Published November 25, 2014

Dietary supplementation with N-carbamylglutamate increases the expression of intestinal amino acid transporters in weaned Huanjiang mini-pig piglets[1]

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ABSTRACT: Weaning is associated with reduced intestinal absorptive capacity in piglets. Our previous study indicated that dietary supplementation with N-carbamylglutamate (NCG) enhanced growth performance and improved intestinal function in weaned piglets. The present study was conducted to test the hypothesis that dietary supplementation with NCG may increase the growth performance of weaned piglets by regulating the expression of intestinal nutrient transporters, thus enhancing nutrient absorption. Twenty-four Huanjiang mini-pig piglets weaned at 21 d of age (3.17 ± 0.21 kg average BW) were randomly assigned to 2 dietary treatments consisting of a basal diet and the basal diet with 0.1% NCG supplementation for a 14-d period with 6 pens per treatment and 1 male and 1 female per pen. On d 14, 1 piglet was randomly selected from each pen for blood and tissue sampling. Dietary NCG supplementation enhanced ($P < 0.05$) growth rate and the efficiency of feed use in weaned Huanjiang mini-pig piglets. The NCG-supplemented diet increased ($P < 0.05$) mRNA expression levels of *Slc6a19*, *Slc7a9*, and *Slc1a1* and the protein abundance of ASCT2, B^0AT1, $b^{0,+}AT$, y^+LAT1, and EAAC1 in the jejunum. Furthermore, the contents of low density lipoprotein, ammonia, urea nitrogen, and AA as well as the activity of alkaline phosphatase in plasma were all altered ($P < 0.05$) by supplementation with NCG. These findings indicate that dietary supplementation with NCG may improve intestinal absorptive function in weaned piglets by increasing the expression of AA transporters in the intestine.

Key words: amino acid transporter, expression, Huanjiang mini-pig piglet, intestine, N-carbamylglutamate, weaning

 J. Anim. Sci. 2013.91:2740–2748
doi:10.2527/jas2012-5795

INTRODUCTION

Weaning is a crucial phase in swine production because piglets must rapidly adapt to dramatic changes in their social and physical environments (van Beers-Schreurs et al., 1998; Moeser et al., 2007). The combined effects of these stressors may change the gastrointestinal conditions and adversely affect the health and welfare of postweaned piglets via a reduction in feed intake and growth performance and an increased susceptibility to diseases (Jones et al., 2001). This is especially critical in modern swine production systems, in which piglets are weaned at around 21 d of age. Early weaning results in villous atrophy and a sustained impairment of intestinal barrier function, which consequently reduces gut digestive and absorptive capacities (Pluske et al., 1997; van Beers-Schreurs et al., 1998).

[1]This work was supported by the National Basic Research Program of China (2012CB124704 and 2013CB127301), National Natural Science Foundation of China (No. 31110103909, 30901040, 30928018, 2013CB127301, and 31101729), and the Chinese Academy of Sciences Visiting Professorship for Senior International Scientists Grant (No. 2011T2S15).

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Received August 29, 2012.
Accepted February 19, 2013.

The intestines, muscles, and liver are the major organs that are involved in the digestion, absorption, and metabolism of dietary nutrients (Jobgen et al., 2006; Wang et al., 2008). The core process for the absorption of dietary AA or peptides is mainly mediated by specific transporters (Bröer, 2008). The *Slc6a19* and *Slc1a5*, *Slc7a9* and *Slc7a7*, *Slc1a1*, and *Slc15a1* have been identified as the major intestinal transporters for neutral, basic, and acidic AA and peptides, respectively (Bröer, 2008). Plasma biochemical variables and tissue AA contents reflect physiological conditions and the nutritional state in animals (He et al., 2009, 2012). In weaned piglets, N-carbamylglutamate (**NCG**) has been reported to enhance intestinal growth and integrity as well as the availability of dietary nutrients for whole BW gain (Liu et al., 2012; Wu et al., 2012). In the present study, we hypothesized that dietary supplementation with NCG would increase the growth performance of weaned Huanjiang mini-pig piglets by regulating nutrient absorption. Therefore, the objective of the present study was to determine the growth performance, the jejunal expression of AA and peptide transporters, and plasma biochemical variables and tissue AA contents in weaned Huanjiang mini-pig piglets fed a control or NCG-supplemented diet.

MATERIALS AND METHODS

The experimental design and procedures in this study were reviewed and approved by the Animal Care and Use Committee of the Institute of Subtropical Agriculture, Chinese Academy of Sciences (Yin et al., 2010b).

Animals and Experimental Treatments

Twenty-four Huanjiang mini-pig piglets were weaned at 21 d of age (3.17 ± 0.21 kg average BW) and randomly assigned to 1 of 2 treatments consisting of a basal diet or the basal diet supplemented with 0.1% NCG for a 14-d period. The basal diet met the NRC (1998) nutrient specifications for 5- to 10-kg BW pigs. There were 6 pens per diet (1 male and1 female/pen). Piglets had free access to feed and drinking water at all times throughout the experimental period. Troughs were weighed back and feed was added daily to determine pen feed intake. The doses of NCG (Changsha Xianlong Biological Technology Co., Ltd., Hunan, China) were based on the results of previous studies with young pigs (Wu et al., 2012).

