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Dietary L-arginine Supplementation Improves Intestinal Function in Weaned Pigs after an *Escherichia coli* Lipopolysaccharide Challenge

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ABSTRACT : This study was conducted to determine whether L-arginine (Arg) supplementation could improve intestinal function in weaned pigs after an *Escherichia coli* lipopolysaccharide (LPS) challenge. Treatments included: i) non-challenged control (CONTR, pigs fed a control diet and injected with sterile saline); ii) LPS-challenged control (LPS, pigs fed the same control diet and challenged by injection with *Escherichia coli* LPS); iii) LPS+0.5% Arg (pigs fed a 0.5% Arg diet and challenged with LPS); and iv) LPS+1.0% Arg (pigs fed a 1.0% Arg diet and challenged with LPS). On d 16, pigs were administrated with LPS or sterile saline. D-xylose was orally administrated at 2 h following LPS challenge, and blood samples were collected at 3 h following LPS challenge. At 6 h post-challenge, pigs were sacrificed and intestinal mucosa samples were collected. Supplementation of Arg attenuated LPS-induced damage in gut digestive and barrier functions, as indicated by an increase in ileal lactase activity, and duodenal and ileal diamine oxidase activities ($p<0.05$). Arg administration also prevented the increase of jejunal malondialdehyde content and the decrease of ileal superoxide dismutase activity by LPS challenge ($p<0.05$). Furthermore, the jejunal nitric oxide level and inducible nitric oxide synthase activity were also improved after Arg supplementation ($p<0.05$). These results indicate that Arg supplementation has beneficial effects in alleviating the impairment of gut function induced by LPS challenge. (**Key Words** : Arginine, Lipopolysaccharide, Weaned Pigs, Intestinal Function)

INTRODUCTION

In pig production, numerous factors such as weaning, infection and stress can lead to intestinal mucosal disruption (Liu et al., 2008a; Wang et al., 2008a, b), which may result in diarrhea and growth retardation of pigs. Therefore, nutritional modulation of the injured gut is very important to the compromised pigs.

L-arginine (Arg), a dibasic amino acid, is traditionally thought of as a nutritionally nonessential amino acid. However, in the last 20 years, Arg has attracted extensive attention because it has been shown to play an important role in various physiological and biological processes (Wu et al., 2004; Wu et al., 2007a, b). Of particular interest, Arg is the substrate for the synthesis of nitric oxide (NO), a key mediator of gut physiological function (Wang et al., 2009), immune response (Han et al., 2008) and neurological function (Orlando et al., 2008). Numerous experiments have shown that Arg is effective in a variety of intestinal injury models (Sukhotnik et al., 2004, 2005; Fotiadis et al., 2007; Spanos et al., 2007; Corl et al., 2008; Liu et al., 2008a; Zhan et al., 2008; Wang et al., 2009). Our previous study showed that dietary supplementation of 0.7% Arg affected microvascular development of the small intestine and improved intestinal morphology of early-weaned pigs (Zhan et al., 2008). At the same time, dietary Arg supplementation alleviated intestinal mucosal disruption induced by *Escherichia coli* lipopolysaccharide (LPS) in weaned pigs, as indicated by ameliorated mucosal morphology, increased cell proliferation and decreased cell apoptosis (Liu et al., 2008a). However, most of these researches in pigs were focused on intestinal structure rather than intestinal function.

In the present experiment, we use a well documented model to induce intestinal injury in weaned pigs by administering *Escherichia coli* LPS (Mercer et al., 1996). Our aim was to investigate whether dietary Arg supplementation could mitigate the impairment of intestinal function and to examine the mechanism(s) of action of Arg in weaned pigs.

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MATERIALS AND METHODS

Animal care and diets

This experiment was approved by the Animal Care and Use Committee of Hubei Province. A total of 72 crossbred pigs (Duroc×Large White×Landrace) weaned at 21±1 d of age (average body weight 5.78±0.26 kg), were randomly allotted into four treatments by body weight. Pigs were housed in 2.50×1.80 m^2 nursery pens equipped with plastics floor. Each treatment was replicated using six pens (three pens of females and three pens of males) with three pigs per pen. Each pen contained a self-feeder and a nipple waterer to provide *ad libitum* access to feed and water. The basal diet (Table 1) was formulated to meet NRC (1998) requirements for all nutrients. The diets were offered in pelleted form. The analysis of crude protein, calcium and phosphorus of diets was conducted according to the procedures of the AOAC (1990). The temperature in the room was maintained at 25 to 27°C by air conditioning.

