

*Full Length Research Paper*

# Effects of L-arginine on intestinal development and endogenous arginine-synthesizing enzymes in neonatal pigs

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**This study aimed to investigate the effects of dietary L-arginine supplementation on the intestinal development of neonatal piglets and the underlying mechanisms. 36 neonatal piglets were randomly allocated into three diet groups: control group (supplemented with 0% L-arginine), 0.4 and 0.8% L-arginine groups. When compared with the control, dietary supplementation with L-arginine decreased ( $P<0.05$ ) blood urea nitrogen (BUN), and improved ( $P<0.05$ ) serum T3 and insulin level of the piglets on day 11. Arginine and its metabolites (citrulline and ornithine) were elevated, additionally, dietary supplementation with 0.8% L-arginine markedly enhanced jejunal villus height, villus area on day 11 and D-xylose absorption rate on day 19. Dietary supplementation with 0.8% L-arginine increased ( $P<0.05$ ) activities of maltose and lactose on day 18, respectively. This effect correlated with profound change in enzyme activities as inducible nitric oxide synthetase (iNOS), glutamine synthetase (GS) and ornithine decarboxylase (ODC) were elevated on day 18. The concentrations of spermine was increased ( $P<0.05$ ) by L-arginine supplementation on day 18. These results collectively suggest that dietary L-arginine supplementation improves protein synthesis and intestinal development of the neonatal pigs, the underlying mechanism includes dietary L-arginine supplementation which regulated the productions of intestinal polyamine in jejunum, and stimulated endogenous arginine-synthesizing enzymes in neonatal piglets.**

**Key words:** Neonatal pig, L-arginine, intestinal development, arginine-synthetases.

## INTRODUCTION

Early weaning has been reported to correlate with villus atrophy, which depresses feed intake and growth performance in piglets (Kelly et al., 1991; Ou et al., 2007). Adequate supply of nutrients from blood and intestine ensured the optimal development of villus (Matheson et al., 2000). The small intestine was suggested to be the major organ to synthesize arginine in neonatal animals (Wu et al., 1995). Low arginine intake associated with

depressed feed intake may be a major reason for increased intestinal epithelial damage in early-weaned pigs.

Previous studies indicated that arginine was strikingly deficient in milk-fed piglets, as the relatively low contents of arginine were found in sow's milk (Wu et al., 2000; O'Quinn et al., 2002). Arginine was therefore considered an essential amino acid for the optimal growth of neonatal pigs (Wu and Knabe, 1994, 1995; Flynn et al., 2000; Wu et al., 2004). Dietary supplementation of L-arginine improved the growth of neonatal pigs (Kim et al., 2004), mainly due to its beneficial effects in enhancing wound healing and angiogenesis, improving protein

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**Table 1.** Composition and nutrient contents of the basal diet (on an as-fed basis).

Ingredient (%)	Ratio (%)
Whole milk power (23% CP)	50.84
Whey protein concentrate (34% CP)	22.00
Plasma protein powder (78% CP)	5.00
Whey power (4% CP)	14.32
Coconut oil	5.00
L-alanine (98%)	1.67
DL-methionine (98%)	0.08
L-threonine (98%)	0.14
L-tryptophan (98%)	0.02
liquid chloride choline (70%)	0.12
Vitamin-mineral premix <sup>1</sup>	0.5
emulsifying agent	0.11
<b>Calculated composition</b>	
Crude protein (%)	26.34
Digestible energy (MJ/kg)	19.25
Fat (%)	15.80
Lactose (%)	32.20
Calcium (%)	1.10
Total phosphorus (%)	0.70
Lysine (%)	2.20
Methionine (%)	0.50
Arginine (%)	0.72

<sup>1</sup>Providing the following (mg/kg powder diet): Fe (as FeSO<sub>4</sub>·7H<sub>2</sub>O), 105; Cu (as CuSO<sub>4</sub>·5H<sub>2</sub>O), 10; Mn (as MnSO<sub>4</sub>·H<sub>2</sub>O), 5; Zn (as ZnSO<sub>4</sub>·7H<sub>2</sub>O), 110; retinyl palmitate, 13.2; cholecalciferol, 1.32; all-rac- $\alpha$ -tocopheryl acetate, 96; menadione sodium bisulfite, 1.50; biotin, 0.24; folic acid, 0.90; nicotinic acid, 60.0; calcium pantothenate, 36; riboflavin, 12; thiamin, 4.5; pyridoxine, 6; cyanocobalamin, 0.06.

anabolism and immune functions as suggested by study in mice (Cremades et al., 2004) and human (Barbul et al., 1990). Biochemically, arginine plays an important role as a substrate for protein synthesis, intermediates in the hepatic urea cycle, as well as precursors for the synthesis of various important metabolic molecules, including nitric oxide (NO), polyamines and creatinine (Wu and Morris 1998; Kim et al., 2007; Flynn et al., 2002; Cynober et al., 1995). Wu and colleague suggested that L-arginine supplementation may boost the growth of young piglet possibly by enhancing the synthesis of NO, proline and polyamines in animals (Wu et al., 2004; Kim et al., 2004). However, it remains unclear whether L-arginine supplementation has other effects besides these metabolic pathways and higher dosage of L-arginine may lead to beneficial effect or detrimental effect.