Sample Collection and Plasma Metabolite Analysis

At 35 d of age, the BW of piglets in individual pens was determined immediately before feeding. One hour after the last meal, 6 piglets (3 males and 3 females) were randomly selected from each treatment group (1 pig/pen) for blood and tissue sampling according to the procedure of Yin et al. (2001). Blood samples were collected into 10-mL heparin-coated tubes via jugular vein puncture and centrifuged at 3,000 × *g* and 4°C for 10 min to recover plasma. Plasma samples were immediately stored at –70°C until required for the analysis of AA and biochemical variables. Piglets were then held under general anesthesia and killed (Yang et al., 2012) by an intravenous (jugular vein) injection of 4% sodium pentobarbital solution (40 mg/kg BW; Kong et al., 2007). Samples (approximately 10 g of each tissue) of the LM, liver, and jejunum (after being cleaned with ice-cold PBS) were collected and immediately frozen in liquid N and stored at –70°C until required for analysis (Tan et al.,2009). Longissimus muscle and liver were analyzed to determine their AA contents, and the jejunum, the major site of AA and peptide absorption (Silk et al., 1985), was analyzed to determine the expression of AA and peptide transporters (mRNA and protein abundances). To measure plasma metabolites, commercial kits (Sino-German Beijing Leadman Biotech Ltd., Beijing, China) for low density lipoprotein (LD9182), high density lipoprotein (HD9173), glucose (GL9211), ammonia (AM6100), triglycerides (TG7160), total protein (TP9020), albumin (LB9010), cholesterol (TC9150), urea nitrogen (BU9121), and alkaline phosphatase (AP9030) and an analyzer (Beckman CX4 Chemistry Analyzer; Beckman Coulter, Brea, CA) were used.

Determination of AA Contents in Plasma, Liver, and Muscle

Plasma AA contents were determined as previously described (Kong et al., 2009b; Yin et al., 2010a). Briefly, 1 mL of plasma and 2.5 mL of 7.5% trichloroacetic acid were mixed thoroughly and centrifuged at 12,000 × *g* and 4°C for 15 min. The supernatant was filtered through a 0.45 μm membrane and then analyzed for AA using an ion-exchange AA analyzer (Hitachi, Tokyo, Japan). To measure the AA content in the muscle and liver, about 0.1 g of ground freeze-dried sample was hydrolyzed in 10 mL of 6 mol/L HCl at 110°C for 24 h. The solution was then adjusted to a volume of 100 mL and 1 mL of the settled solution was used for further analysis after 10-fold dilution. The solution was filtered through a 0.45-μm membrane before analysis (Kong et al., 2009a).

Extraction of RNA and cDNA Synthesis

Approximately 100 mg of tissue from each sample was pulverized in liquid N according to the method of Zhou et al. (2012; Yang and Yin, 2013). Total RNA

was isolated from homogenate using the TRIZOL reagent (100 mg tissue per 1 mL Trizol; Invitrogen, Carlsbad, CA). The integrity of RNA was checked by 1% agarose gel electrophoresis stained with 10 μg/mL ethidium bromide. The quality and quantity of RNA was determined by UV spectroscopy using a spectrophotometer (NanoDrop ND-1000; Thermo Fisher Scientific, Wilmington DE). The RNA (10 g) was treated with DNase I (Invitrogen) according to the manufacturer's instructions, and the treated RNA was then quantified using a spectrophotometer (NanoDrop ND-1000; Thermo Fisher Scientific), and 1 μg of DNA free RNA was used for reverse transcription and the PCR. First-strand cDNA was synthesized with Oligo (dT) 20 and Superscript II reverse transcriptase (Invitrogen).

Table 1. Primers used for real-time PCR analysis

Genes	Primers	Sequences (5′-3′)	Size
Slc1a5	Forward	GATTGTGGAGATGGAGGATGTGG	128 bp
	Reverse	TGCGAGTGAAGAGGAAGTAGATGAGA	
Slc6a19	Forward	TCTGTCCACAACAACTGCGAG	206 bp
	Reverse	CAGCGAAGTTCTCCTGCGTC	
Slc7a9	Forward	GAACCCAAGACCACAAATC	180 bp
	Reverse	ACCCAGTGTCGCAAGAAT	
Slc7a7	Forward	AGGAGAACCCACAGATTAGC	113 bp
	Reverse	GCGGAGGAGGAGAAGAA	
Slc1a1	Forward	GGCACCGCACTCTACGAAGCA	177 bp
	Reverse	GCCCACGGCACTTAGCACGA	
Slc15a1	Forward	CATCGCCATACCCTTCTG	144 bp
	Reverse	TTCCCATCCATCGTGACATT	
GAPDH	Forward	AAGGAGTAAGAGCCCCTGGA	140 bp
	Reverse	TCTGGGATGGAAACTGGAA	

