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ORIGINAL ARTICLE

Dietary supplementation with L-arginine or *N*-carbamylglutamate enhances intestinal growth and heat shock protein-70 expression in weanling pigs fed a corn- and soybean meal-based diet

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Abstract This study determined effects of dietary supplementation with L-arginine (Arg) or *N*-carbamylglutamate (NCG) on intestinal health and growth in early-weaned pigs. Eighty-four Landrace × Yorkshire pigs (average body weight of 5.56 ± 0.07 kg; weaned at 21 days of age) were fed for 7 days one of the three isonitrogenous diets: (1) a corn- and soybean meal-based diet (CSM), (2) CSM + 0.08% NCG (0.08%), and (3) CSM + 0.6% Arg. There were four pens of pigs per diet (7 pigs/pen). At the end of a 7-day feeding period, six piglets were randomly selected from each treatment for tissue collections. Compared with the control group, Arg or NCG supplementation increased ($P < 0.05$): (1) Arg concentrations in plasma, (2) small-intestinal growth, (3) villus height in duodenum, jejunum and ileum, (4) crypt depth in jejunum and ileum, (5) goblet cell counts in intestinal mucosae, and (6) whole-body weight gain in pigs. Real-time polymerase chain reaction and western blotting analyses revealed that both mRNA and protein levels for heat shock protein-70 (HSP70) were higher ($P < 0.05$) in the intestinal mucosae of Arg- or NCG-supplemented pigs than in the control group. Furthermore, the incidence of diarrhea in the NCG group was 18% lower ($P < 0.01$) than that in the control group. Collectively, these results indicate that dietary supplementation with 0.6% Arg or 0.08% NCG enhances intestinal HSP70 gene expression, intestinal growth and integrity, and the availability of dietary nutrients for whole-body weight gain in postweaning pigs fed a CSM-based diet. Thus, Arg or NCG is a functional ingredient in the weaning diet to improve nutrition, health, and growth performance of these neonates.

Keywords L-Arginine · *N*-carbamylglutamate · Growth performance · Diarrhea · Intestinal morphology · Weaned pigs

Abbreviations

Arg	L-Arginine
CSM	Corn- and soybean meal-based diet
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
HSP70	Heat shock protein 70
IEL	Intraepithelial leukocytes
NAG	*N*-Acetylglutamate
NCG	*N*-carbamylglutamate
NO	Nitric oxide
RT-PCR	Real-time polymerase chain reaction

Introduction

L-Arginine (Arg) is a nutritionally essential amino acid for young pigs, particularly under stressful conditions (Kim

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and Wu 2009; Wu et al. 2004a, 2007a). As a substrate for the synthesis of nitric oxide (NO), polyamines, creatine, agmatine, and protein (Eklou-Lawson et al. 2009; Wu and Morris 1998), Arg plays versatile roles in treating many developmental and health problems in humans and animals (Wu et al. 2009). Increasing evidence shows that inadequate exogenous and endogenous provision of Arg to 7–21-day-old pigs fed milk protein (which is deficient in Arg) is a major factor limiting their maximum growth (Kim and Wu 2004; Wu et al. 2007a; Yao et al. 2008). Additionally, Arg deficiency can result in hyperammonemia, impaired vasodilation of blood vessels, as well as intestinal and immunological dysfunction (Rhoads and Wu 2009; Tan et al. 2009a; Wu et al. 2004a).

Using the young pig as an excellent animal model for studying infant nutrition (Deng et al. 2009; Elango et al. 2009; Suryawan et al. 2009; Yin et al. 2009b), Wu et al. (2004b) discovered that a low level of mitochondrial N-acetylglutamate (NAG) limits in vivo intestinal Arg synthesis in sow-reared piglets. Therefore, oral administration of *N*-carbamylglutamate (NCG), which is a safe and metabolically stable analog of NAG (Gessler et al. 2010; Wu 2009), increases the endogenous synthesis of Arg, plasma Arg concentration, muscle protein synthesis, and whole-body growth in milk-fed piglets (Frank et al. 2007; Wu et al. 2004b; Yao et al. 2008). Although these findings provide the proof of principle for an important role of Arg in neonatal nutrition and growth, little is known about the effects of supplementing Arg or NCG to a plant protein-based diet for weanling pigs on intestinal health and growth.

