dong et al. (2015). DiHA supplementation efficiently increased the activities of jejunal amylase and ileal amylase and trypsin in IUGR piglets. The disaccharidases (lactase, sucrase and maltase) are considered as the major brush border enzymes of enterocytes, which are responsible for the final stages of breaking down and absorbing the nutrients (Silva et al., 2010). The carbohydrates are degraded into monosaccharide (mainly glucose) by disaccharideases, which are the main energy source of the body (Jéquier, 1994; Chotinsky et al., 2001). Intestinal glucose absorption is mediated by specialized nutrient transporters SGLT1 and GLUT2, which transport glucose to the enterocytes and then enter into the blood circulation (Schmitt et al., 2016; Röder et al., 2014). Na^+-K^+-ATPase (sodium pump), a membrane-bound enzyme, maintains the Na^+ and K^+ gradients across the plasma membrane of animal cells and therefore regulates the cell function (Gaudet and Brochu, 2009). Intestinal mucosal Na^+-K^+-ATPase is involved in the absorption of glucose which provides energy for the transmembrane transport of SGLT1 by decomposing ATP (Charney and Donowitz, 1978). AKP is an enzyme existing in intestinal mucosa, which has a pivotal role in intestinal homeostasis and glucose absorption (Pramanik et al., 1994; Jean-Paul, 2010). In the present study, IUGR piglets showed lower activities of intestinal lactase and sucrase, jejunal AKP and ileal Na^+-K^+-ATPase. The concentrations of GLUT2 and SGLT1 were also decreased in the intestine of IUGR weaned piglets. Previous research demonstrated that the activities of Na^+-K^+-ATPase and AKP were decreased in IUGR rats (Iida, 1986). IUGR led to the dysfunction in digestive system, as well as the alteration in the mRNA expressions of some nutrient transporters. Gene expressions of intestinal nutrient transporters for glucose (*Glut2, Sglt1*), fatty acids (*Fabp2, Fasn*) and amino acids (*Snat2, Pept1*) were decreased in IUGR weaned piglets. Diet supplemented with DiHA enhanced the activities of lactase, maltase and AKP in the jejunum and Na^+-K^+-ATPase activity in the ileum. The concentrations of jejunal GLUT2 and ileal SGLT1, and the mRNA expressions of nutrient transporters were increased after DiHA treatment in IUGR piglets. These results may indicate that DiHA relieved the impaired digestion and absorption of intestine in IUGR piglets by ameliorating the digestive enzyme capacity and nutrient transporter function. It is reported that DiHA has anti-inflammation activity and immunomodulation effect. So we speculated that DiHA supplementation may improve intestinal health by increasing intestinal immune function and decreasing intestinal inflammatory response, finally enhancing the digestion and absorption of intestine and growth performance in IUGR piglets. The working mechanism of DiHA need further study.

5. Conclusions

In conclusion, data from dose dependent experiment showed that the optimal dose of DiHA in the piglet diets is 80 mg/kg. The results obtained in experiment 2 indicated that the inclusion of DiHA in IUGR piglets could alleviate the reduced growth performance by improving the development of intestine and increasing intestinal digestion and absorption function during the first 4 wk post-weaning. Our findings may be helpful in the development of new nutritional strategies for IUGR infants to protect intestinal health in early life.

CRediT authorship contribution statement

Y. Niu: Conceptualization, Formal analysis, Investigation, Writing - review & editing, Project administration. **Y.W. Zhao:** Formal analysis. **J.T. He:** Formal analysis. **M.M. Shen:** Formal analysis. **Z.D. Gan:** Formal analysis. **L.L. Zhang:** Funding acquisition. **T. Wang:** Conceptualization, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

All authors approve the submission of this manuscript and declare no conflict of interest. The manuscript has not been published previously, and not under consideration for publication elsewhere.

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