Relative Quantification of mRNA Expression of AA and Peptide Transporters

A software (Oligo 6.0; Molecular Biology Insights, Cascade, CO) was used to design primers for neutral (*Slc6a19* and *Slc1a5*), basic (*Slc7a9* and *Slc7a7*), and acidic (*Slc1a1*) AA and peptide (*Slc15a1*) transporters (Table 1). After the quantity of cDNA was determined by a spectrophotometer (NanoDrop ND-1000; Thermo Fisher Scientific), real-time quantitative PCR analyses were performed (ABI 7900HT Fast Real-Time PCR System; Applied Biosystems, Carlsbad, CA) with a total volume of 10 μL containing 5 ng of cDNA, 5 μL SYBR Green mix, 0.2 μL ROX Reference Dye (50x), and 0.2 μL each of forward and reverse primers. After predenaturation (10 s at 95°C), 40 cycles of amplification were conducted, where each cycle consisted of 95°C for 5 s and 60°C for 20 s, followed by a melting curve program (60 to 99°C with a heating rate of 0.1°C/s and fluorescence measurement). In each sample, the amplification of glyceral dehyde-3-phosphate dehydrogenase (**GAPDH**) was used to normalize the expression of the selected genes, and no difference in cycle threshold (**Ct**) values was observed between control and NCG-treated piglets. The mRNA expression abundance (**A**) of target genes was calculated as $A = 2[^{Ct(GAPDH) - Ct(target)}]$. The efficiency of real-time reverse-transcription PCR was determined by the amplification of a dilution series of cDNA according to the equation $[10]^{(-1/slope)}$. Target mRNA and GAPDH mRNA are amplified with comparable efficiencies (Bustin et al., 2009; Wang et al., 2009; Liu et al.,2012). In negative controls, cDNA was replaced by water.

Quantification of the Protein Amounts of AA and Peptide Transporters

The frozen samples were powdered under liquid N and lysed in radioimmunoprecipitation assay buffer [150 m*M* NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, and 50 m*M* Tris-HCl at pH 7.4 plus a protease inhibitor cocktail (5892791001; Roche, Shanghai, China)]. After centrifugation at 10,000 × *g* and 4°C for 10 min, the protein concentration in the supernatant fluid was determined using an assay (Bicinchoninic Acid Assay; Beyotime Biotechnology, Beijing, China). All samples were adjusted to an equal protein concentration and then diluted with 2x loading buffer [0.63 mL of 0.5 *M* Tris-HCl (pH 6.8), 0.42 mL 75% glycerol, 0.125 g SDS, 0.25 mL β-mercaptoethanol, 0.2 mL 0.05% solution of bromophenol blue, and 1 mL water] to a final volume of 2.5 mL and heated in boiling water for 5 min. After the solution was cooled with ice, it was used for Western blot analysis (Yang and Yin, 2012; Fu et al., 2013).

The denatured proteins were separated using SDS-PAGE (10% gradient gel) and transferred to poly vinylidene fluoride (**PVDF**) membranes (Millipore, Billerica, MA) overnight at 12 V using an apparatus (Bio-Rad Transblot; Hercules, CA). The membranes were blocked in 5% fat-free milk in Tris-Tween buffered saline (**TTBS**; 20 m*M* Tris/150 m*M* NaCl, pH 7.5, and 0.1% Tween-20) for 3 h and then incubated with ASCT2, B^0AT1, $b^{0,+}AT$, y^+LAT1, EAAC1, PEPT1, or β-actin antibody (Table 2) at 4°C overnight with gentle rocking. After being washed 3 times with TTBS, the membranes were incubated at room temperature for 2 h with horseradish peroxidase-linked secondary antibodies. Finally, the membranes were washed with TTBS and then developed (Supersignal West Dura Extended Duration Substrate; Pierce, Rockford, IL). The images were detected by chemiluminescence (Applygen Technologies Inc., Beijing, China). For size estimation, a prestained protein standard (10–160 kDa, SM0671; Fermentas, Vilnius, Lithuania) was used. Multiple exposures were obtained for each Western blot to ensure the linearity of chemiluminescence

Table 2. Antibodies used for Western blot analyses

Antiprotein	Gene	Catalog number	Dilution
Primary antibodies			
ASCT2	*Slc1a5*	sc130963[1]	1:500
B^0AT1	*Slc6a19*	sc160811[1]	1:1,000
$b^{0,+}AT$	*Slc7a9*	ab40033[2]	1:1,000
y^+LAT1	*Slc7a7*	sc34551[1]	1:500
EAAC1	*Slc1a1*	ab78395[2]	1:1,000
PEPT1	*Slc15a1*	sc19917[1]	1:500
β-actin	β-*actin*	sc47778[1]	1:1,000
Secondary antibodies			
Chicken IgG		14-24-06[3]	1:3,000
Rabbit IgG		sc-2004[1]	1:3,000
Goat IgG		sc-2768[1]	1:3,000
Mouse IgG		ab6789[2]	1:3,000

