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ORIGINAL ARTICLE

Effects of dietary L-lysine intake on the intestinal mucosa and expression of CAT genes in weaned piglets

Liuqin He · Huansheng Yang · Yongqing Hou · Tiejun Li · Jun Fang · Xihong Zhou · Yulong Yin · Li Wu · Martin Nyachoti · Guoyao Wu

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Abstract The objective of this study was to evaluate effects of dietary L-lysine on the intestinal mucosa and expression of cationic amino acid transporters (CAT) in weaned piglets. Twenty-eight piglets weaned at 21 days of age (Duroc × Landrace × Yorkshire; 6.51 ± 0.65 kg body weight) were assigned randomly into one of the four groups: Zein + LYS (zein-based diet + 1.35 % supplemental lysine), Zein − LYS (zein-based diet), NF (nitrogen-free diet), and CON (basal diet). The experiment lasted for 3 weeks, during which food intake and body weight were recorded. At the end of the trial, blood was collected from the jugular vein of all pigs, followed by their euthanasia. Dietary supplementation with lysine enhanced villus height and crypt depth in the jejunum ($P < 0.05$). Jejunal mRNA levels for the $b^{0,+}$-AT, y^+LAT1 and CAT1 genes were greater ($P < 0.05$) in the Zein + LYS group than in the control, and the opposite was observed for CAT1. Dietary content of lysine differentially affected intestinal CAT expression to modulate absorption of lysine and other basic amino acids. Thus, transport of these nutrients is a key regulatory step in utilization of dietary protein by growing pigs and lysine in the diet influences the expression of amino acid transporters in the small intestine.

Keywords Pigs · Digestibility · Cationic amino acids · Intestinal mucosa · Transporters

Abbreviations
AA Amino acids
CAT Cationic amino acid transporters

Introduction

Establishing optimal requirements for amino acids (AA) is critically important for maximizing performance of weaned piglets (Kim et al. 2005). Diets for piglets should be formulated to meet requirements for both nutritionally essential and non-essential AA in an ideal pattern (Wu

L. He · H. Yang · T. Li (✉) · Y. Yin (✉) · L. Wu · G. Wu
Key Laboratory of Agroecology in Subtropical Region, Scientific Observing and Experimental Station of Animal Nutrition and Feed Science in South-Central China, Ministry of Agriculture, Hunan Engineering and Research Center for Animal and Poultry Sciences, Institute of Subtropical Agriculture, Research Center for Healthy Breeding of Livestock and Poultry, The Chinese Academy of Science, Changsha 410125, Hunan, China
e-mail: tjli@isa.ac.cn

Y. Yin
e-mail: yinyulong@isa.ac.cn

Y. Hou · Y. Yin
Hubei Key Laboratory of Animal Nutrition and Feed Science, Wuhan Polytechnic University, Wuhan 430023, China

J. Fang
College of Bioscience and Biotechnology, Hunan Agricultural University, Changsha 410128, China

X. Zhou
Key Laboratory of Molecular Animal Nutrition, Institute of Feed Science, Zhejiang University, Hangzhou 310012, Zhengjiang, China

M. Nyachoti
Department of Animal Science, University of Manitoba, Winnipeg, Canada R3T 2N2, MB

G. Wu
Department of Animal Science, Texas A&M University, College Station, 77843 College Station, TX, USA

et al. 2013). Lysine is typically the first limiting AA in swine diets (Gatrell et al. 2013; Wu 2010a) and, therefore, it is important to understand how dietary lysine is absorbed by the small intestine of piglets (He et al. 2009; Li et al. 2009, 2011a, b; Wu et al. 2009).

Amino acids are transported by neutral, acidic, or basic AA transporters (Kanai and Endou 2003; Seow et al. 2004; Wu 2013a). Cationic amino acid transporters (CAT) are widely distributed in tissues and play an important role in the transport of arginine, histidine, lysine, and ornithine to regulate their homeostasis (Russell et al. 2003). The different carrier proteins mediating transport of cationic AA include Na^+-independent systems y^+, y^+L, $b^{0,+}$, and b^+, as well as the Na^+-dependent system $B^{0,+}$ (Mann et al. 2003; Wu et al. 2009). There are many reports which indicated that the importance of CAT in the small intestine to maintain homeostasis of basic AA and overall protein nutrition in the body (Bröer et al. 2000).