Therefore, the current work was conducted to investigate the effects of Arg or NCG on intestinal morphology and expression of heat shock protein-70 (HSP70), diarrhea, and growth performance in early-weaned pigs. These neonates were used because they developed intestinal atrophy during the first week postweaning due to malnutrition, inflammation, and oxidative stress (Lalles et al. 2009; Wang et al. 2008; Wu et al. 1996b). HSP70 was studied because it is a major member of a highly conserved family of HSP proteins (Beckmann et al. 1990) and plays a key role in protecting the gastrointestinal tract from injury (Haynes et al. 2009; Hou et al. 2010; Malyshev et al. 1995).

Materials and methods

Animals and treatment

Eighty-four Landrace × Yorkshire piglets (average body weight of 5.56 ± 0.07 kg, mean ± SEM; weaned at 21 days of age) were fed for 7 days one of the three diets: (1) a corn- and soybean meal-based diet (CSM) (Table 1); (2) CSM + 0.6% Arg; (3) CSM + 0.08% NCG (0.08%). To ensure the same content of nitrogen in the three experimental diets, diets 1 and 3 were supplemented with 1.23% L-alanine at the expense of cornstarch. There were four pens of pigs per diet (7 pigs/pen). Pigs had free access to drinking water and their respective diets. NCG was provided by Institute of Subtropical Agriculture, the Chinese Academy of Sciences, Hunan, China. Arg and L-alanine were obtained from Ajinomoto Inc. (Tokyo, Japan). The doses of Arg and NCG were based on previous studies with young pigs fed an Arg-supplemented diet (Tan et al. 2009a) and orally administered with NCG (Wu et al. 2004b), respectively, to avoid any adverse effects of excessive Arg on the gut (Grimble 2007) and other organs (Wink and Mitchell 1998). The basal diet met the National Research Council (1998)-recommended requirements of nutrients for young pigs. This study was performed in accordance with the Chinese guidelines for animal welfare and approved by the Animal Care and Use Committee of the Institute of Subtropical Agriculture, the Chinese Academy of Sciences.

Table 1 Composition of the basal diet for weanling pigs (as-fed basis)

Ingredients	Content (%)	Nutrient levels	Content
Corn (8% CP; <13% H_2O)	50.32	DE (MJ/kg)	14.21
Soybean meal (43% CP)	24.2	Crude protein (%)	20.0
Fish meal (CP 65%)	6	Calcium (%)	0.71
Whey (100%)	9	Available	0.48
Corn starch	1.23	Phosphorus (%)	
Cream (50% fat)	6	L-Lysine (%)	1.35
Limestone	0.5	L-Methionine (%)	0.39
Monocalcium phosphate	1	L-Threonine (%)	0.91
NaCl	0.2	L-Arginine (%)	1.09
Flavor	0.06		
Mineral–vitamin premix[a]	1		
L-Lysine·HCl	0.31		
L-Methionine	0.06		
L-Threonine	0.12		
Total	100		

[a] Providing the following per kg diet: $CuSO_4 \cdot 5H_2O$ 19.8 mg; KI 0.20 mg; $FeSO_4 \cdot 7H_2O$ 400 mg; $NaSeO_3$ 0.56 mg; $ZnSO_4 \cdot 7H_2O$ 359 mg; $MnSO_4 \cdot H_2O$ 10.2 mg; vitamin K (menadione) 5 mg; vitamin B_1 2 mg; vitamin B_2 15 mg; vitamin B_{12} 30 μg; vitamin A 5,400 IU; vitamin D_3 110 IU; vitamin E 18 IU; choline chloride 80 mg; antioxidants 20 mg; Fungicide 100 mg

Sample collection

At 28 days of age, 1 h after the last meal, eight piglets were randomly selected from each treatment (2 pigs/pen) for tissue collections. Blood samples (10 ml) were obtained

from the jugular vein into heparinized tubes, followed immediately by centrifugation at 3,000×*g* for 10 min at 4°C (Yin et al. 2009a). The supernatant fluid (plasma) was collected and immediately stored at −20°C for biochemical analyses. Immediately after blood sampling, pigs were anaesthetized with an intravenous injection of sodium pentobarbital (50 mg/kg BW) and bled by exsanguination (Yin et al. 2009a). The entire intestine was rapidly removed, thoroughly fluxed with sterile saline to remove luminal digesta, and then dissected free of mesenteric attachments, weighted, and placed on a smooth, cold surface tray. Duodenum, jejunum, and ileum were obtained as described by Deng et al. (2009). Samples of intestinal segments were taken for the assessment of intestinal morphology (Wu et al. 1996b). Goblet cell numbers within the intestinal mucosa were determined using toluidine blue-periodic acid Schiff-stained intestinal sections (Dunsford et al. 1991) and expressed per 100-μm length of villus.