[1]Santa Cruz Biotechnology, Inc. (Santa Cruz, CA).
[2]Abcam, Cambridge, UK.
[3]KPL, Gaithersburg, MD.

signals. Western blots were quantified by measuring the intensity of correctly sized bands software (AlphaImager 2200; Alpha Innotech Corporation, CA). For PEPT1 and $b^{0,+}AT$, more than 1 band was observed because polyclonal antibodies were used, and the bands were identified based on their molecular weight.

Statistical Analysis

All data were subjected to a *t* test (SAS Inst. Inc., Cary, NC). Data are presented as means ± SEM, and *P*-values < 0.05 were used to indicate statistical significance.

Table 3. Effects of dietary supplementation with N-carbamylglutamate (NCG) on growth performance of weaned Huanjiang mini-pig piglets[1,2]

	Dietary treatment		
Item	Control	NCG	*P*-value
BW, kg			
Initial	3.2 ± 0.2	3.2 ± 0.1	0.817
Final	4.3 ± 0.3	4.5 ± 0.2	0.524
ADFI, g			
d 0 to 7	139 ± 15	139 ± 7	0.997
d 7 to 14	207 ± 14	205 ± 15	0.917
d 0 to 14	173 ± 14	172 ± 13	0.948
ADG, g/d			
d 0 to 7	60 ± 6	57 ± 8	0.834
d 7 to 14	98 ± 2	124 ± 6	0.021
d 0 to 14	79 ± 4	91 ± 2	0.034
G:F, g/g			
d 0 to 7	0.467 ± 0.026	0.415 ± 0.049	0.373
d 7 to 14	0.582 ± 0.039	0.723 ± 0.042	0.034
d 0 to 14	0.523 ± 0.010	0.597 ± 0.029	0.049

[1]NCG = diet with 0.1% N-carbamylglutamate.
[2]*n* = 6.

RESULTS

Growth Performance and Plasma Biochemical Variables

During the first week (d 0 to 7), feeding the NCG-supplemented diet had no effect on ADFI, ADG, or G:F in weaned piglets (Table 3). During the second week (d 7 to 14), dietary supplementation with NCG improved ADG ($P = 0.021$) and G:F ($P = 0.034$) in weaned piglets but had no effect on ADFI. Consumption of the NCG-supplemented diet for 2 wk (d 0 to 14) increased ADG ($P = 0.034$) and G:F ($P = 0.049$) in piglets but had no effect on feed intake compared with the control group. Dietary supplementation with NCG also increased the activity of alkaline phosphatase ($P = 0.048$) and decreased the plasma contents of low density lipoprotein ($P = 0.007$), ammonia ($P = 0.043$), and urea N ($P = 0.023$) in weaned piglets (Table 4). There were no differences in the plasma contents of high density lipoprotein, glucose, triglycerides, total protein, albumin, or cholesterol between the control and NCG-supplemented groups.

Amino Acid Contents in Plasma, Liver, and Skeletal Muscle

The contents of AA in the plasma, liver, and muscle of weaned Huanjiang mini-pig piglets are shown in Table 5. Piglets that were fed the NCG-supplemented diet had greater plasma contents of Cys ($P = 0.022$), Val ($P = 0.040$), Met ($P = 0.031$), Ile ($P = 0.047$), Phe ($P = 0.034$), and Arg ($P = 0.026$) and decreased contents of Pro ($P = 0.044$), Glu ($P = 0.028$), Gly ($P = 0.007$), and Ala ($P = 0.015$) compared with the control. No differences in the AA contents of the liver and muscle were observed between the control and the NCG-supplemented groups.

Expression of mRNA and Protein Amounts of AA and Peptide Transporters

The mRNA abundance of *Slc6a19*, *Slc7a9*, and *Slc1a1* in the jejunum of the NCG-supplemented piglets were greater ($P < 0.05$) than those in the control group (Fig. 1). There were no differences in the mRNA expression of *Slc1a5*, *Slc7a7*, or *Slc15a1* between the control and NCG-supplemented groups. Dietary supplementation with NCG increased ($P < 0.05$) the protein abundance of ASCT2, B^0AT1, $b^{0,+}AT$,y^+LAT1, and EAAC1 in the jejunum but had no effect on the protein abundance of PEPT1 (Fig. 2).