Experimental design

Four treatments were as follows: i) non-challenged control (CONTR). The pigs were fed a basal diet and injected with sterile saline; ii) LPS-challenged control (LPS). The pigs were fed the same basal diet and challenged with *Escherichia coli* LPS; iii) LPS+0.5% Arg treatment. The pigs were fed a 0.5% Arg diet and challenged with LPS; and iv) LPS+1.0% Arg treatment. The pigs were fed an 1.0% Arg diet and challenged with LPS. Our previous study demonstrated that 0.5% and 1.0% Arg supplementation attenuated weight loss induced by *Escherichia coli* LPS challenge in weaned pigs. Thus, the doses of 0.5% and 1.0% Arg were chose in the current study. To obtain isonitrogenous diets, we supplemented 1.72%, 0.86% and 0% glycine (purity >99%; Ajinomoto, Japan) to the control, 0.5% Arg and 1.0% Arg diets, respectively. At 08.00 h of d 16, pigs were administrated intraperitoneally with *Escherichia coli* LPS at 100 μg/kg body weight or an equivalent amount of sterile saline. At 10.00 h (2 h post-challenge), one pig per pen was given d-xylose solution at a dose of 500 mg/kg BW via oral gavage. At 11.00 h (3 h post-challenge), the blood samples were collected. At 14.00 h (6 h post-challenge), one pig per pen was killed. The same pigs were used for the d-xylose test and slaughtering. The LPS (*Escherichia coli* serotype 055: B5, Sigma Chemical Inc., St. Louis, MO, USA) was dissolved in sterile 0.9% NaCl solution (500 mg LPS/litre saline). The d-xylose (Sigma Chemical Inc., St. Louis, MO, USA) solution was prepared as a 10% solution in deionized water. To avoid the potential effects of LPS-induced food intake reduction on gastrointestinal characteristics, the pigs selected for the d-xylose test and slaughtering were individually transferred to an adjacent cage at 20.00 h of d 15, and were fasted until slaughter.

Table 1. Ingredient composition of the basal diet (as-fed basis)

Item	%
Ingredient	
Maize	56.40
Soyabean meal, dehulled (46.5% CP)	22.40
Wheat middling	5.00
Fish meal	3.60
Soy protein concentrate	1.40
Fat powder[1]	2.00
Whey powder	3.00
Glycine[2]	1.72
Cornstarch[2]	0.28
Acidifier[3]	0.20
Dicalcium phosphate	1.22
Limestone	0.94
NaCl	0.34
L-lysine·HCl (78.8% lysine)	0.27
DL-methionine (99% threonine)	0.10
L-threonine (98% threonine)	0.08
Butylated hydroquinone	0.05
Vitamin and mineral premix[4]	1.00
Nutrient composition	
Digestible energy[5, 6] (MJ/kg)	13.60
Crude protein[7]	20.30
Calcium[7]	0.80
Total phosphorus[7]	0.70
Total lysine[6]	1.28
Total methionine+cysteine[6]	0.65
Total arginine[6]	1.28

[1] A rumen-stable fat powder, purchased from Berg+Schmidt, German.
[2] In the 0.5% Arg diet, 1.72% glycine and 0.28% cornstarch were replaced by 0.5% Arg, 0.86% glycine and 0.64% cornstarch. In the 1.0% Arg diet, 1.72% glycine and 0.28% cornstarch were replaced by 1.0% Arg and 1.0% cornstarch. All diets were isonitrogenous.
[3] A compound acidifier including lactic acid and phosphoric acid, provided by Wuhan Fanhua Biotechnology Company, Wuhan, China.
[4] The vitamin and mineral premix (defatted rice bran as carrier) provided the following amounts per kilogram of complete diet: retinol acetate, 2,700 μg; cholecalciferol, 62.5 μg; dl-α-tocopheryl acetate, 20 mg; menadione, 3 mg; vitamin B_{12}, 18 μg; riboflavin, 4 mg; niacin, 40 mg; pantothenic acid, 15 mg; choline chloride, 400 mg; folic acid, 700 μg; thiamin, 1.5 mg; pyridoxine, 3 mg; biotin, 100 μg; Zn, 80 mg ($ZnSO_4 \cdot 7H_2O$); Mn, 20 mg ($MnSO_4 \cdot 5H_2O$); Fe, 83 mg ($FeSO_4 \cdot H_2O$); Cu, 25 mg ($CuSO_4 \cdot 5H_2O$); I, 0.48 mg (KI); Se, 0.36 mg ($Na_2SeO_3 \cdot 5H_2O$).
[5] Based on diets containing cornstarch.
[6] Calculated. [7] Analyzed.