Complex relationships exist among neutral and cationic AA transporters (François et al. 2004). However, no studies have examined effects of dietary intake of L-lysine on expression of CAT in the small intestine of developing pigs, particularly in response to the feeding of zein as the exclusive source of protein. Therefore, the present study was conducted with weaned pigs to fill in this gap of knowledge and also to evaluate effects of dietary L-lysine intake on growth performance, as well as intestinal mucosal morphology and CAT expression.

Materials and methods

The experimental design and procedures of this study were reviewed and approved by the Animal Care and Use Committee of the Institute of Subtropical Agriculture, The Chinese Academy of Sciences.

Animals and dietary treatment

Twenty-eight piglets (offspring of Duroc × Landrace × Yorkshire) with body weight (BW) of 6.51 ± 0.65 kg (mean ± SD) were weaned at 21 days of age and were assigned randomly to one of the four dietary treatments (7 pigs/treatment). The young pigs were housed individually in cages and were allowed for a 1-week period of adaptation before being weighed and randomly allocated to dietary treatments (Table 1). Dietary treatments consisted of a basal diet (CON) formulated to meet nutrient specifications for weaned piglets (NRC 1998), two diets containing zein (Wujiang Bache Drug Company, Jiangsu, China) supplemented with 1.35 % lysine (the Zein + LYS group) or without lysine (the Zein − LYS group), and a N-free (NF) diet (Table 1). The zein-containing diets were formulated to contain similar nutrient levels (except for lysine in the Zein − LYS diet) to those in the CON group, but the sources of dietary protein were different from the CON group (Table 1). Zein contained the following nutrients (%, as-fed basis): dry matter, 94.73; crude protein, 85.2; Lys, 0.08; His, 1.91; Arg, 1.43; Val, 2.90; Ile, 3.00; and Leu, 19.2. Pigs were fed their respective diets for 3 weeks, during which feed intake and BW were measured weekly.

Table 1 Ingredient and calculated nutrient composition of the experimental diets (as-fed basis)

Item	Diet treatment[a]			
	Zein + LYS	Zein − LYS	NF	CON
Ingredient, %				
Corn starch	49.05	50.40	72.40	–
Zein	22.00	22.00	–	–
Corn	–	–	–	57.34
Soybean meal, 43.8 % CP	–	–	–	14.89
Saccharose	15.00	15.00	15.00	15.00
Soy oil	4.00	4.00	4.00	4.00
Limestone	1.00	1.00	1.00	1.00
Salt	0.50	0.50	0.50	0.50
Titanium dioxide	0.10	0.10	0.10	0.10
GB2	4.00	4.00	4.00	4.00
Vitamin-mineral premix[b]	1.00	1.00	1.00	1.00
$CaHPO_4$	2.00	2.00	2.00	2.00
L-Lysine·HCl	1.35	–	–	0.17
Calculated composition				
Digestible energy, MJ/kg	17.18	17.26	17.14	17.24
Crude protein, %	20.20	20.30	1.60	20.20
Lys, %	1.35	0.00	0.00	1.35
Met, %	0.30	0.30	0.00	0.30
Met + Cys, %	0.65	0.65	0.00	0.65
Thr, %	1.05	1.05	0.00	1.05
Trp, %	0.25	0.25	0.00	0.25
Ca, %	0.80	0.80	0.80	0.80
P, %	0.69	0.69	0.69	0.69

[a] Dietary treatments: Zein + LYS, diet containing zein with L-lysine supplementation; Zein − LYS, diet containing zein without L-lysine supplementation; *NF* nitrogen-free diet; CON, basal diet