Growth performance and diarrhea

Body weights of individual pigs were measured immediately before feeding at the beginning and end of the trial. Feed consumption by pigs in each pen was recorded daily. Average daily gain (ADG) and average daily feed intake (ADFI) were calculated for all pigs (Kong et al. 2009). The number of pigs with diarrhea was recorded daily throughout the study. The severity of diarrhea was evaluated using the fecal consistency score system (Kong et al. 2007; Marquardt et al. 1999). Fecal consistency scores (0 normal; 1 soft feces; 2 mild diarrhea; 3 severe diarrhea) were determined by two trained persons with no prior knowledge of dietary treatment allocation. The incidence of diarrhea was calculated as (the total number of pigs with diarrhea/the total number of all experimental pigs) × 100%. Diarrhea index was calculated as total score of diarrhea/total number of pigs.

Determination of amino acids in plasma

Plasma (0.5 ml) was deproteinized with 0.5 ml of 1.5 mM $HClO_4$, followed by addition of 0.25 ml of 2 M K_2CO_3 (Li et al. 2009b). The neutralized extract was analyzed for amino acids using high-performance liquid chromatography (Wu and Meininger 2008). This method involved the pre-column derivatization of amino acids with *o*-phthaldialdehyde and fluorescence detection. Amino acids in samples were quantified on the basis of known amounts of standards (Sigma Chemicals, St. Louis, MO, USA). Proline and cysteine/cystine in plasma were analyzed as described by Wu and Knabe (1994).

Real-time PCR for HSP70 in the ileal mucosa

Total RNA was extracted from the ileal mucosa using a guanidinium isothiocyanate method (Trizol™ reagent, Gibco BRL, Berlin, Germany) and treated with DNAse according to the manufacturer's instructions. mRNA levels for HSP70 were determined by a standard real-time polymerase chain reaction (RT-PCR) method as previously described (Tan et al. 2009a; Yu et al. 2007). The primer pairs for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and HSP70 are presented in Table 2. GAPDH was used as the housekeeping gene, whose mRNA levels in the ileum did not differ among the three groups of pigs. The RT-PCR conditions were: 30 s denaturation at 94°C, 30 s annealing at 56°C, and 30 s extension at 72°C for 30 cycles. The relative quantification of gene amplification by RT-PCR was performed using cycle threshold (C_t) values. The comparative C_t value method was employed to quantitate expression levels for HSP70 relative to those for GAPDH as described by Fu et al. (2006).

Western blot for HSP70 protein in the ileal mucosa

Western blotting analysis of HSP70 protein was performed as described previously (Yao et al. 2008). Briefly, 100 mg of the ileal mucosal sample was homogenized in 6 volumes of buffer A (20 mM HEPES, pH 7.4, 100 mM KCl, 0.2 mM EDTA, 2 mM EGTA, 1 mM dithiothreitol, 50 mM NaF, 50 mM *β*-glycerolphosphate, 0.1 mM phenylmethylsulfonyl fluoride, 1 mM benzamidine, 0.5 mM sodium vanadate, and 1 μM microcystin LR) and centrifuged at 10,000×g for 10 min at 4°C. The supernatant fluid was aliquoted into microcentrifuge tubes, and its protein content was quantified using a detergent compatible protein assay kit (Bio-Rad, Hercules, CA, USA). Aliquots of 10 μg protein for each sample were mixed with a one-fifth volume of the

Table 2 Primers for HSP70 and GADPH

Target gene	Primer sequences	Products (bp)	Annealing temperature (°C)
HSP70	F: gccctgaatccgcagaata	152	59
	R: tccccacggtaggaaacg		
GADPH	F: actcactcttctacctttgatgct	100	59
	R: tgttgctgtagccaaattca		