Sample collection

At 3 h after LPS or saline injection, blood samples from the pigs given d-xylose solution were collected into heparinized vacuum tubes (Becton Dickinson vacutainer Systems, Franklin Lakes, NJ) and centrifuged (3,500×g for

10 min) to separate plasma. Plasma from each pig was stored at -80°C until analysis of d-xylose content.

At 6 h after LPS or saline injection, one pig per pen (six pigs per treatment) was humanely killed by an intramuscular injection of sodium pentobarbital solution (40 mg/kg BW). The small intestine was dissected free of the mesenteric attachment and placed on a chilled stainless steel tray. The 10-cm segments at 25, 50, and 75% of the total intestinal length were removed from the intestine to represent samples for duodenum, jejunum and ileum, respectively. The segments were opened lengthways and washed with ice-cold PBS. The mucosa was scraped off, immediately frozen in liquid nitrogen and then stored at -80°C.

The mucosa samples were thawed, weighed and suspended in ice-cold NaCl solution (10:1, vol/wt). The mucosa was homogenized with a tissue homogenizer for 45 seconds, followed by centrifuging at 3,000 rpm for 15 min. The supernatant was collected for further analysis of disaccharidases, diamine oxidase (DAO), malondialdehyde (MDA), superoxide dismatase (SOD), nitric oxide (NO), total nitric oxide synthase (tNOS) and inducible nitric oxide synthase (iNOS). Protein concentration of mucosal homogenates was measured by the method of Lowry (1951).

Intestinal mucosal disaccharidase activity

Disaccharidase activity was assayed according to the method of Hou et al. (2006). In brief, a volume of 100 μl diluted supernatant was dispensed into a glass test tube and incubated with respective substrate (lactose, sucrose or maltose) (100 μl, 0.056 mol/L) at 37°C for 60 min. The reaction was terminated by submerging the tubes in boiling water for 2 min. The tubes were cooled and the glucose concentration was determined (Sigma Diagnostics, St. Louis, MO). Enzyme activity is expressed as U/mg protein.

Plasma d-xylose content

The d-xylose absorption test was carried out according to the method described by Mansoori et al. (2009). 50 μl of the collected plasma was added to 5 ml of phloroglucinol (Sigma Chemical Inc., St. Louis, MO, USA) colour reagent solution and heated at 100°C for 4 min. The samples were allowed to cool to room temperature in a water bath. Xylose standard solutions were prepared by dissolving d-xylose in saturated benzoic acid in deionised water, to make 0, 0.7, 1.3, 2.6 mmol/L, respectively. They were added to colour reagent solution alongside the samples. The absorbance of all samples and standard solutions were measured, using a spectrophotometer (Model 6100, Jenway LTD., Felsted, Dunmow, CM6 3LB, Essex, England, UK), set at 554 nm. The standard solution of 0 mmol/L d-xylose was considered as blank.

Intestinal mucosal diamine oxidase activity

DAO activity was assayed according to the method of Peng et al. (2004). In brief, in the final volume of 3.8 ml, the assay mixture contained 3 ml phosphate buffer (0.2 M, pH 7.2), 0.1 ml (4 μg) horseradish peroxidase solution (Sigma Chemical Inc., St. Louis, MO, USA), 0.1 ml of o-dianisidine methanol solution (500 μg o-dianisidine, Sigma Chemical Inc., St. Louis, MO, USA), 0.5 ml intestinal mucosal homogenates or the diluted DAO standard solutions (2.5, 5, 10, 20, 40 and 80 mg/ml DAO, Sigma Chemical Inc., St. Louis, MO, USA), 0.1 ml of substrate solution (175 μg cadaverine dihydrochloride, Sigma Chemical Inc., St. Louis, MO, USA). After being mixed, the mixture was incubated in a water bath at 37°C for 30 min, then the OD value at 436 nm was recorded after resting for 5 min in the air. The DAO contents were calculated according to the standard curve. The results were calculated as U/mg protein.

Intestinal mucosal malondialdehyde and superoxide dismatase

The lipid peroxidation product malonedialdehyde (MDA) was measured by the thiobarbituric acid reaction using a commercial kit (Nanjing Jiancheng Biological Product, Nanjing, China) according to the manufacturer's recommendations. The results were calculated as nmol/mg protein. Superoxide dismutase (SOD) activity was evaluated by inhibition of nitroblue tetrazolium reduction by superoxide anion generated by the xanthine/xanthine oxidase system using a commercial assay kit (Nanjing Jiancheng Biological Product, Nanjing, China). The results were expressed as U/mg protein.