In an attempt to unveil the underlying mechanisms of L-arginine supplementation and young piglet growth, we carried out this study with wider range of dosages and started during early neonatal period. We found that

dietary arginine supplementation can prevent intestinal atrophy in early-weaned pigs, in addition to the impact on nitrogen metabolism as reported previously. The dosage-dependent data suggested that higher arginine supplementation has more substantial effect on some but not all aspects of analysis, which may be of potential interest in applying such practice in livestock industry of China and the world.

## MATERIALS AND METHODS

Experimental procedures in this study were approved by Animal Experimental Committee of Guangdong Institute of Animal Science. 36, 4-day-old healthy male neonatal piglets (Duroc, Landrace x Largininee White) were randomly assigned to three treatment groups, with four replicates of three piglets each. Every replicate of piglets were housed in a pen in an air-conditioned room with ambient temperature of 32 ± 1°C and constant humidity. The basal milk-powder diets were formulated to meet required NRC 1998 minimal levels for 3 to 5 kg piglets (Table 1). Levels of exogenous L-arginine supplemented in diets of the three treatments were set at 0 (control group), 0.4 and 0.8% (on the basis of milk replacer powder). Appropriate amounts of alanine were added to formulate isonitrogenous diets. The diets were mixed with water at a ratio of 1:4 freshly before feeding, and provided to the piglets every 3 h from 06:00 a.m and 12:00 p.m. Piglets had easy access to water supplied by the semiautomatic device. Experimental piglets were observed to become accustomed to the feeding method quickly and consumed each meal with no spillage. Feed intake (as-fed basis) was calculated in accordance with the weight difference in feed trough before and after feeding. Body weights (BW) of all piglets were individually measured on day 1, 11 and 18 of the experiment. Average daily gain (ADG), average daily feed intake (ADFI) and feed : gain (F : G) ratio were calculated.

## Sample collection

Blood samples of all piglets were obtained from anterior vena cava and both serum and plasma were separately kept for further determination on day 11 and 18 of the experiment. Respectively, on day 11 and 18 of the experiment, one pig out of each experimental replicate was randomly selected and slaughtered after intraperitoneal injection of sodium pentobarbital (50 mg/kg BW). The small intestine from the pyloric sphincter to the ileocolonic junction was rapidly removed by cutting along the mesenteric border on an ice-cold metal plate and divided into three segments. The segment of small intestine proximal to the ligament of Treitz was designated as the duodenum, with the stomach being removed. The remainder of the small intestine was divided into 2 equal portions; the proximal half was designated the jejunum and the distal half, the ileum (Burrin et al., 2000). From the midline of each region, a 10 cm piece was slit along its length and mucosa was removed by gentle scraping with a glass slide. These samples were rapidly frozen in liquid nitrogen for analysis of the activities of disaccharidases (maltase, lactase and sucrase). On day 19 of the experiment, a D-xylose test was conducted on the remaining piglets. D-Xylose was orally administered to the piglets at dose of 1 ml/kg BW, blood samples were collected from anterior vena cava 2 h later for the determination of D-xylose.

## Analysis procedures

Blood hormones (insulin, IGF-1, T<sub>3</sub> and T<sub>4</sub>) were measured through

**Table 2.** Effect of dietary L-arginine supplementation on growth performance of piglets\*.

Item	Control	0.4% Arginine	0.8% Arginine
<b>Body weight (kg)</b>			
Day 1	2.07±0.01	1.99±0.07	2.08±0.08
Day 11	3.28±0.12	3.36±0.04	3.37±0.03
Day 18	5.54±0.32	5.79±0.09	5.76±0.19
<b>Daily weight gain (kg/d)</b>			
Days 1 to 11	0.12±0.01	0.14±0.01	0.13±0.01
Days 12 to 18	0.32±0.02	0.34±0.01	0.34±0.11
Days 1 to 18	0.22±0.01	0.24±0.01	0.24±0.04
<b>Daily feed intake (kg/d)</b>			
Days 1 to 11	0.12±0.01	0.12±0.01	0.12±0.01
Days 12 to 18	0.26±0.01	0.26±0.02	0.27±0.04
Days 1 to 18	0.19±0.01	0.19±0.01	0.19±0.01
<b>Feed : gain ratio</b>			
Days 1 to 11	0.99±0.12	0.86±0.03	0.91±0.02
Days 12 to 18	0.82±0.05	0.75±0.05	0.79±0.02
Days 1 to 18	0.86±0.04	0.78±0.04	0.82±0.01

\*Data are means ± SD (n = 4). Values in a row with different superscripts differ ( $P < 0.05$ ).