DISCUSSION

This study investigated the effects of dietary supplementation with NCG on the expression of AA and peptide transporters in the jejunum of weaned Huanjiang mini-pig piglets. The present results confirmed previous findings that dietary supplementation with NCG increases the growth rate and alters the plasma contents of urea N and ammonia in piglets (Frank et al., 2007) and further demonstrated that the increased growth rate was accompanied by alterations of the plasma contents of some AA (i.e., Glu, Gly, Ala, Cys, Val, Met, Ile, Phe, Arg, and Pro), increased efficiency of feed conversion, and the expression of some AA transporters. These findings indicated that dietary supplementation with NCG may improve the absorptive functions in weanling piglets by increasing the expression of AA transporters in the intestine.

Weaning is associated with reduced feed consumption by piglets (Miller et al., 1986), which decreases the intake of nutrients, including AA. Notably, weaned piglets often experience intestinal dysfunction and atrophy (Gu et al., 2002). A recent study showed that dietary supplementation with NCG enhanced intestinal growth and Heat shock 70 kDa protein (**HSP70**) expression and increased villus height and crypt depth in the small intestine (Wu et al., 2010, 2013a,b), thus increasing the absorption area in the intestine in weaned piglets. Supplementation with NCG also increased the number of goblet cells, which 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). Dietary AA and peptides are absorbed via their respective transporter systems. The neutral, basic, and acidic systems are the 3 main systems for AA absorption in the intestine and are involved in transporting all neutral AA, cationic AA with cysteine, and glutamate and aspartate, respectively (Bröer, 2008). The B^0AT1 and ASCT2, $b^{0,+}AT$ and y^+LAT1, and EAAC1 are, respectively, the major members of these systems. In the present study, supplementation with NCG increased the efficiency of feed efficiency and the expression of some AA transporters in weaned Huanjiang mini-pig piglets, which indicates that NCG not only improves the health

Table 4. Effects of dietary supplementation with N-carbamylglutamate (NCG) on plasma biochemical variables in weaned Huanjiang mini-pig piglets[1,2]

	Dietary treatment		
Item	Control	NCG	*P*-value
Alkaline phosphatase, units/L	302.3 ± 11.3	342.6 ± 15.1	0.048
Low density lipoprotein, mmol/L	0.81 ± 0.04	0.63 ± 0.03	0.007
High density lipoprotein, mmol/L	0.78 ± 0.13	0.62 ± 0.06	0.311
Glucose, mmol/L	6.92 ± 0.35	6.86 ± 0.61	0.858
Ammonia, μmol/L	87.13 ± 1.67	82.40 ± 0.91	0.043
Triglycerides, mmol/L	0.48 ± 0.05	0.56 ± 0.09	0.463
Total protein, g/L	51.08 ± 2.68	52.23 ± 2.45	0.758
Albumin, g/L	29.80 ± 1.31	29.38 ± 1.36	0.830
Cholesterol, mmol/L	1.62 ± 0.18	1.51 ± 0.12	0.624
Urea nitrogen, mmol/L	3.97 ± 0.13	3.35 ± 0.15	0.023

[1]NCG = diet with 0.1% N-carbamylglutamate.

[2]$n = 6$.

Table 5. Effects of dietary supplementation with N-carbamylglutamate (NCG) on AA contents of plasma, liver, and muscle in weaned Huanjiang mini-pig piglets[1,2]