Intestinal mucosal nitric oxide and nitric oxide synthase

NO content, and tNOS and iNOS activities were analyzed using commercial assay kits (Nanjing Jiancheng Biological Product, Nanjing, China). Nitrate and nitrite (NOx) were measured as oxidized stable end products of NO and the total nitrite level in the sample was determined according to the method described by Zhang et al. (2008). Results of NO content were calculated as μmol/g protein. tNOS and iNOS activities were measured directly by catalyzing Arg method (Zhang et al., 2008). Results of tNOS and iNOS activities were calculated as U/mg protein.

Statistical analysis

All data were subjected to ANOVA appropriate for randomized complete block design by using the GLM procedure of SAS (SAS Institute, Cary, NC, USA). The differences among group means were compared using Duncan Multiple Comparison based on the variance derived from ANOVA. In addition, orthogonal contrast was used to compare the treatment effects of the LPS pigs fed the basal

Table 2. Effects of Arg supplementation on intestinal mucosal disaccharidase activities (U/mg protein) of weaned pigs after 6 h *Escherichia coli* LPS challenge[1]

Item	CONTR	LPS	LPS+0.5% Arg	LPS+1.0% Arg	SEM	Orthogonal contrast[2]
Lactase						
Duodenum	4.303	4.056	4.638	3.755	0.822	0.824
Jejunum	5.914	6.266	5.290	6.386	0.959	0.595
Ileum	2.313^{b}	1.540^{a}	2.205^{b}	1.828^{ab}	0.309	0.033
Suctase						
Duodenum	4.168	4.220	3.085	3.981	0.682	0.217
Jejunum	7.594^{b}	5.046^{a}	5.078^{a}	6.681^{ab}	1.127	0.330
Ileum	4.659^{b}	2.855^{a}	3.761^{ab}	3.939^{ab}	0.704	0.134
Maltase						
Duodenum	7.964	8.163	8.914	8.268	1.837	0.785
Jejunum	11.820	10.176	10.822	10.949	2.033	0.671
Ileum	8.347^{b}	5.412^{a}	6.726^{ab}	6.553^{ab}	0.965	0.090

SEM = Standard error of the mean.

[1] Values are least-square means (n = 6). CONTR (non-challenged control) = Pigs fed a control diet and injected with sterile saline; LPS (LPS challenged control) = Pigs fed the same control diet and challenged with *Escherichia coli* LPS; LPS+0.5% Arg = Pig fed a 0.5% Arg diet and challenged with LPS; LPS+1.0% Arg = Pig fed a 1.0% Arg diet and challenged with LPS.

[2] Orthogonal contrast was used for comparing LPS+0.5% Arg and LPS+1.0% Arg pigs against LPS pigs.

[a, b] Values within a row with different letters differ ($p<0.05$).

diet vs. those fed diets supplemented with different Arg levels. Differences were considered as significant when $p<0.05$.

RESULTS

Intestinal mucosal disaccharidase activity

The data for disaccharidase activities are presented in Table 2. The LPS pigs had lower ileal lactase (33% lower), suctase (39% lower) and maltase (35% lower) activities, and jejunal suctase (34% lower) activities compared to the CONTR pigs ($p<0.05$). Compared to the LPS pigs, the LPS+0.5% Arg pigs had significant higher ileal lactase activity (43% higher, $p<0.05$). Compared to both the LPS pigs and the CONTR pigs, the ileal suctase and maltase activities in the LPS+0.5% pigs, and the ileal lactase, suctase and maltase activities, and jejunal suctase activity in LPS+1.0% Arg pigs, were not different ($p>0.05$). Further orthogonal contrast also showed that Arg supplementation increased ileal lactase activity in pigs challenged with LPS ($p<0.05$).

Plasma d-xylose content

The data for d-xylose content are shown in Table 3. Compared to the CONTR pigs, LPS pigs showed a decrease in plasma d-xylose content (46% lower, $p<0.05$). Arg supplementation did not affect plasma d-xylose content compared to the LPS pigs ($p>0.05$).