Radio-immuno method using reagent kits (Tianjin JiuDing Bioengineering Co. Ltd). Blood urea nitrogen (BUN) was determined by automatic biochemistry analyzer (Beckman Instruments, Fullerton, CA) at 340 nm using the assay kits from Beckman Coulter Inc (Fullerton, CA). Plasma concentrations of arginine, glutamate, proline, glutamine, citrulline, ornithine, lysine, threonine, valine, histidine and alanine were analyzed by amino-acid autoanalyzer (L-8900, Hitachi). Nitric oxide synthetase (NOS), NO, disaccharidases and serum D-xylose were determined using colorimetric methods with a spectrophotometer (Biomate 5, Thermo Electron Corporation, Rochester, NY). The Assays were conducted using the assay kits purchased from Nanjing Jiancheng Institute of Bioengineering (Nanjing, Jiangsu, China) and the procedures were followed accordingly. Activities of argininosuccinate synthetase (ASS), pyrroline-5-carboxylate synthetase (P5CS) and spermine were determined using swine enzyme-linked immunosorbent assay kits (Adlitteram Diagnostic Laboratories, USA).

### Small intestinal morphology

Two centimeters segment of the jejunum was collected immediately after slaughter and processed as previously described (Jensen et al., 2001). In brief, the samples were fixed with 10% neutral buffered formalin for 24 h, and trimmed to prepare paraffin-embedded block for histological slides. Three cross sections (5 µm thick) of each intestinal segment were stained with hematoxylin and eosin following standard protocol. The 10 straightest villus and their associated crypts from each segment were quantified. The villus height was measured from the tip to the base, and then the crypt depth was measured from the base of the villus to the base of the crypt. The villus area was calculated according to the published method (Frankel et al., 1993).

### Statistical analysis

Data were presented as means ± SD. Statistical analyses were performed using the general linear model procedures of SPSS (version 11.5, SPSS Institute). Statistical comparisons were done by ANOVA followed by the post-hoc Newman-Keuls multiple range test. Difference was considered significant when  $P$  value  $< 0.05$ .

## RESULTS

### Growth performance

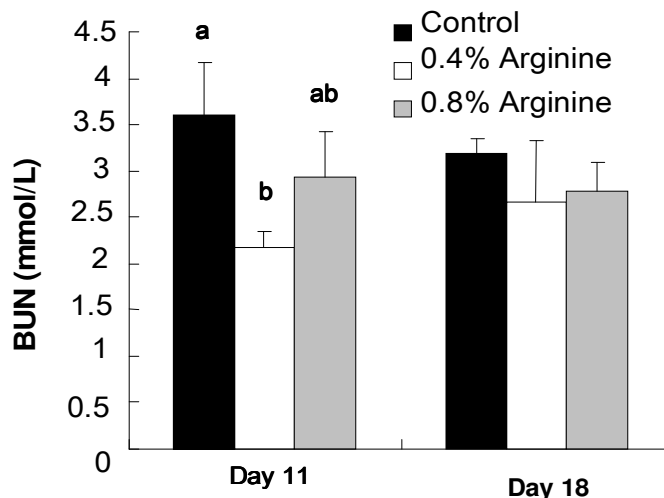
The effect of dietary L-arginine supplementation on growth performance is summarized in Table 2. When compared with the control group, arginine supplementation did not improve BW, ADG and ADFI, but slightly decreased F : G ratio of the piglets throughout the experiment ( $P > 0.05$ ).

### Blood urea nitrogen (BUN)

BUN levels of the piglets of 0.4 and 0.8% arginine groups were 39.4 ( $P < 0.05$ ) and 18.3% ( $P < 0.05$ ), both lower than that of the control group on day 11 (Figure 1). No obvious difference was observed among BUN contents of the three groups at day 18 (Figure 1).

### Blood hormones

Blood  $T_3$  levels of piglets obviously increased with



**Figure 1.** Effect of dietary arginine supplementation on serum urea nitrogen levels of piglets. Bars represent the means  $\pm$  SD. Within an experimental phase, means without a common letter differ ( $P < 0.05$ ).