	Dietary treatment								
	Liver, %		Muscle, %		Plasma, μmol/L		*P*-value		
Item	Control	NCG	Control	NCG	Control	NCG	Liver	Muscle	Plasma
Asp	204.7 ± 8.7	203.9 ± 10.9	238.3 ± 2.8	240.1 ± 4.9	46.5 ± 3.2	42.4 ± 2.8	0.995	0.760	0.769
Thr	99.5 ± 4.6	100.0 ± 5.6	119.9 ± 1.5	119.3 ± 2.8	371.5 ± 16.4	381.4 ± 18.9	0.944	0.871	0.798
Ser	92.3 ± 4.4	89.5 ± 7.3	100.6 ± 1.4	97.7 ± 2.7	161.1 ± 12.4	137.6 ± 17.6	0.751	0.363	0.490
Glu	312.8 ± 13.8	294.4 ± 25.2	423.1 ± 6.2	430.2 ± 8.9	509.0 ± 17.3	457.2 ± 15.2	0.534	0.530	0.028
Gly	119.6 ± 4.8	118.8 ± 4.0	117.4 ± 1.5	119.5 ± 1.5	1,147.4 ± 28.5	944.5 ± 20.6	0.902	0.333	0.007
Ala	123.8 ± 5.7	126.4 ± 1.2	143.0 ± 1.7	144.4 ± 2.1	651.7 ± 25.2	571.2 ± 18.7	0.659	0.627	0.015
Cys	65.4 ± 1.7	65.5 ± 0.8	71.6 ± 0.3	71.0 ± 0.9	138.4 ± 12.5	163.4 ± 8.0	0.924	0.537	0.022
Val	146.5 ± 5.5	146.1 ± 4.3	147.8 ± 1.7	151.1 ± 2.1	324.8 ± 18.0	362.9 ± 17.5	0.963	0.266	0.040
Met	56.9 ± 2.4	58.5 ± 1.1	59.6 ± 0.8	59.3 ± 0.9	52.3 ± 8.7	69.3 ± 4.5	0.553	0.770	0.031
Ile	113.4 ± 4.4	112.2 ± 4.2	125.9 ± 2.2	129.4 ± 2.6	123.4 ± 11.8	151.7 ± 14.1	0.840	0.337	0.047
Leu	210.0 ± 8.9	208.9 ± 7.0	208.1 ± 2.7	209.3 ± 4.5	178.1 ± 17.0	190.4 ± 18.3	0.927	0.828	0.771
Tyr	89.8 ± 3.8	92.9 ± 2.6	104.5 ± 7.2	88.0 ± 10.8	176.2 ± 19.3	163.3 ± 15.0	0.527	0.232	0.833
Phe	165.8 ± 3.9	163.1 ± 4.8	121.0 ± 1.2	119.9 ± 2.0	101.0 ± 6.9	121.9 ± 5.6	0.676	0.646	0.034
Lys	190.4 ± 7.8	191.9 ± 6.9	217.4 ± 4.5	220.5 ± 5.4	251.7 ± 17.4	243.7 ± 9.5	0.884	0.666	0.681
His	73.2 ± 2.8	72.5 ± 2.5	114.1 ± 3.2	112.0 ± 5.5	120.5 ± 7.6	111.1 ± 7.4	0.853	0.754	0.667
Arg	131.8 ± 6.5	134.3 ± 3.1	157.7 ± 2.3	160.0 ± 3.6	169.0 ± 14.2	203.8 ± 12.4	0.743	0.609	0.026
Pro	171.0 ± 11.5	165.6 ± 3.4	166.1 ± 1.7	168.5 ± 2.0	639.4 ± 24.8	515.9 ± 18.3	0.672	0.375	0.044

[1]NCG = diet with 0.1% N-carbamylglutamate

[2]$n = 6$.