Intestinal mucosal diamine oxidase activity

The data for DAO activity are shown in Table 3. LPS injection decreased DAO activity in ileum (45% lower) compared to the CONTR pigs ($p<0.05$). Relative to LPS pigs, dietary supplementation of 0.5% Arg significantly

Table 3. Effects of Arg supplementation on plasma d-xylose content (mmol/L), and intestinal mucosal diamine oxidase (DAO) activity (U/mg protein) of weaned pigs after 6 h *Escherichia coli* LPS challenge[1]

Item	CONTR	LPS	LPS+0.5% Arg	LPS+1.0% Arg	SEM	Orthogonal contrast[2]
Plasma d-xylose	0.178^{b}	0.096^{a}	0.108^{a}	0.108^{a}	0.032	0.562
DAO						
Duodenum	0.175^{a}	0.153^{a}	0.323^{b}	0.156^{a}	0.024	0.001
Jejunum	0.220	0.244	0.288	0.246	0.079	0.641
Ileum	0.311^{b}	0.172^{a}	0.307^{b}	0.278^{b}	0.047	0.005

SEM = Standard error of the mean.

[1] Values are least-square means (n = 6). CONTR (non-challenged control) = Pigs fed a control diet and injected with sterile saline; LPS (LPS challenged control) = Pigs fed the same control diet and challenged with *Escherichia coli* LPS; LPS+0.5% Arg = Pig fed a 0.5% Arg diet and challenged with LPS; LPS+1.0% Arg = Pig fed a 1.0% Arg diet and challenged with LPS.

[2] Orthogonal contrast was used for comparing LPS+0.5% Arg and LPS+1.0% Arg pigs against LPS pigs.

[a, b] Values within a row with different letters differ ($p<0.05$).

Table 4. Effects of Arg supplementation on malondialdehyde (MDA) content (nmol/mg protein) and superoxide dismatase (SOD) activity (U/mg protein) in intestinal mucosa of weaned pigs after 6 h *Escherichia coli* LPS challenge[1]

Item	CONTR	LPS	LPS+0.5% Arg	LPS+1.0% Arg	SEM	Orthogonal contrast[2]
MDA						
Duodenum	0.163[a]	0.305[b]	0.225[ab]	0.227[ab]	0.046	0.090
Jejunum	0.233[a]	0.327[b]	0.214[a]	0.164[a]	0.043	0.002
Ileum	0.161	0.211	0.202	0.176	0.305	0.424
SOD						
Duodenum	10.275[a]	11.260[a]	11.658[a]	14.624[b]	1.404	0.172
Jejunum	16.370	12.834	15.695	13.316	2.029	0.271
Ileum	18.968[a]	13.055[b]	14.280[b]	18.240[a]	1.697	0.010

SEM = Standard error of the mean.

[1] Values are least-square means (n = 6). CONTR (non-challenged control) = Pigs fed a control diet and injected with sterile saline; LPS (LPS challenged control) = Pigs fed the same control diet and challenged with *Escherichia coli* LPS; LPS+0.5% Arg = Pig fed a 0.5% Arg diet and challenged with LPS; LPS+1.0% Arg = pig fed a 1.0% Arg diet and challenged with LPS.

[2] Orthogonal contrast was used for comparing LPS+0.5% Arg and LPS+1.0% Arg pigs against LPS pigs.

[a, b] Values within a row with different letters differ ($p<0.05$).

increased DAO activity in duodenum (111% higher) and ileum (78% higher) ($p<0.05$). and 1.0% Arg supplementation significantly increased DAO activity in ileum (62% higher) ($p<0.05$). Compared to the CONTR pigs, the pigs in both LPS+0.5% and 1.0% Arg groups maintained the duodenal and ileal DAO activity. Moreover, the LPS+0.5% pigs had even higher duodenal DAO activity relative to the CONTR pigs (85% higher, $p<0.05$). Further orthogonal contrast also indicated that Arg supplementation increased duodenal ($p = 0.001$) and ileal ($p<0.01$) DAO activity in pigs challenged with LPS.

Intestinal mucosal malondialdehyde and superoxide dismatase

The data for MDA content and SOD activity are presented in Table 4. Following LPS injection, a significant increase in MDA content was seen in duodenum (87% higher) and jejunum (40% higher) compared to the CONTR pigs. Dietary supplementation of 0.5% Arg significantly decreased jejunal MDA content (35% lower) compared to LPS pigs. The duodenal MDA content did not differ in the LPS+0.5% pigs compared to both the LPS pigs and the CONTR pigs ($p>0.05$). Supplementation of 1.0% Arg also exerted similar effects on MDA content. The results of orthogonal contrast indicated that Arg administration decreased jejunal MDA content in pigs injected with LPS ($p<0.01$).