**Table 3.** Effect of dietary L-arginine supplementation on serum hormone levels of piglets\*.

Item	Control	0.4% Arginine	0.8% Arginine
<b>T<sub>3</sub> (ng/ml)</b>			
Day 11	0.43 $\pm$ 0.02 <sup>a</sup>	0.81 $\pm$ 0.06 <sup>b</sup>	0.90 $\pm$ 0.13 <sup>b</sup>
Day 18	0.64 $\pm$ 0.08 <sup>a</sup>	0.97 $\pm$ 0.05 <sup>b</sup>	0.87 $\pm$ 0.02 <sup>b</sup>
<b>T<sub>4</sub> (ng/ml)</b>			
Day 11	55.03 $\pm$ 2.23	53.95 $\pm$ 2.84	64.16 $\pm$ 3.91
Day 18	50.44 $\pm$ 3.57	44.35 $\pm$ 3.60	49.78 $\pm$ 2.76
<b>IGF-1 (ng/ml)</b>			
Day 11	8723.2 $\pm$ 1254.1	9511.6 $\pm$ 2703.1	9494.8 $\pm$ 957.3
Day 18	12858.4 $\pm$ 548.8	14166.6 $\pm$ 752.0	13158.9 $\pm$ 593.8
<b>Insulin</b>			
Day 11	8.46 $\pm$ 1.46 <sup>a</sup>	21.24 $\pm$ 3.28 <sup>ab</sup>	25.79 $\pm$ 7.04 <sup>b</sup>
Day 18	6.93 $\pm$ 0.43	7.51 $\pm$ 0.76	6.73 $\pm$ 1.53

\*Data are means  $\pm$  SD (n = 4). Values in a row with different superscripts differ ( $P < 0.05$ ).

arginine supplementation in contrast to those of control piglets on both days 11 and 18 ( $P < 0.05$ ; Table 3). No significant difference in blood T<sub>4</sub> or IGF-1 content was seen among groups at either experimental phase, although, arginine supplementation did tend to increase blood IGF-1 at both experimental periods. 0.8% arginine group possessed prominently higher level of blood insulin than the control on day 11 ( $P < 0.05$ ).

### Small intestinal morphology

Jejunal villus height ( $P < 0.05$ ) and villus areal ( $P < 0.05$ ) of

piglets were significantly higher in 0.8% arginine group than the control group on day 11. Jejunal crypt depth was notably higher in 0.4% arginine group than the other two groups on day 18 ( $P < 0.05$ , Table 4).

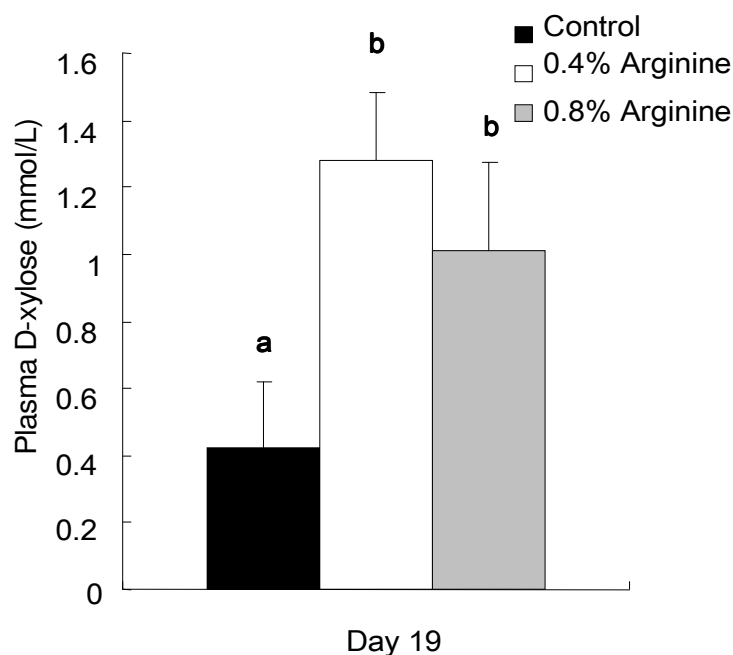
### D-xylose absorption

Two hours after oral gavage of D-xylose, contents of plasma D-xylose of 0.4 and 0.8% arginine groups increased by 200 ( $P < 0.05$ ) and 140% ( $P < 0.05$ ), respectively than the control group (Figure 2).

**Table 4.** Effect of dietary L-arginine supplementation on jejunum histomorphology of piglets\*.

Item	control	0.4% arginine	0.8% arginine
<b>Villus height (<math>\mu\text{m}</math>)</b>			
Day 11	364.9 $\pm$ 19.3 <sup>a</sup>	414.1 $\pm$ 21.6 <sup>ab</sup>	464.7 $\pm$ 11.6 <sup>b</sup>
Day 18	452.7 $\pm$ 20.3	501.0 $\pm$ 33.3	479.2 $\pm$ 41.5
<b>Crypt depth (<math>\mu\text{m}</math>)</b>			
Day 11	160.9 $\pm$ 16.8	181.9 $\pm$ 8.5	184.5 $\pm$ 3.0
Day 18	133.6 $\pm$ 4.6 <sup>a</sup>	181.4 $\pm$ 6.3 <sup>b</sup>	154.0 $\pm$ 9.7 <sup>a</sup>
<b>Villus areal (<math>\mu\text{m}^2</math>)</b>			
Day 11	138702 $\pm$ 7244 <sup>a</sup>	180169 $\pm$ 14027 <sup>ab</sup>	205545 $\pm$ 15023 <sup>b</sup>
Day 18	184193 $\pm$ 12304	187220 $\pm$ 11190	167949 $\pm$ 8587

\*Data are means  $\pm$  SD (n = 4). Values in a row with different superscripts differ ( $P < 0.05$ ).