F forward; *R* reverse

sample buffer (0.35 M Tris–Cl, pH 6.8, 10% SDS, 30% glycerol, 9.3% dithiothreitol, and 0.175 mM bromophenol blue) and heat-denatured by boiling for 5 min. Separated proteins were transferred to polyvinylidene difluoride membranes (Immobilon-P, Millipore, Bedford, MA, USA) overnight at 4°C and then incubated with a blocking solution (0.05% Tween 20, 50 mM Tris, pH 8.0, 150 mM NaCl, and 5% powdered non-fat milk) for 1 h. Membranes were incubated for 2 h at room temperature with a monoclonal HSP70 antibody diluted at 1:3,000 dilution (Stressgen Bioreagents, British Columbia, Canada). After the first antibody was stripped off, the same blot was treated with a rabbit polyclonal anti β-actin antibody diluted 1:5,000 (Stressgen Bioreagents, British Columbia, Canada). The membranes were incubated with an appropriate peroxidase-labeled secondary antibody prepared in PBS-Tween 20. Membranes were then washed and incubated for 2 h at room temperature with a goat anti-rabbit secondary antibody (horseradish peroxidase-conjugated goat anti-mouse; Zhongsan gold bridge, China) at a 1:5,000 dilution. Primary antibody binding was visualized using an enhanced chemiluminescence kit (Pierce, Rockford, IL, USA) and Hyperfilm-MP (Amersham International, Buckinghamshire, UK). The intensities of proteins on the membranes were quantified using the Alpha Innotech 8800 image station (San Leandro, CA, USA) equipped with the FLUORCHEM software.

Statistical analysis

Values are presented as the mean ± SEM. The normality of data was tested using the Shapiro–Wilk test in SAS (version 8.1; SAS Institute, Cary, NC, USA). Log transformation of variables was performed when variance of data was not homogenous among treatment groups (Fu et al. 2010). Results were statistically analyzed using one-way ANOVA (SAS Institute, NC, USA), with the pen and the pig as the experimental unit for feed intake and other variables, respectively. The Duncan's multiple range test was used to compare differences among the treatment groups (Steel et al. 1997). The incidence of diarrhea was analyzed using the Chi-Square test in SAS. Probability values ≤ 0.05 were taken to indicate statistical significance.

Results

Effects of Arg and NCG on growth performance and diarrhea

The basal diet included 9% high-quality whey, 6% cream, 6% fish meal, and 0.06% flavor to increase feed intake by weanling pigs fed a corn- and soybean meal-based diet. Compared with the control group, supplementing either Arg or NCG to the weaning diet for 7 days increased ($P < 0.05$) the daily weight gain of pigs without affecting their feed intake (Table 3). There were no differences ($P > 0.05$) in ADG or ADFI between the Arg and NCG groups (Table 3). The intestinal weights of the pigs in the Arg and NCG groups were heavier ($P < 0.05$) than those in the control group (Table 3).

Approximately 18% of piglets had diarrhea within 1 week postweaning (Fig. 1). Dietary supplementation with NCG decreased ($P < 0.01$) the incidence of diarrhea and the diarrhea index by 66 and 61%, respectively (Fig. 1). Either the incidence of diarrhea or the diarrhea index did not differ ($P > 0.05$) between the Arg and NCG groups. At the dose used, Arg and NCG supplementation had no adverse effects on the intestine or the whole body.

Effects of Arg and NCG on intestinal morphology and goblet cell numbers

Data on intestinal morphology are summarized in Table 4. Dietary supplementation with Arg or NCG enhanced ($P < 0.05$) villus height in the duodenum, jejunum, and ileum, as well as crypt depth in the jejunum and ileum of weanling pigs. Ratios of villus height to crypt depth did not differ ($P > 0.05$) among the three groups of pigs. Goblet cells were readily detected in the small intestine (Fig. 2). Either Arg or NCG supplementation increased ($P < 0.05$) the numbers of goblet cells in the jejunum and ileum of weanling pigs (Table 4). All of the measured intestinal variables did not differ ($P > 0.05$) between the Arg and NCG groups.

Effects of NCG and Arg on concentrations of amino acids in plasma

Compared with the control group, dietary supplementation with Arg or NCG increased ($P < 0.05$) concentrations of

Table 3 Effects of dietary supplementation with arginine or NCG on the growth performance of weanling pigs

Variables	Control	Arg diet	NCG diet
Initial body weight (kg)	5.56 ± 0.08	5.57 ± 0.12	5.55 ± 0.09
Final body weight (kg)	5.75 ± 0.09[a]	6.24 ± 0.16[b]	6.30 ± 0.13[b]
Average daily gain (g)	36.6 ± 4.6[a]	78.6 ± 5.8[b]	93.3 ± 4.9[b]
Daily feed intake (g/day)	178 ± 11	192 ± 10	181 ± 15
Intestinal weight (g)	401 ± 19[a]	490 ± 20[b]	513 ± 37[b]