A significant decrease in SOD activity was observed in ileum (31% lower) in LPS pigs compared to CONTR pigs ($p<0.05$). 1.0% Arg supplementation resulted in a significant increase in SOD activity in duodenum (30% higher) and ileum (40% higher) compared to LPS pigs ($p<0.05$). Supplementation of 0.5% Arg had no effect on SOD activity relative to LPS pigs ($p>0.05$). The results of orthogonal contrast showed that Arg supplementation increased ileal SOD activity in pigs challenged with LPS ($p = 0.01$).

Intestinal mucosal nitric oxide and nitric oxide synthase

The data for NO content, and tNOS and iNOS activities are shown in Table 5. LPS challenge decreased NO content in jejunum (32% lower) and ileum (39% lower) compared to CONTR pigs ($p<0.05$). Compared to LPS pigs, 0.5% Arg supplementation significantly increased jejunal NO content by 46% ($p<0.05$). The ileal NO content in the LPS+0.5% pigs, and the jejunal and ileal NO content in the LPS+1.0% pigs, did not differ compared to both the LPS pigs and the CONTR pigs ($p>0.05$). The results of orthogonal contrast indicated that Arg supplementation increased jejunal NO content ($p<0.05$) in pigs challenged with LPS.

LPS administration resulted in a significant decrease in tNOS and iNOS activities in jejunum (31% lower for tNOS; 41% lower for iNOS) and ileum (43% lower for tNOS; 43% lower for iNOS) compared to CONTR pigs ($p<0.05$). Compared to both the LPS pigs and the CONTR pigs, the jejunal tNOS activity, and jujunal and ileal iNOS activities in the LPS+0.5%, and jejunal and ileal tNOS activities, and ileal iNOS activity in the LPS+1.0% pigs were not different ($p>0.05$). The results of orthogonal contrast indicated that Arg supplementation increased jejunal iNOS activity ($p<0.05$) in pigs challenged with LPS.

DISCUSSION

In the current experiment, to explore whether dietary Arg supplementation could mitigate the impairment of intestinal function in weaned pigs, we used a well-documented model for inducing intestinal injury in weaned pigs by administrating *Escherichia coli* LPS (Mercer et al., 1996). LPS, a powerful toxin produced by gram negative bacteria, induces symptoms of acute bacterial infection including fever, anorexia, somnolence and inactivity (Liu et

Table 5. Effects of Arg supplementation on nitric oxide (NO) content (μmol/g protein) and nitric oxide synthase (NOS) activity (U/mg protein) in intestinal mucosa of weaned pigs after 6 h *Escherichia coli* LPS challenge[1]

Item	CONTR	LPS	LPS+0.5% Arg	LPS+1.0% Arg	SEM	Orthogonal contrast[2]
NO						
Duodenum	5.230	3.359	3.946	3.695	0.862	0.506
Jejunum	5.752^{b}	3.894^{a}	5.687^{b}	4.425^{ab}	0.637	0.019
Ileum	5.592^{b}	3.408^{a}	4.637^{ab}	4.714^{ab}	0.795	0.094
tNOS						
Duodenum	1.880	1.425	1.492	1.767	0.239	0.183
Jejunum	2.281^{b}	1.564^{a}	1.942^{ab}	1.770^{ab}	0.251	0.109
Ileum	2.366^{b}	1.347^{a}	1.678^{a}	1.896^{ab}	0.301	0.121
iNOS						
Duodenum	1.368	1.142	1.053	1.089	0.225	0.714
Jejunum	1.962^{b}	1.151^{a}	1.545^{ab}	1.478^{a}	0.213	0.035
Ileum	1.743^{b}	0.986^{a}	1.315^{ab}	1.231^{ab}	0.287	0.223

SEM = Standard error of the mean; tNOS, total NOS; iNOS, inducible NOS.

[1] Values are least-square means (n = 6). CONTR (non-challenged control) = Pigs fed a control diet and injected with sterile saline; LPS (LPS challenged control) = Pigs fed the same control diet and challenged with *Escherichia coli* LPS; LPS+0.5% Arg = Pig fed a 0.5% Arg diet and challenged with LPS; LPS+1.0% Arg = Pig fed a 1.0% Arg diet and challenged with LPS.

[2] Orthogonal contrast was used for comparing LPS+0.5% Arg and LPS+1.0% Arg pigs against LPS pigs.