**Figure 2.** Effect of dietary arginine supplementation on intestinal permeability of piglets. Bars represent the means  $\pm$  SD. Within an experimental phase, means without a common letter differ ( $P < 0.05$ ).

### Levels of jejunal disaccharidases

In contrast to the control and 0.4% arginine groups, the activity of maltases in jejunal mucosa was notably elevated ( $P < 0.05$ ) in 0.8% arginine treatment on day 18 (Table 5). Both 0.4 ( $P < 0.05$ ) and 0.8% ( $P < 0.05$ ) arginine groups showed at least 2-fold higher contents of lactase in jejunal mucosa than the control group on day 11. Strikingly, the enhancing effect of arginine decreased on day 18, although, there was still significant effect between two arginine supplementation groups and the control group. Additionally, 0.8% arginine group also showed

significant increase as compared to 0.4% arginine group. Concentrations of jejunal mucosa sucrase did not differ among the three groups on day 11 as well as day 18. Sucrase level significantly increased from 0.88 on day 11 to 3.55 on day 18 in the control group, while this change was not observed in arginine group with almost the same level of sucrase from day 11 to 18.

### Concentration of plasma amino acids

In comparison with the control group, 0.4 and 0.8%

**Table 5.** Effect of dietary L-arginine supplementation on disaccharidases activities in jejunal mucosa of piglets (U/mg\_prot)\*.

Item	Control	0.4% Arginine	0.8% Arginine
<b>Maltases</b>			
d 11	5.21±2.14	4.81±1.25	7.50±2.27
d 18	9.77±0.75 <sup>a</sup>	9.78±1.29 <sup>a</sup>	13.36±1.29 <sup>b</sup>
<b>Lactase</b>			
d 11	5.26±1.49 <sup>a</sup>	22.67±7.02 <sup>b</sup>	20.20±4.97 <sup>b</sup>
d 18	3.41±1.12 <sup>a</sup>	4.66±0.84 <sup>a</sup>	6.94±1.2 <sup>b</sup>
<b>Sucrase</b>			
d 11	0.88±0.38	3.70±1.52	4.43±1.56
d 18	3.55±0.19	2.49±0.74	2.42±0.83

\* Data are means ± SD (n = 4). Values in a row with different superscripts differ ( $P < 0.05$ ).

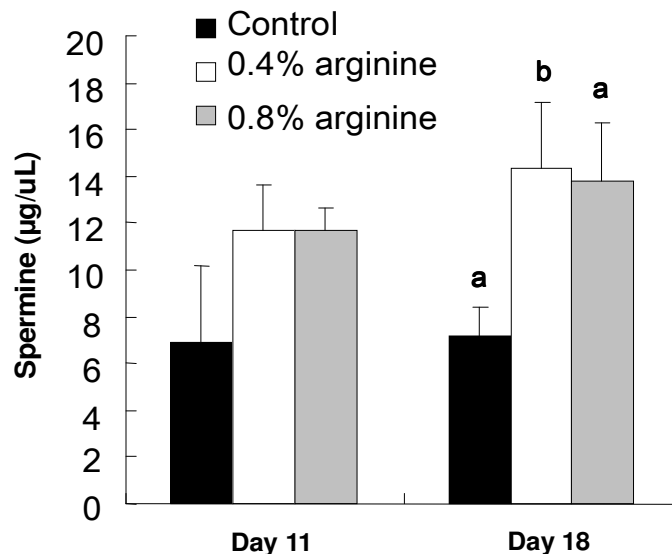
**Table 6.** Effect of dietary L-arginine supplementation on concentrations of plasma amino acid in piglets\*.