[b] Supplied per kilogram of finished feed: vitamin A, 10,800 IU; vitamin D3, 4,000 IU; vitamin E, 40 IU; vitamin K3, 4 mg; vitamin B1, 6 mg; vitamin B2, 12 mg; vitamin B6, 6 mg; vitamin B12, 0.05 mg; biotin, 0.2 mg; folic acid, 2 mg; niacin, 50 mg; D-Calcium pantothenate, 25 mg; Fe, 100 mg as ferrous sulfate; Cu, 150 mg as copper sulfate; Mn, 40 mg as manganese oxide; Zn, 100 mg as zinc oxide; I, 0.5 mg as potassium iodide; and Se, 0.3 mg as sodium selenite

Tissue sample collection

At the end of the trial when pigs were 42-day-old, blood was collected from their jugular vein, followed by euthanasia, as described previously (Geng et al. 2011; Yin et al. 2010c). After euthanasia, the entire intestine and viscera were rapidly removed and dissected free of mesenteric attachments and placed on a smooth, cold surface tray (Hou et al. 2012; Wang et al. 2008). Intestinal segments (10 cm) were obtained from the proximal jejunum and the distal ileum, which were then thoroughly flushed with sterile saline (Yang et al. 2011; Zhou et al. 2011), immediately frozen in liquid nitrogen, and stored at −80 °C for analysis of gene expression or fixed in 10 % neutral buffered formalin for examination of intestinal morphology.

Intestinal morphology

Formalin-fixed jejunum and ileum samples were embedded in paraffin; cross sections of the segments were cut approximately 5 mm thick using a microtome and stained with hematoxylin and eosin (Wu et al. 1996). In each section, villus height and crypt depth were measured using a light microscope with a computer-assisted morphometric system (Yao et al. 2011). Villus height was defined as the distance from the villus tip to the crypt mouth, and crypt depth from the crypt mouth to the base (Zhou et al. 2011).

RNA extraction and cDNA synthesis for b^{0+}-AT, CAT1, and y + LAT1 in the intestine

Intestinal and liver tissue samples were pulverized in liquid nitrogen. Total RNA was isolated from about 100 mg of the powder using TRIZOL reagent (Invitrogen, Carlsbad, CA, USA) and treated with DNase I (Invitrogen) according to the manufacturer's instructions. Total RNA was reversed into cDNA using a SuperScript First-Strand Synthesis System kit (Invitrogen). Polymerase chain reaction amplification was performed in a total volume of 50 μl including Taq DNA polymerase and specific primers. Primers for $b^{0,+}$-AT, CAT-1, y^+LAT-1, and β-actin were designed with Primer 5.0 (Wang et al. 2009c). The following sequences of PCR primer pairs are used: forward:5′-GA ACCCAAGACCACAAATC-3′, reverse: 5′-ACCCAGTG TCGCAAGAAT-3′ for $b^{0,+}$-AT (180 bp); forward: 5′-CA TCAAAAACTGGCAGCTCA-3′, Reverse: 5′-TGGTAG CGATGCAG TCAAAG-3′ for CAT-1 (185 bp); forward: 5′-TTTGTTATGCGGAACTGG-3′, reverse: 5′-AAAGG TGATGGCAATGAC-3′ for y^+LAT1 (180 bp); forward: 5′-GGATGCAGA AGGAGAT CACG-3′, reverse: 5′-ATC TGCTGGAAGGTGGACAG-3′ for β-actin (145 bp).