[a, b] Values within a row with different letters differ ($p<0.05$).

al., 2008b). In addition, LPS causes various morphologic changes in the gut, such as villus atrophy, submucosal edema, epithelial vacuolation, frank hemorrhage and necrosis (Liu et al., 2008a). Moreover, the LPS-induced morphologic alterations also results in alterations of intestinal function, such as altered secretory and absorptive functions (Kruzel et al., 2000), and increased mucosal permeability (Fink et al., 1991). In addition to the direct effect of LPS on intestine, LPS may cause indirectly gut injury through reduced feed intake. It has been reported that feed intake is correlated with intestinal characteristics (Verdonk et al., 2001). In this study, the pigs were fasted during 6 h LPS challenge (i.e. 6 h before slaughter), which excludes the potential effect of LPS-induced feed intake reduction on gastrointestinal characteristics.

Many studies have shown that Arg protected the intestinal mucosa morphology from damage in a number of gut injury models (Sukhotnik et al., 2004, 2005; Fotiadis et al., 2007; Spanos et al., 2007; Zhan et al., 2008; Wang et al., 2009). Our previous research also showed that dietary supplementation of 0.5% and 1.0% Arg alleviated intestinal morphologic alterations (villus atrophy and crypt hyperplasia) induced by LPS in weaned pigs (Liu et al., 2008a). However, little research was conducted to determine the effect of Arg on intestinal function in weaned pigs. In the current study, we hypothesized that Arg supplementation might alleviate the impairment of intestinal function induced by LPS in weaned pigs.

Activities of mucosal disaccharidases including lactase, sucrase, and maltase have long been used as indicators of mucosa maturation and gut digestive function in pigs (Hampson and Kidder, 1986). In the present study, Arg supplementation increased ileal lactase activity in pigs challenged with LPS, which indicates that Arg supplementation protected the intestinal digestive function from damage caused by the LPS challenge. Currently, there are very few studies on the regulation of intestinal digestive function through dietary Arg supplementation. Interestingly, Míguez et al. (2004) reported that maltase and sucrase activities in the intestinal mucosa were elevated in streptozotocin-induced diabetic rats and were restored to control levels after dietary Arg supplementation. It has been reported that Arg can stimulate the secretion of insulin, growth hormone and glucagon (Wu et al., 2000; Yao et al., 2008). Of them, insulin can promote mucosa maturation, and increase mucosa disaccharidase activities in neonatal pigs (Zheng et al., 1999). Therefore, it is possible that Arg supplementation attenuated the decrease of disaccharidase activities partially by stimulating insulin release.

Intestinal absorption capacity has routinely been assessed by the d-xylose absorption test in man and animals (Semrad, 2005). Until now, no studies were conducted to determine the effect of Arg administration on intestinal absorptive function. In the present study, Arg supplementation did not alleviate the decrease of plasma d-xylose content induced by LPS injection, which indicates that Arg supplementation does not improve intestinal absorption ability.

Intestinal barrier function can be commonly assessed by many indices such as DAO activity (Peng et al., 2004), bacterial translocation (Gao et al., 2006), and intestine mucosal permeability (Peng et al., 2004). Of them, DAO is

an intracellular enzyme with high activity existing in intestinal mucosa in both human beings and all other mammalians. It is a relatively stable marker of intestinal mucosa integrity (Zhang et al., 2002). The activity of DAO in intestinal mucosa decreases when its cells are injured. Thus, the DAO activity of intestinal mucosa can reflect the changes in intestinal mucosa integrity and barrier function (Zhang et al., 2002). In the current study, relative to LPS pigs, 0.5% Arg increased DAO activity in duodenum and ileum, and 1.0% Arg increased DAO activity in ileum. These results indicate that Arg supplementation alleviated the injury of intestinal barrier function induced by LPS challenge. In agreement with our findings, Corl et al. (2008) reported that Arg supplementation improving intestinal permeability via a mammalian target of rapamycin/$p70^{S6k}$-independent mechanism in piglet rotavirus enteritis. In addition, Gurbuz et al. (1998) reported that Arg-enriched diets prevented bacterial translocation induced by radiation-induced enteritis in rat.