Item	Control	0.4% Arginine	0.8% Arginine
<b>Arginine</b>			
Day 11	57.44±5.06 <sup>a</sup>	89.08±6.96 <sup>b</sup>	92.17±10.05 <sup>b</sup>
Day 18	72.75±4.66 <sup>a</sup>	129.00±21.59 <sup>b</sup>	137.67±11.48 <sup>b</sup>
<b>Aspartate</b>			
Day 11	21.75±1.11	25.75±5.12	29.75±5.54
Day 18	18.50±2.66 <sup>a</sup>	24.75±2.7 <sup>ab</sup>	30.50±2.25 <sup>b</sup>
<b>Citrulline</b>			
Day 11	76.25±8.64 <sup>a</sup>	115.25±8.14 <sup>b</sup>	77.75±8.19 <sup>a</sup>
Day 18	95.25±7.43	99.25±6.93	91.00±18.87
<b>Glutamate</b>			
Day 11	163.00±13.68 <sup>a</sup>	305.00±40.14 <sup>b</sup>	300.75±28.25 <sup>b</sup>
Day 18	247.00±49.79	306.75±46.96	350.50±32.72
<b>Ornithine</b>			
Day 11	54.25±7.73	52.50±4.73	58.00±8.75
Day 18	56.75±6.34 <sup>a</sup>	95.75±14.94 <sup>b</sup>	111.00±11.1 <sup>b</sup>
<b>Proline</b>			
Day 11	55.25±2.32	43.00±2.27	51.75±13.36
Day 18	38.25±5.91 <sup>a</sup>	72.50±14.23 <sup>b</sup>	78.50±3.20 <sup>b</sup>

\*Data are means ± SD (n = 4). Values in a row with different superscripts differ ( $P < 0.05$ ).

arginine addition improved plasma concentrations of arginine respectively, by 29.53 ( $P < 0.05$ ) and 59.84% ( $P < 0.05$ ) on day 11, and 77.32 ( $P < 0.05$ ) and 89.24% ( $P < 0.05$ ) on day 18 (Table 6). Plasma citrulline level was higher in 0.4% arginine group than in the other two on day 11 ( $P < 0.05$ ), while it reduced to the similar level of the other two groups on day 18, suggesting some change

of citrulline metabolism at early stage. Contents of plasma ornithine ( $P < 0.05$ ) were notably raised by both 0.4 and 0.8% arginine supplementation than the control treatment only on day 18, but not on day 11. In comparison with the control group, both 0.4 and 0.8% arginine increased the level of plasma glutamate ( $P < 0.05$ ) on day 11, and those of plasma ornithine



**Figure 3.** Effect of dietary L-arginine supplementation on spermine concentrations in jejunal mucosa of piglets. Bars represent the means  $\pm$  SD. Within an experimental phase, means without a common letter differ ( $P < 0.05$ ).

**Table 7.** Effect of dietary L-arginine supplementation on concentration of NO in small intestinal mucosa of piglets (U/mgprot)\*.

Item	Control	0.4% Arginine	0.8% Arginine
<b>Day 11</b>			
Duodenum	0.36 $\pm$ 0.02 <sup>b</sup>	0.60 $\pm$ 0.11 <sup>a</sup>	0.34 $\pm$ 0.05 <sup>b</sup>
Jejunum	0.37 $\pm$ 0.04	0.31 $\pm$ 0.04	0.30 $\pm$ 0.03
Ileum	0.40 $\pm$ 0.06	0.45 $\pm$ 0.07	0.29 $\pm$ 0.04
<b>Day 18</b>			
Duodenum	0.26 $\pm$ 0.05	0.46 $\pm$ 0.21	0.52 $\pm$ 0.25
Jejunum	0.19 $\pm$ 0.03	0.22 $\pm$ 0.02	0.26 $\pm$ 0.07
Ileum	0.25 $\pm$ 0.02	0.22 $\pm$ 0.03	0.21 $\pm$ 0.03

\*Data are means  $\pm$  SD (n = 4). Values in a row with different superscripts differ ( $P < 0.05$ ).

( $P < 0.05$ ) and proline ( $P < 0.05$ ) on day 18.

### Contents of spermine in jejunal mucosa

As revealed in Figure 3, no noteworthy difference in jejunal mucosa spermine contents was observed among the three groups on day 11. As compared with the control and 0.8% arginine groups, concentration of spermine in jejunal mucosa of piglets was raised by 0.4% arginine supplementation at day 18 ( $P < 0.05$ ).

### Concentration of NO in small intestinal mucosa

The level of NO in the duodenal mucosa of piglets in

0.4% arginine group was higher than the other two on day 11 ( $P < 0.05$ ). Contents of duodenal NO in 0.4 and 0.8% arginine groups was respectively, 81.25 ( $P > 0.05$ ) and 101.95% ( $P > 0.05$ ) higher than the control group on day 18. No obvious difference in NO concentration was observed in jejunum or ileum at either experimental phase (Table 7).