Quantification of mRNA levels was performed using real-time PCR analysis

The quality of RNA was assessed by 1 % agarose gel electrophoresis, and stained with 10 μg/mL ethidium bromide. In all samples, RNA had an OD260:OD280 ratio between 1.8 and 2.0. Synthesis of the first strand of cDNA was performed using Oligo(dT)-20 and Superscript II reverse-transcriptase (Invitrogen). Primers for porcine $b^{0,+}$-AT, CAT-1, and y^+LAT1 cDNA sequences were designed to generate amplification products. β-actin was used as an internal reference gene to normalize target gene transcript levels. Real-time PCR was performed using the SYBR Green detection kit, containing $MgCl_2$, dNTP, and Hotstar Taq polymerase. An aliquot (1 μL) of cDNA template solution was added to a total volume of 10 μL containing 5 μL of the SYBR Green mix, and 0.4 μL each of forward and reverse primers. The following protocol was used: (i) pre-denaturation program (30 s at 95 °C); (ii) amplification and quantification program, repeated 40 cycles (5 s at 95 °C, 30 s at 60 °C); and (iii) a melting curve program (extension at 72 °C). The relative quantification of gene amplification by RT-PCR was performed using cycle threshold (Ct) values (Fu et al. 2006). The comparative Ct value method was employed to quantitate mRNA levels for $b^{0,+}$-AT, CAT-1 and y^+LAT-1 relative to those for β-actin as described by Yin et al. (2011).

Statistical analysis

Data are presented as the mean ± SEM. One-way analysis of variance was used to analyze statistically experimental data using SAS 8.0 (Yin et al. 2004; Zhou et al. 2011). Differences among treatment means were determined by the Student–Newman–Keuls multiple comparison (Wei et al. 2012). Probability values <0.05 were taken to indicate statistical significance.

Results

Effects of diets with different L-lysine levels on growth performance

There were no differences ($P > 0.05$) in initial BW among the four groups of piglets (Table 2). However, compared with the control group, pigs fed the other three diets had lower ($P < 0.05$) final BW, lower feed intake, and lower growth performance. Moreover, the final BW of pigs in the Zein − LYS group was the lowest among all the study animals. In particular, the average daily gain (ADG) and average daily feed intake (ADFI) differed among the four dietary treatments ($P < 0.05$). Compared with the NF

Table 2 Effect of diet with or without lysine on growth performance of weaning piglets

Items	Diet treatment[A]			
	Zein + LYS	Zein − LYS	NF	CON
Initial weight (kg)	6.56 ± 0.15	6.49 ± 0.14	6.54 ± 0.12	6.48 ± 0.16
Final weight (kg)	5.82 ± 0.14^{b}	5.58 ± 0.17^{b}	6.41 ± 0.30^{b}	9.82 ± 0.43^{a}
ADG (g/d)	−35.2 ± 0.01^{c}	−43.3 ± 0.01^{c}	−6.19 ± 0.02^{b}	159.1 ± 1.40^{a}
ADFI (g/d)	330 ± 24^{c}	285 ± 13^{c}	414 ± 6.6^{b}	556 ± 33^{a}

Means with different superscript letters within a row differ ($P < 0.05$). Values are ± SEM, $n = 7$

[A] Dietary treatments: Zein + LYS, diet containing zein with L-lysine supplementation; Zein − LYS, diet containing zein without L-lysine supplementation; *NF* nitrogen-free diet; CON, basal diet

group, ADG, and ADFI in the Zein + LYS and Zein − LYS groups were decreased substantially ($P < 0.05$). Pigs in the CON group had normal growth, but the ADG was negative in the Zein + LYS, Zein − LYS and NF groups (Table 2).

Effects of diets with different L-lysine levels on intestinal morphology

Data on intestinal morphology are summarized in Fig. 1a and representative staining of jejunal and ileal mucosae is shown in Fig. 1b. In the control group, the jejunum and ileum in pigs fed the Zein + LYS diet had higher villus height ($P < 0.05$), whereas the ileal villus height in the NF fed pigs was similar to that in the control pigs. Pigs fed the Zein + LYS diet had the deepest crypts compared to pigs in all other treatments, whereas pigs fed the Zein − LYS had the shallowest crypts ($P < 0.01$). There were no differences ($P > 0.05$) in crypt depth between NF and the control piglets (Fig. 1a). Mucosal thickness in the jejunum was not influenced by dietary treatment. In the ileum, there were significant differences in villus height, mucosal thickness, and crypt depth between the control group and each of the other treatment groups ($P < 0.05$). Compared with control group, villus height in pigs fed the Zein + LYS and NF diets was decreased by 35.1 and 42.8 % ($P < 0.05$), respectively. Jejunal mucosal thickness was reduced by 40.8 % ($P < 0.05$) in NF fed pigs, compared with the control group. Pigs in the NF diet had a lower crypt depth than those fed the Zein − LYS diet (Fig. 1b).