It is well known that oxidative stress is one of major factors contributing to intestinal injury (Fang et al., 2002; Liu et al., 2007). Usually, antioxidative ability of mucosa is evaluated by SOD activity and MDA content (Liu et al., 2007). SOD is one of important antioxidases that scavenge oxygen-free radicals, and MDA is an end-product of free radical chain reaction and lipid peroxidation (Liu et al., 2007). In the current study, consistent with attenuated impairment of intestinal function caused by the LPS challenge, a decrease of jejunal MDA content and an increase of duodenal and ileal SOD activity were also observed after 0.5% or 1.0% Arg supplementation. In agreement with our findings, Liu et al. (2003) reported that Arg protected intestine barrier function through reducing intestinal lipid peroxidation and increasing antioxidant enzyme in rat with obstructive jaundice. In addition, Fotiadis et al. (2007) also reported that Arg decreased serum MDA levels, and attenuated intestinal mucosa damage in a rat model of intestinal ischaemia/reperfusion injury. In the current study, it is possible that feeding pigs with Arg-supplemented diet reduced gut function injury partially by reducing oxidative stress.

Arg is converted to NO and citrulline by the enzyme NOS (Kohli et al., 2004). There are three known isoforms of the enzyme, all of which are expressed in the intestine: neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS) (Wang et al., 2009). eNOS and nNOS are constitutively expressed, so they together are called constitutive NOS (cNOS). The cNOS generate relatively a small amount of NO in the small intestine (Mercer et al., 1996). In contrast, the iNOS generates much larger quantities of NO (Mercer et al., 1996). Generally, the production of NO by cNOS is of critical importance in the maintenance of intestinal mucosal integrity (Wang et al., 2009). The production of NO by iNOS, on the other hand, can have both beneficial and deleterious effects depending upon the precise conditions under which it is expressed (Wang et al., 2009). Although the role of iNOS in mediating intestinal inflammation was previously controversial, the results from many experimental models of colitis in iNOS-deficient mice suggest that iNOS also plays an important role in mucosal inflammation (Wang et al., 2009).

Generally, iNOS is stimulated to produce a large amount of NO in a variety of cell types in response to endotoxin and various cytokines (Mercer et al., 1996). However, in the current study, it is surprising that NO content and iNOS activity in jejunum and ileum were decreased following LPS challenge. The reasons might be that over-production of reactive oxygen species induced by LPS challenge can disrupt the integrity of the endothelium, and decrease NOS activity, and consequently decrease NO production (Rhoden et al., 2001; Liu et al., 2005). In addition, the effect of LPS challenge on intestinal NO production, tNOS and iNOS activities might be time-dependent. In our study, regretfully, only one time-point (6 h) for collecting intestinal samples was used. It might not be able to adequately reflect the dynamic changes of intestinal NO, tNOS and iNOS. If we could use more time-points, it would provide adequate information to confirm the roles of intestinal NO, tNOS and iNOS in weaned pigs after LPS challenge, which awaits further experiments.

In our study, Arg supplementation increased jejunal NO content and iNOS activity of the LPS-challenged pigs. Our findings indicate that the increase of mucosal NO production after Arg supplementation are mainly due to the increase of activity in iNOS, which further indicates the beneficial effect of NO produced by iNOS. Similarly, Schleiffer and Raul (1996) showed that pretreatment with Arg improved intestinal recovery after ischaemic damage in rats by an NOS dependent mechanism. In the current study, it is possible that feeding pigs with Arg-supplemented diet reduced gut function injury by increasing NO production due to iNOS.

NO might attenuate intestinal injury through scavenging the oxygen-derived free radical superoxide anion and exert antioxidative activity (Chander and Chopra, 2005). In this study, LPS caused an increase in mucosal MDA content and a decrease of mucosal SOD activity. LPS-induced oxidative stress was associated with impaired gut digestive, absorptive and barrier functions. Moreover, there was a significant decrease in mucosal NO level and iNOS activity in LPS pigs. Treatment with Arg attenuated LPS-induced lipid peroxidation and increased SOD activity. Furthermore, the NO level, iNOS activity and gut functional damage were improved. So, the protective effects of Arg on intestinal function are partially associated with the increased antioxidative ability as a result of increasing NO

production by iNOS.

Of course, besides the antioxidative ability, NO can protect intestinal function via the other mechanisms such as improving intestinal blood supply by its vasodilatory action (Chander and Chopra, 2005), diminishing the leukocyte adhesion, neutrophil infiltration and the formation of inflammatory mediators (Kobayashi et al., 1995), which indicates that NO may play a pivotal role in Arg attenuating the impairment of intestinal function in our study.

In conclusion, dietary supplementation of Arg exerts beneficial effects in improving intestinal function in weaned pigs after LPS challenge. NO may play a pivotal role in Arg improving intestinal function.

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