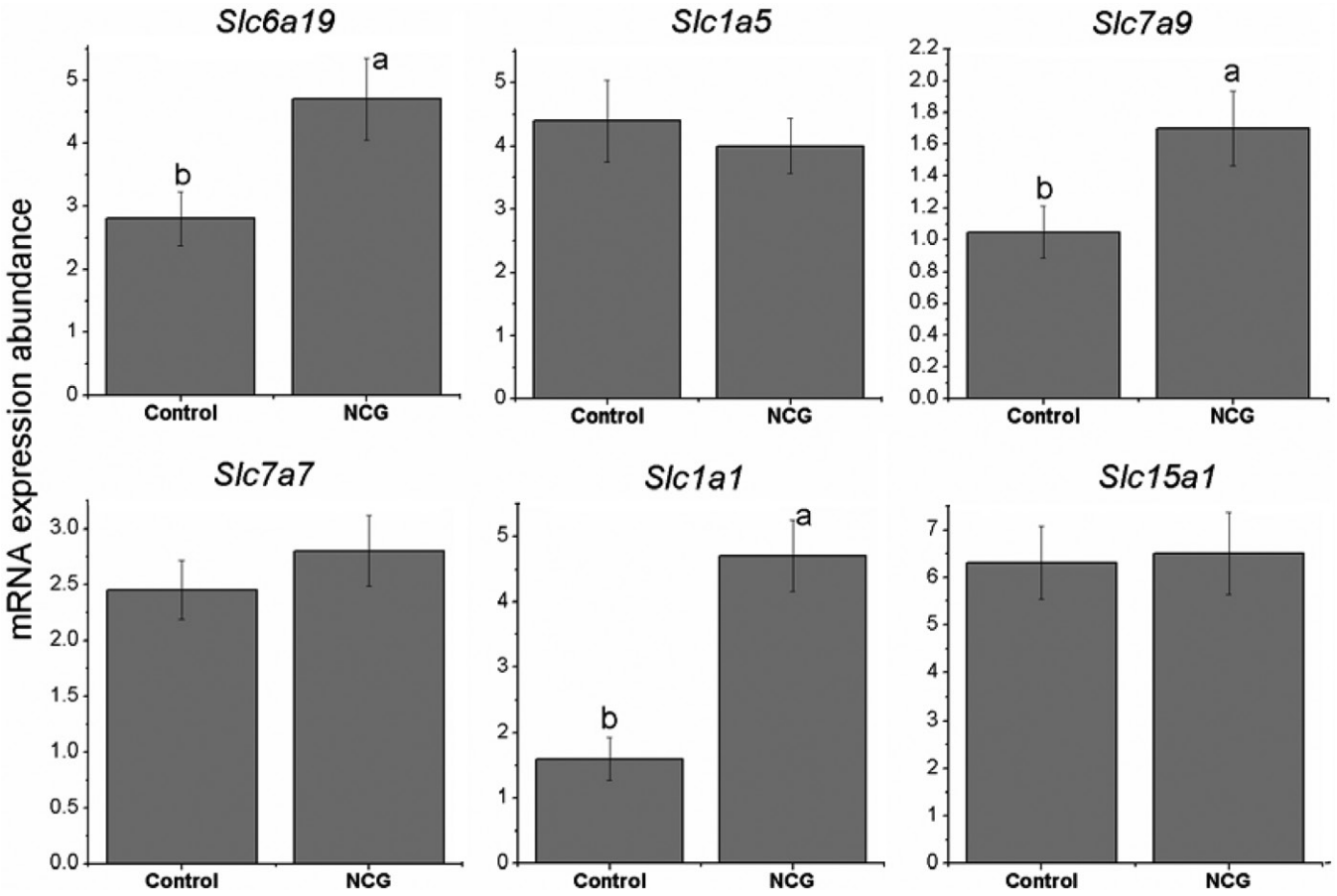

Figure 1. Effects of dietary supplementation with N-carbamylglutamate (NCG) on mRNA expression of jejunal AA and peptide transporters in weaned Huanjiang mini-pig piglets. The mRNA expression abundances of *Slc6a19*, *Slc1a5*, *Slc7a9*, *Slc7a7*, *Slc1a1*, and *Slc15a1* were normalized using *GAPDH* as an internal control. [a,b]Within a variable, values with different superscripts differ ($P < 0.05$). Data are expressed as means ± SEM; $n = 6$.

and growth of the intestine, but may also ameliorate the absorptive functions of weaned piglets. Supplementation with NCG increased mRNA expression of *Slc6a19*, *Slc7a9*, and *Slc1a1* and protein abundance of ASCT2, B^0AT1, $b^{0,+}AT$,y^+LAT1, and EAAC1 in the jejunum of weaned Huanjiang mini-pig piglets. The differences between mRNA expression and protein abundance of the regulated genes may be due to the differences in the translational mechanisms of these genes. Further studies are needed to test the mechanism involved in gene regulation and to determine whether or not other genes related to nutrient absorption are also regulated by supplementation with NCG.

Supplementation with NCG has been reported to enhance the growth performance of both nursing and weaned piglets (Frank et al., 2007). For example, when nursing piglets were given a creep feed supplemented with NCG, plasma Arg and ST contents were increased as was protein synthesis in skeletal muscle, which is likely the primary mechanism that underlies the increased growth performance of NCG-fed piglets (Frank et al., 2007). Furthermore, Wu et al. (2007) suggested that the enhanced growth associated with dietary supplementation with NCG may be mediated through improved intestinal function, nutrition, and health in weaned piglets. Because supplementation with NCG enhanced the efficiency of feed conversion and intestinal AA transporters in the present study, the observed improvement in growth performance because of supplementation with NCG may be caused by the enhancement of intestinal nutrient uptake.

Arginine is not only an essential building block for tissue protein but also serves as an important precursor for the synthesis of creatine, polyamines, and nitric oxide (Wu and Morris, 1998; Kong et al., 2012). However, dietary supplementation with high doses of Arg cause a reduction in the absorption of other indispensable AA (e.g., Lys and Trp) that share the same transport systems in pig enterocytes (Wu et al., 2004, 2007). Therefore, it is possible that supplementation with NCG may be a viable nutritional strategy for increasing Arg or citrulline in suckling piglets. Arginine has been shown to promote wound healing (MacKay and Miller, 2003; Witte and Barbul, 2003) and has been reported to enhance intestinal epithelial cell proliferation and migration, which is associated with the repair of damaged intestinal villi (Rhoads et al., 2008; Tan et al., 2010). Because weaning