Expression of $b^{0,+}$-AT, y^{+}LAT1, and CAT1 mRNA in the small intestine of piglets fed different diets

Changes in $b^{0,+}$-AT, y + LAT1, and CAT1 mRNA levels in the small intestine of piglets are shown in Table 3. The abundance of $b^{0,+}$-AT, y^{+}LAT-1, and CAT-1 mRNA in the jejunum was higher ($P < 0.05$) in the control group than in the other three treatment groups, except for y^{+}LAT-1 in pigs fed the Zein + LYS diet. In the treatment groups other than the control group, pigs fed the Zein + LYS diet had greater ($P < 0.05$) values for $b^{0,+}$-AT and y^{+}LAT-1 than pigs fed the Zein − LYS and NF diets. No differences were detected between the Zein − LYS and NF dietary treatment. There were significant differences ($P < 0.01$) among treatment groups for the CAT-1 mRNA abundance. Especially, the expression of CAT-1 mRNA abundance in Zein + LYS group was the lowest. Compared with the NF group, pigs fed the Zein + LYS and Zein − LYS diets had 61.8 and 29.4 % lower levels of intestinal CAT-1 mRNA, respectively. Interestingly, $b^{0,+}$-AT and y^{+}LAT-1 mRNA were detected mostly in the jejunum (Table 2).

In the ileum, there were no difference ($P > 0.05$) in the abundance of $b^{0,+}$-AT mRNA levels among dietary treatments (Table 3). However, y^{+}LAT-1 or CAT-1 mRNA levels in the control group were different from the three other groups ($P < 0.05$). Compared with the control, y^{+}LAT-1 and CAT-1 mRNA abundances in the Zein + LYS, Zein − LYS and NF groups were lower ($P < 0.05$). No differences were detected in y^{+}LAT-1 or CAT-1 mRNA levels among pigs fed the Zein + LYS, Zein − LYS and NF diets ($P > 0.05$).

Expression of $b^{0,+}$-AT, y + LAT1, and CAT1 mRNA in the different intestinal segments of pigs

Data on $b^{0,+}$-AT, y + LAT1, and CAT1 mRNA levels in different segments of the small intestine are shown in Figs. 2, 3, and 4. The abundance of $b^{0,+}$-AT mRNA (Fig. 2) differed among the four groups ($P < 0.05$); however, there were no differences among dietary treatments in the ileum ($P > 0.05$). In the jejunum, the abundance of $b^{0,+}$-AT in pigs fed the Zein + LYS diet was higher ($P < 0.05$) compared to those fed the Zein − LYS and NF diets, but values were similar between the Zein − LYS and NF groups. Likewise, jejunal y^{+}LAT1 mRNA abundance in the Zein + LYS group was the highest, and there were significant differences among the four groups ($P < 0.05$) (Fig. 3). Additionally, y^{+}LAT1 mRNA abundance differed in the ileum ($P < 0.05$), and was higher in the Zein + LYS