Values are expressed as mean ± SEM, $n = 28$ piglets for body weights and average daily gains, $n = 4$ pens (7 pigs per pen) for daily feed intake, and $n = 8$ for intestinal weights per treatment

[a, b] Means within a row with different letters differ ($P < 0.05$)

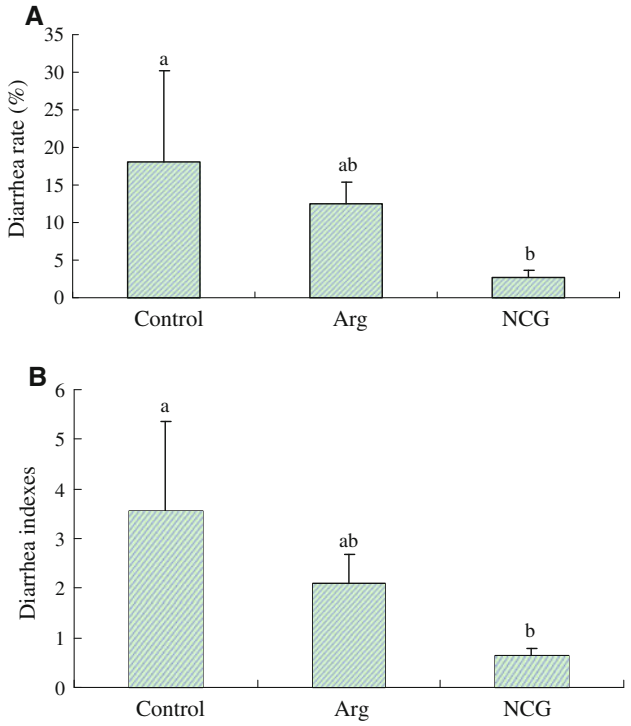

Fig. 1 Effects of arginine and NCG on the incidence of diarrhea (**a**) and the diarrhea index (**b**) in weanling pigs. Values are expressed as mean ± SEM, $n = 28$. [a,b]Means with different letters differ ($P < 0.05$)

arginine, ornithine, and proline in the plasma of weanling piglets (Table 5). NCG, but not Arg, also enhanced ($P < 0.05$) citrulline concentrations in plasma. Concentrations of all other measured amino acids in plasma did not differ among the three groups of pigs (data not shown).

HSP70 gene expression in the ileal mucosa

Real-time PCR analysis showed that the mRNA levels for HSP70 were 46 and 61% higher ($P < 0.05$) in the ileal mucosa of the Arg- and NCG- supplemented piglets, respectively, compared with the control group (0.337 ± 0.025, 0.493 ± 0.049, and 0.544 ± 0.023 for the control, Arg, and NCG groups, respectively). Dietary supplementation with Arg or NCG increased ($P < 0.05$) protein levels for HSP70 in the ileal mucosa (Fig. 3). Either mRNA or protein levels for HSP70 in the ileal mucosa did not differ ($P > 0.05$) between the Arg- and NCG-supplemented pigs.

Discussion

Arg plays important roles in regulating both growth and metabolic function in pigs (Blachier et al. 2010; Flynn et al. 2009; Tan et al. 2009b). Arg deficiency results in growth retardation, intestinal and reproductive dysfunction,

Table 4 Effects of dietary supplementation with arginine or NCG on intestinal morphology in weanling pigs

Variables	Control	Arg diet	NCG diet
Villus height (μm)			
Duodenum	343 ± 20[a]	466 ± 16[b]	425 ± 17[ab]
Jejunum	300 ± 16[a]	424 ± 13[b]	416 ± 16[b]
Ileum	326 ± 14[a]	410 ± 10[b]	418 ± 11[b]
Crypt depth (μm)			
Duodenum	224 ± 13	256 ± 12	258 ± 12
Jejunum	191 ± 8[a]	252 ± 14[b]	260 ± 10[b]
Ileum	196 ± 7[a]	248 ± 11[b]	245 ± 10[b]
Villus height:crypt depth			
Duodenum	1.48 ± 0.08	1.83 ± 0.12	1.65 ± 0.11
Jejunum	1.56 ± 0.11	1.77 ± 0.18	1.61 ± 0.08
Ileum	1.72 ± 0.09	1.66 ± 0.11	1.84 ± 0.06
Goblet cells (per 100-μm length of villus)			
Duodenum	4.67 ± 0.68	6.50 ± 0.78	5.33 ± 1.01
Jejunum	3.83 ± 0.67[a]	5.00 ± 0.88[b]	5.67 ± 0.68[b]
Ileum	7.33 ± 0.95[a]	10.2 ± 0.83[b]	9.83 ± 0.98[b]