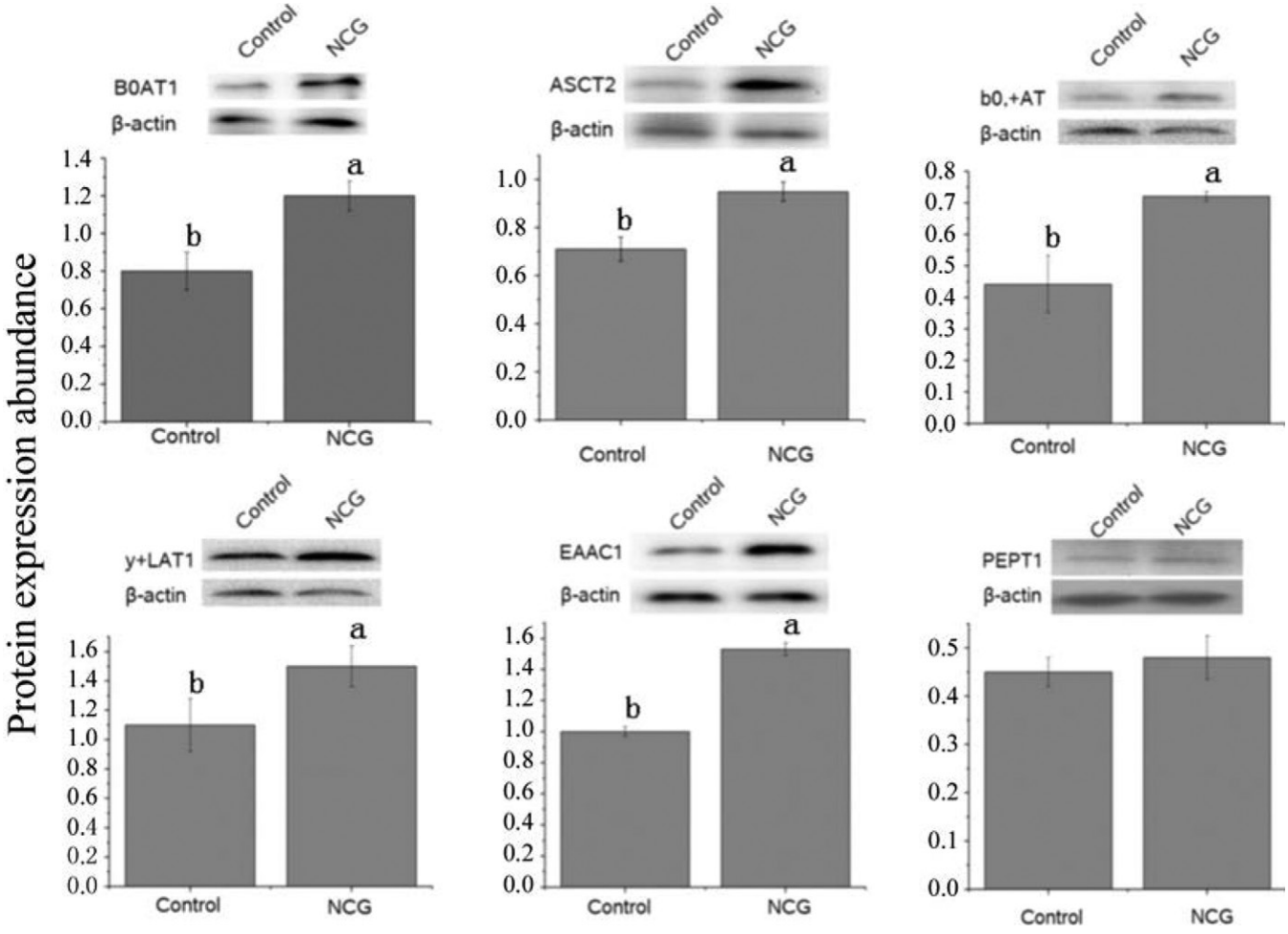

Figure 2. Effects of dietary supplementation with N-carbamylglutamate (NCG) on the protein abundance of jejunal AA and peptide transporters in weaned Huanjiang mini-pig piglets. The protein abundances of ASCT2, B^0AT1, $b^{0,+}AT$,y^+LAT1, EAAC1, and PEPT1 were normalized using β-actin as an internal control. [a,b]Within a variable, values with different superscripts differ ($P < 0.05$). Data are expressed as means ± SEM; $n = 6$.

results in the sustained impairment of intestinal barrier function and dietary supplementation with Arg and NCG enhances the integrity of the intestinal barrier (Pluske et al., 1997; van Beers-Schreurs et al., 1998; Wu et al., 2010), NCG may regulate the expression of AA transporters in the jejunum of weaned piglets by facilitating the repair of intestinal villi.

The NCG is a metabolically stable analog of N-acetylglutamate, which plays an important role in regulating Arg synthesis by activating carbamylphosphate synthase (Cohen and Grisolia, 1950; Wu et al., 2004). In the present study, supplementation with NCG in weaned piglets clearly increased the plasma content of Arg. This increase was accompanied by decreases in the concentrations of Glu and Pro, which are precursors for the synthesis of Arg in enterocytes of piglets (Wu et al., 2004). In agreement with a previous report that piglets fed a NCG-supplemented diet had a lower plasma content of ammonia (Frank et al., 2007), dietary supplementation with NCG in the present study reduced the concentrations of plasma ammonia and urea N in weaned piglets. These results indicated that NCG-treated weaned piglets have greater rates of net protein synthesis and a lower rate of AA catabolism. Those results indicate that, by regulating the intestinal expression of nutrient transporters, NCG may enhance the absorption of nutrients, which further results in metabolic and physiological changes and increases the efficiency of feed use and growth performance of weaned piglets.

In conclusion, supplementation of weaned pigs with NCG increased growth rate and feed efficiency of Huanjiang mini-pig piglets. This response was likely mediated through regulation of the expression of some of the genes related to nutrient absorption. These findings indicate that NCG may serve as a functional ingredient in the diet to improve the intestinal absorptive capacity in weaned piglets.

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