### Activities of NOS, ASS and P5CS in jejunal mucosa

0.8 and 0.4% arginine treatment prominently increased jejunal NOS level ( $P < 0.05$ ) against the control group on day 18 (Table 8), but no difference was observed on day 11 among three groups. In comparison with the control group, 0.4% arginine supplementation elevated jejunal

**Table 8.** Effect of dietary L-arginine supplementation on enzyme activities in jejunal mucosa of piglets\*.

Item	Control	0.4% Arginine	0.8% Arginine
<b>NOS (U/mgprot)</b>			
Day 11	0.33±0.02	0.33±0.02	0.32±0.01
Day 18	0.28±0.01 <sup>a</sup>	0.38±0.04 <sup>ab</sup>	0.44±0.05 <sup>b</sup>
<b>ASS (mg/ml)</b>			
Day 11	8.19±1.53 <sup>a</sup>	20.43±0.85 <sup>b</sup>	17.24±5.33 <sup>ab</sup>
Day 18	5.05±0.83	6.16±0.48	8.32±2.18
<b>P5CS (mg/ml)</b>			
Day 11	330.10±13.15	325.57±6.48	396.52±43.41
Day 18	395.39±20.42 <sup>a</sup>	462.53±12.26 <sup>b</sup>	402.00±24.36 <sup>ab</sup>

\* Data are means ± SD (n = 4). Values in a row with different superscripts differ ( $P < 0.05$ ). NOS, Nitric oxide synthetase; ASS, argininosuccinate synthetase; P5CS, pyrroline-5-carboxylate synthetase.

ASS activity on day 11 ( $P < 0.05$ ), while 0.8% arginine group showed no obvious difference from the other two groups. However, the effects of arginine supplementation seemed to be dampened on day 18 as similar level of ASS was found in three groups. Jejunal P5CS activity was improved by 0.4% arginine treatment on day 18 ( $P < 0.05$ ) against the control group, while 0.8% arginine treatment had no significant effect at either stage.

## DISCUSSION

In this study, we systematically analyzed the effects of arginine supplementation on growth performance of young piglets, intestine morphology, enzymes related with arginine metabolism and some parameters related to amino acid metabolism. Our result demonstrates that dietary arginine supplementation did not significantly improve BW, ADG, ADFI and feed conversion rate of neonatal piglets, which is contradictory to previous studies showing that dietary arginine supplementation, improved feed conversion rate, daily gain and BW of the weaned or early-weaned piglets (Southern and Baker, 1983). This discrepancy may be due to the different dose of arginine supplementation and the time course of piglet growth, in additional to different species of piglet studied. However, we did observe that blood urea nitrogen of both arginine groups were diminished through the experiment (Figure 1) on day 11, indicating the improvement of protein utilization efficiency *in vivo* at early stage (Sherry et al., 1978). Arginine was reported to be effective in stimulating the secretion of insulin and growth hormone (Flynn et al., 2000; Wu and Morris, 1998; Yao et al., 2008). In this study, arginine treatment tended to increase blood IGF-1 at both experimental periods (no significance,  $P > 0.05$ ), and 0.8% arginine administration significantly raised blood insulin on day 11. The results also reveal that blood  $T_3$  levels on both days 11 and 18

were increased by arginine (Table 3). These findings indicate that hormone secretion might partly contribute to the enhancing tissue protein synthesis and the growth-promoting effect of arginine supplementation.

In early infancy, the small intestine undergoes a period of rapid growth and development associated with multiple alterations in intestinal structure and function (Butzner and Gall, 1990). Reduction of villus height was reported to retard the growth of weaned piglets (Cera et al., 1988). Our work reveal that jejunal villus height and villus area of piglets were improved by 0.8% arginine supplementation on day 11, and jejunal crypt depth was notably greater in 0.4% arginine group on day 18 than the control group, which were consistent with previous findings that 0.7% dietary L-arginine supplementation enhanced intestinal villus height on day 6 and 10 of experiment (Zhan et al., 2008).

Absorption rate of D-xylose in both arginine groups were markedly higher than control group by the end of the experiment (Figure 2), indicating that arginine supplementation improved the intestinal capability in both digestion and absorption in neonatal piglets. Moreover, in contrast with the control group, both 0.4 and 0.8% arginine addition improved lactase level in jejunal mucosa on day 11, and 0.8% arginine supplementation elevated the activities of maltase and lactase in jejunal mucosa on day 18 (Table 5). Nevertheless, concentrations of small jejunum mucosa sucrase did not differ among the three groups on day 11 as well on day 18; it may change the pattern of sucrase level from day 11 to 18 period. These results show that the absorption efficiency of macromolecules and lactose of the intestinal mucosa was largely enhanced by arginine supplementation. As Shulman et al. (2005) reported that changes in lactose absorption related primarily to lactase activity, previous work in miniature pigs also suggested that disaccharidase-specific activity together with changes in small-intestinal mucosal growth contributed to disaccharide



digestibility (Redel et al., 1997). Our results indicate that arginine contributed to the maturational change in disaccharides digestion and absorption by improving the activities of lactase and maltase in the intestine.