Values are mean ± SEM, $n = 8$ per treatment

[a, b] Means within a row with different letters differ ($P < 0.05$)

impaired immune and neurological development, hyperammonemia, and even death (He et al. 2009; Wu et al. 2004a, 2009). Because milk protein contains a low content of protein (Davis et al. 1994; Wu and Knabe 1994), supplementing 0.2 and 0.4% Arg to a dried milk diet (containing 19.2% crude protein and 0.75% Arg; similar to the protein and Arg content in sow's milk) numerically increased weight gain by 43 and 93%, respectively, in 7–14-day-old piglets weaned at 3–4 days of age (Leibholz 1982). Subsequently, Kim and Wu (2004) demonstrated that dietary supplementation with 0.2 and 0.4% Arg dose-dependently enhanced Arg concentrations in plasma (30 and 61%), reduced ammonia concentrations in plasma (20 and 35%), and increased weight gains (28 and 66%) in 7–21-day-old pigs artificially reared on a milk feeding system. Most recently, Tan et al. (2009a) found that supplementing 0.6% Arg to a whey- and cream-based diet maximally improved growth performance of piglets weaned at 21 days of age. Similar findings were also reported by Hernandez et al. (2009). These results indicate that dietary supplementation with Arg enhances protein deposition and the efficiency of utilization of dietary amino acids in milk-fed young pigs. Extending these observations, we reported here that supplementing Arg to a corn- and soybean meal-based diet promoted the intestinal and whole-body growth of weanling piglets (Table 3).

Weaning is associated with stress, a cortisol surge, and induction of intestinal arginase expression in piglets (Lalles et al. 2009; Wu et al. 2000a, b). The extensive degradation