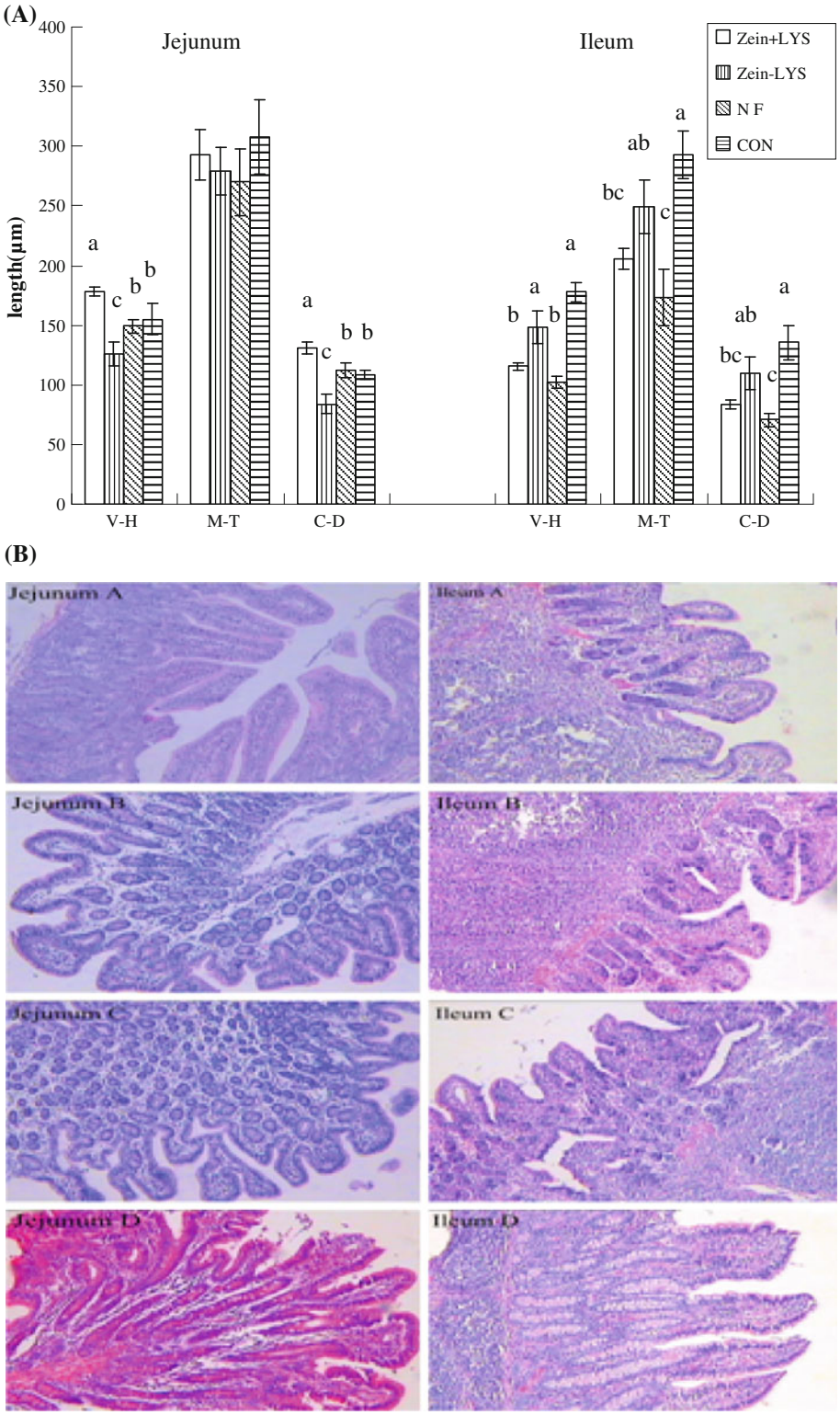

Fig. 1 **a** Effects of diets with different levels of L-lysine on intestinal morphology. Villus height (V–H), mucosal thickness (M-T), and crypt depth (C-D) were measured. Values are expressed as mean ± SEM, $n = 7$. Mean values with *different letters* differ ($P < 0.05$). Pigs in the Zein + LYS group were fed zein plus added L-lysine. Pigs in the Zein − LYS Group were fed zein without L-lysine supplementation. Pigs in the NF group were fed a nitrogen-free diet. Pigs in the control group were fed a basal diet. **b** Representative staining of the jejunal and ileum mucose in piglets. A was the Zein + LYS group, B was the Zein − LYS group, C was the NF group, D was the control group. Villus height, mucosal thickness and crypt depth from animals had a simple columnar epithelium smoothly covering the underlying lamina propria, as seen in the sections for the jejunum and the ileum

group than in the Zein − LYS and NF groups. CAT1 mRNA abundance was significantly influenced ($P < 0.05$) by dietary treatment such that values for the NF group were higher than those for the Zein + LYS and Zein − LYS groups (Fig. 4).