Arginine family amino acids, including arginine, glutamine, glutamate, proline, aspartate, asparagine, ornithine and citrulline, were reported to be interconvertible via complex interorgan metabolism in most mammals, including pigs (Wu et al., 2007). The small intestine is an essential organ for the inter-conversion of arginine family in pigs (Wu, 1997). Ornithine and citrulline were two key amino acids involved in the endogenous synthesis of arginine in the enterocyte. Citrulline and arginine, which were derived from glutamine, glutamate and proline in the small intestine of pigs, could be utilized for arginine synthesis by extrahepatic tissues and cells (Wu et al., 1995, 2007). Plasma arginine concentration was believed to be the most sensitive indicator of *in vivo* arginine status in neonates, including piglets (Batshaw et al., 1984; Johnson et al., 1972). In a research conducted in female mice, deficiency of dietary arginine reduced the concentration of plasma arginine, citrulline and ornithine (Cremades et al., 2004). On the contrary, it has been reported that 0.48% dietary arginine supplementation significantly increased plasma arginine of the 28-day-old weaning piglets (Southern et al., 1983). Our study demonstrates that plasma content of arginine was dose-dependently elevated by arginine treatment on both days 11 and 18 of the experiment (Table 6), which was consistent with the findings of Kim et al. (2004) and Wilkinson et al. (2004).

Arginine, as well as citrulline, which were synthesized from glutamine, glutamate and proline in enterocytes (Wu and Knabe, 1994; Wu et al., 1995), could be utilized for arginine synthesis by extrahepatic tissues and cells (Wu et al., 1995, 2007). Like the arginine, plasma content of citrulline was elevated by arginine treatment on day 11, indicating the possible improvement of *de novo* arginine synthesis (Wu and Knabe, 1995). ASS was found to be a key enzyme which converted citrulline to arginine (Morris, 2004). Our work reveals that 0.4% arginine supplementation elevated jejunal ASS activity on day 11 as well as P5CS level on day 18 against the control group, which was consistent with the changing trend of plasma citrulline of the piglets.

As an important precursor of arginine synthesis in the enterocyte of neonatal piglets (Wu and Knabe, 1994; Wilkinson et al., 2004; Murphy et al., 1996), plasma glutamate was concordantly raised in the arginine groups on day 11, meanwhile, plasma ornithine, proline and aspartate levels were dose-dependently raised by arginine on day 18, which was consistent with the previous findings (Southern et al., 1983). Taken together, arginine supplementation improved the absorption of arginine, as well as the endogenous synthesis of arginine in the neonatal pigs. In addition, the longer period of arginine treatment (18 day) offered, the better availability of arginine was

observed.

Apart from being an important precursor of arginine synthesis, ornithine also played a key role in synthesizing polyamines. As widely distributed organic cations, polyamines were involved in macromolecular synthesis as well as cell proliferation and differentiation in mammalian cell systems (Hoshiai et al., 1981; Morrison and Seidel, 1995; Wu et al., 2000; Teixeira et al., 2002). Polyamines were considered essential for endothelial cell proliferation (Morrison and Seidel, 1995). Spermine is a polyamine involved in cellular metabolism found in all eukaryotic cells; it plays a significant role in cell proliferation and differentiation, especially in mucosal epithelial cells of the small intestine (Tapiero and Mathé, 2002). This study illustrates that jejunal spermine concentration was raised by 0.4% arginine treatment on day 18 in contrast to the other two groups (Figure 3), which was believed to further improve the structural development and function of the intestine (Hampson and Kidder, 1986).

NO is a major endothelium-derived relaxing factor that plays an important role in regulating and maintaining vascular function (Tangphaoa et al., 1999). As a lipophilic molecule, NO diffuses easily into adjacent smooth muscle cells and activates soluble guanylate cyclase signaling pathway, which lead to vasodilation (Furchgott and Zawadzki, 1980; Stuehr, 2004). It has been reported that physiological variations of plasma arginine could influence endothelial NO production, thereby modifying vascular tone and platelet function (Tangphaoa et al., 1999). Arginine was catalyzed by NOS to form endothelial NO in the small intestine (Moncada and Higgs, 1993; Berkowitz et al., 2003). In our study, 0.4% arginine supplementation improved the level of NO in the duodenal mucosa of piglets on day 11, and both 0.4 and 0.8% arginine addition tended to improve duodenal NO content on day 18. These findings were in line with those of Clarkson et al. (1996), Kharitonov et al. (1995) and Urschel et al. (2007). Likewise, the content of jejunal NOS was obviously increased by 0.8% arginine supplementation on day 18 (Table 8).

In conclusion, our study demonstrates that dietary arginine supplementation was capable of improving protein synthesis and intestinal development, stimulating enzymes synthesis of intestinal mucosa, thereby promoting growth performance of the piglets. In all factors considered, the 0.8% arginine administration exhibited a slightly better effect in promoting the growth of piglets as compared to 0.4% arginine group, which has already showed many beneficial effects.

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