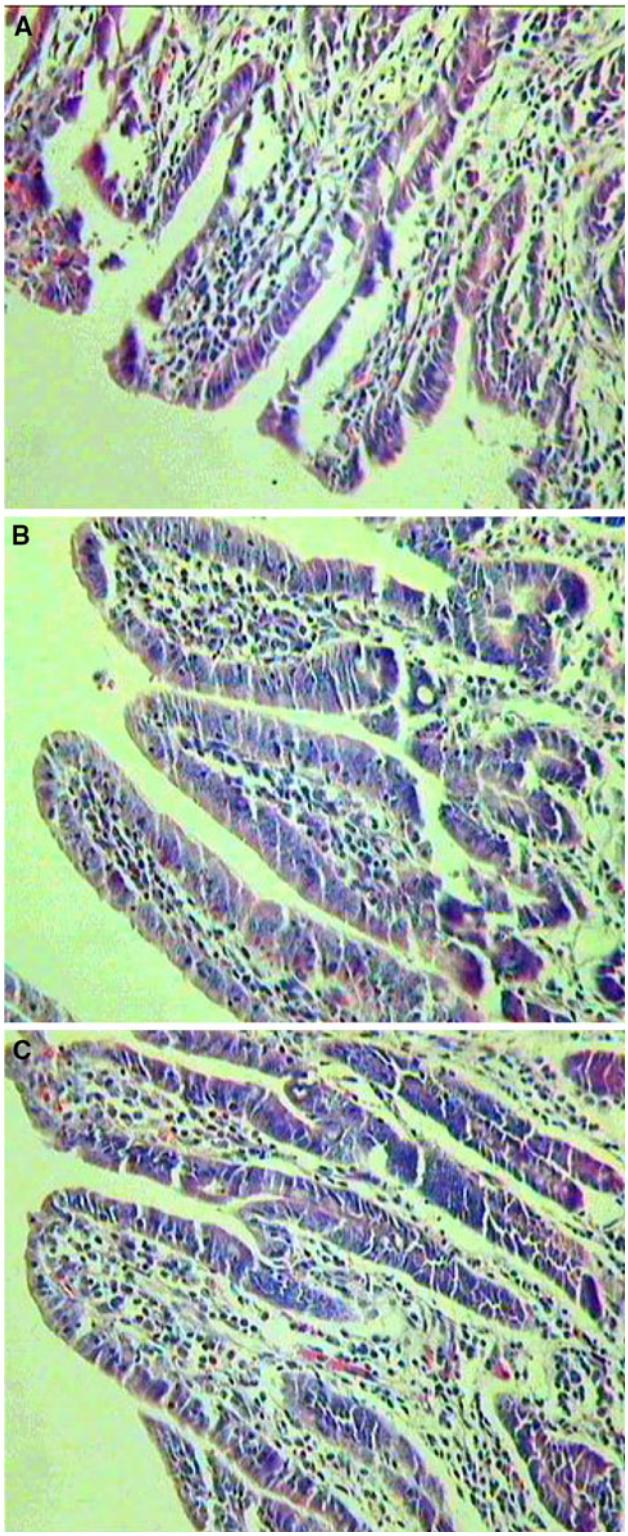

Fig. 2 Number of goblet cells in the jejunal mucosa of weanling pigs. Representative staining of jejunal goblet cells in control (**a**), Arg (**b**), and NCG (**c**) groups of pigs

of enteral Arg by arginase in the small-intestinal mucosa (Wu et al. 1996a) and luminal microbes of weaned pigs (Chen et al. 2009; Kyriakidis and Tiligada 2009) may

Table 5 Effects of dietary supplementation with arginine or NCG on concentrations of amino acids in the plasma of weanling pigs

Item	Control (μmol/L)	Arg diet (μmol/L)	NCG diet (μmol/L)
Arginine	283 ± 8^{a}	361 ± 13^{b}	388 ± 12^{b}
Ornithine	104 ± 4^{a}	135 ± 6^{b}	124 ± 7^{b}
Citrulline	60 ± 3^{a}	68 ± 5^{a}	82 ± 4^{b}
Proline	304 ± 9^{a}	402 ± 12^{b}	365 ± 9^{b}

Values are expressed as mean ± SEM, $n = 8$ pigs per treatment

[a, b] Means within a row with different superscript letters differ ($P < 0.05$)

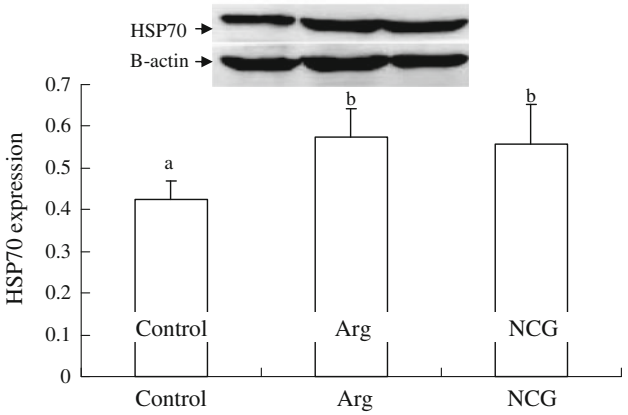

Fig. 3 HSP70 protein levels in the jejunal mucosa of weanling pigs. HSP70 protein in the jejunal mucosa was determined by the western blot analysis. Values are expressed as mean ± SEM, $n = 8$. [a, b]Means within different letters differ ($P < 0.05$)

greatly reduce the entry of Arg from feed into the portal circulation (Bergen and Wu 2009; Wu et al. 2007b). The substantial loss of Arg via intestinal catabolism (Wu et al. 1996a), coupled with reduced feed intake, lowers the circulating levels of Arg in weanling pigs (Wu et al. 2009). For example, we found that concentrations of Arg in plasma were 0.080 ± 0.009 and 0.147 ± 0.012 mM (mean ± SEM, $n = 10$; $P < 0.01$), respectively, in 23-day-old pigs weaned at 21 day of age to a corn- and soybean meal-based diet without animal products and in 23-day-old sow-reared piglets (Wang et al. 2008). Because of Arg deficiency in piglets during the first days postweaning, the control group of pigs gained little weight between 21 and 28 days of age (Table 3). Notably, dietary supplementation with 0.6% Arg was highly effective in increasing Arg provision to weanling pigs fed a corn- and soybean meal-based diet, therefore preventing Arg deficiency in these neonates (Tables 3, 5). At 28 days of age (day 7 post-weaning), piglets consumed substantially more food than the previous days, resulting in plasma Arg concentration >200 μM at 1 h after feeding (Table 5).