Discussion

Amino acids are essential nutrients for tissue protein synthesis and other metabolic functions in animals (Guo et al. 2008; Teng et al. 2010; Wang et al. 2005; Wu 2009,

Table 3 Effects of different diets on intestinal $b^{0+,}$-AT, y^+LAT1, and CAT1 mRNA relative abundance of piglets

Item	Diet treatment[A]				
	Zein + LYS	Zein − LYS	NF	CON	*P* value
Jejunum					
$b^{0,+}$-AT	0.56 ± 0.03^{b}	0.15 ± 0.01^{c}	0.14 ± 0.02^{c}	1.00 ± 0.18^{a}	0.008
y^+LAT-1	1.25 ± 0.12^{a}	0.36 ± 0.02^{b}	0.40 ± 0.03^{b}	1.00 ± 0.19^{a}	0.009
CAT-1	0.13 ± 0.01^{d}	0.24 ± 0.04^{c}	0.34 ± 0.02^{b}	1.00 ± 0.08^{a}	0.006
Ileum					
$b^{0,+}$-AT	0.83 ± 0.10	0.92 ± 0.16	1.14 ± 0.08	1.00 ± 0.03	0.082
y^+LAT-1	0.61 ± 0.04^{b}	0.55 ± 0.03^{b}	0.54 ± 0.04^{b}	1.00 ± 0.01^{a}	0.047
CAT-1	0.12 ± 0.01^{b}	0.21 ± 0.02^{b}	0.22 ± 0.02^{b}	1.00 ± 0.19^{a}	0.022

Means with different superscript letters within a row differ ($P < 0.05$). Values are mean ± SEM, $n = 7$

[A] Dietary treatments: Zein + LYS, diet containing zein with L-lysine supplementation; Zein − LYS, diet containing zein without L-lysine supplementation; *NF* nitrogen-free diet; CON, basal diet

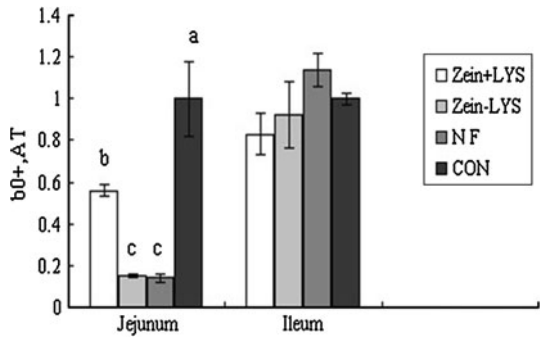

Fig. 2 Abundance of $b^{0+,}$-AT mRNA in the small intestine. Values are expressed as mean ± SEM, $n = 7$. Mean values with *different letters* differ ($P < 0.05$). Pigs in the Zein + LYS group were fed zein plus added L-lysine. Pigs in the Zein − LYS Group were fed zein without L-lysine supplementation. Pigs in the NF group were fed a nitrogen-free diet. Pigs in the control group were fed a basal diet

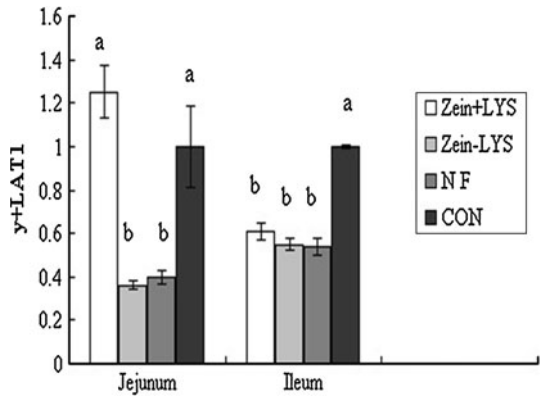

Fig. 3 Abundance of y^+LAT1 mRNA in the small intestine. Values are expressed as mean ± SEM, $n = 7$. Mean values with *different letters* differ ($P < 0.05$). Pigs in the Zein + LYS group were fed zein plus added L-lysine. Pigs in the Zein − LYS Group were fed zein without L-lysine supplementation. Pigs in the NF group were fed a nitrogen-free diet. Pigs in the control group were fed a basal diet