Another novel and significant result of the current study is that supplementing NCG to the weanling diet was as effective as Arg in improving growth performance of young pigs (Table 3). NCG promotes the conversion of glutamine/glutamate and proline into citrulline and arginine in piglet enterocytes (Wu et al. 2004b). Thus, concentrations of both citrulline and arginine were elevated in NCG-supplemented pigs (Table 5). In this study, whey, cream, and fish meal were included in the basal diet to increase the feed intake of weanling piglets by 28% (Table 3) compared with weanling pigs fed to a corn- and soybean meal-based diet without animal proteins (Wang et al. 2008); therefore, amounts of glutamine, glutamate, and proline from the enteral diet were augmented to provide more substrates for intestinal synthesis of citrulline and arginine via pyrroline-5-carboxylate synthase and proline oxidase (Wu 1997, 1998). Because whey, cream, and fish meal are expensive ingredients, it would be important to determine in future studies whether directly supplementing citrulline or Arg to low-cost diets (e.g., corn- and soybean meal-based diets without animal proteins) would be economically more advantageous and nutritionally more efficient in feeding weanling piglets than adding NCG to costly diets.

Polyamines are physiologically important products of Arg degradation in cells, including enterocytes of the intestinal villus and crypt (Wang et al. 2009b; Wu et al. 2000a, b) because these substances are essential for DNA and protein synthesis (Blachier et al. 2007; Tan et al., 2009b; Wu et al. 2009). Because weaning stress causes intestinal atrophy and dysfunction (Gu et al. 2002; Hampson 1986; Wang et al. 2009a), Arg nutrition may play a more prominent role in maintaining gut integrity in weanling pigs than in sow-reared neonates (Han et al. 2009; Wu et al. 2009). In support of this view, supplementing Arg or NCG to weanling piglets markedly enhanced villus height and crypt depth of the small intestine, as well as ameliorated gut atrophy (Table 4). Additionally, Arg or NCG supplementation increased the numbers of goblet cells in the intestinal mucosa (Table 4). These cells secrete mucins, trefoil peptides, and other bioactive molecules to create a physical barrier at the mucosal surfaces of the intestine (Koninkx et al. 1988; Law et al. 2007; Wang et al. 2007). The underlying mechanisms for the beneficial effects of Arg or NCG may involve polyamine synthesis (Wu et al. 2000b), Arg signaling in the intestine (Rhoads and Wu 2009), balance among basic amino acids (Baker 2009), and expression of oxidative defense proteins (Palii et al. 2009; Stipanuk et al. 2009; Tan et al. 2010). Like other intestinal mucosal cells and vascular endothelial cells (Zhan et al. 2008), goblet cells may depend on Arg and its metabolites for proliferation and differentiation.

Intracellular HSP70, which is expressed in the gastrointestinal tract (David et al. 2002), plays a key role in protecting the intestinal mucosa (Otaka et al. 2006). Proteomics studies revealed that Arg deprivation decreased HSP expression and increased the cellular susceptibility to apoptosis in intestinal Caco-2 cells (Lenaerts et al. 2007). An anti-oxidative effect of Arg has also been reported for other cell types, including skeletal muscle (Ma et al. 2010), adipose tissue (Jobgen et al. 2009), and endothelial cells (Li et al. 2009a). Results of our current study demonstrated that intestinal HSP70 expression at both mRNA and protein levels was enhanced in response to dietary supplementation with Arg or NCG (Fig. 3), as reported for piglets supplemented with α-ketoglutarate (Hou et al. 2010). Because NCG itself did not affect HSP70 protein levels in porcine enterocytes (our unpublished data), it is likely that this agent exerts its effect through the synthesis of citrulline, Arg, or NO in intestinal mucosal cells. In this regard, it is noteworthy that NO has been shown to increase HSP70 mRNA levels in the hypothalamus of sucrose-fed rats during acute restraint stress (Otaka et al. 2006), as well as HSP70 protein levels in rat heart and liver (Malyshev et al. 1995). Furthermore, Manucha and Valles (2008) reported that physiological levels of NO promoted a HSP70-mediated resistance to obstruction-induced cell death by inhibiting a mitochondrial apoptotic pathway. Because the diet and drinking water for piglets contained nitrite and nitrate, we could not determine their concentrations in plasma or the intestine as an indicator of systemic or local NO synthesis (Jobgen et al. 2007). However, based on the published evidence (Wu and Meininger 2002), a 28–37% increase in circulating levels of Arg (Table 5) is expected to stimulate NO production by both intestinal mucosal cells and the whole body of Arg- or NCG-supplemented piglets.

In summary, results of this study indicate that dietary supplementation with 0.6% Arg or 0.08% NAG enhances intestinal growth and HSP70 expression, prevents intestinal dysfunction, ameliorates weaning stress, and promotes growth performance in weanling pigs. Arg or NCG is a functional ingredient in the diet for these neonates to improve nutrition as well as gut integrity and health.

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