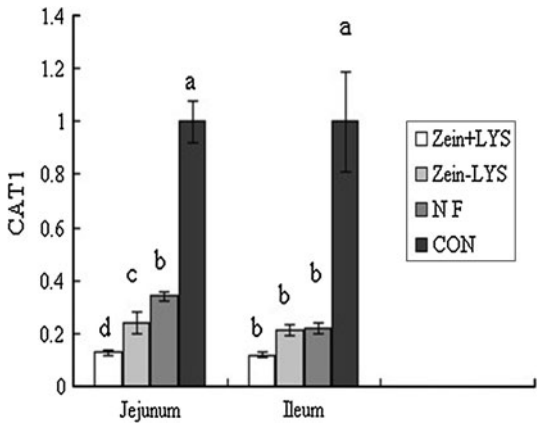

Fig. 4 Expression abundance of CAT1 mRNA in the small intestine. Values are expressed as mean ± SEM, $n = 7$. Mean values with *different letters* differ ($P < 0.05$). Pigs in the Zein + LYS group were fed zein plus added L-lysine. Pigs in the Zein − LYS Group were fed zein without L-lysine supplementation. Pigs in the NF group were fed a nitrogen-free diet. Pigs in the control group were fed a basal diet

2010b). In this study, we used zein as a protein source. Zein, a natural protein in corn, is severely deficient in basic AA (particularly lysine), compared with common feedstuffs for animal diets (Li et al. 2011a). Our results indicate that, compared with the NF and Zein − LYS groups, dietary supplementation with lysine influenced expression of the genes for transport of basic AA to affect intestinal morphology and regulate growth performance. Thus, an ideal dietary pattern for the weaned piglets can increase the efficiency of protein synthesis (Deng et al. 2010; Li et al. 2011b; Rezaei et al. 2013a, b); otherwise, feeding diets deficient in lysine will increase the oxidation of other AA to CO_2, water, ammonia, and urea (Li et al. 2008; Wu 2010b). In the present study, growth performance of piglets in the Zein + LYS and Zein − LYS groups was lower

than that in the NF group, which might be caused by different sources of diets. Notably, zein was the only protein source that may be imbalanced in the composition of AA (Table 1). Meanwhile, there are reports that excess AA are usually not stored in the body and are subjected to irreversible loss via oxidation and production of nitrogenous metabolites (Deng et al. 2010; Gattas and Silva 2012; Yin et al. 2010a, b, c) and, therefore, AA must be supplied daily through the diet (Wu 2009). Furthermore, growth performance of piglets feeding nitrogen-free diet can maintain weight gain in a short-term, but the components of the gain is likely fat rather than protein. In the NF group, the diet contained a large amount of corn starch which could provide energy and precursors for lipid synthesis.

The small intestine has a main function for nutrient absorption and transport. A normal structure of the small-intestinal mucosa is necessary for optimal growth as well as nutrient digestion and absorption (Rezaei et al. 2011; Sukhotnik et al. 2005; Yao et al. 2012). The present study shows that dietary supplementation with lysine attenuated an increase in villus height and crypt depth in the jejunum, but in the ileum lysine supplementation decreased mucosal thickness, villus height and crypt depth. Some studies have shown that degradation of lysine and other basic AA in the small intestine plays a role in modulating the intestinal villus, crypt, and mucosal thickness (Wang et al. 2009b) because their metabolites are essential for DNA and protein synthesis (Blachier et al. 2007; Tan et al. 2009; Wu et al. 2009). Because intestinal epithelial cells do not degrade lysine (Chen et al. 2007, 2009), microbes in the lumen of the intestine are likely responsible for lysine catabolism in the gut (Dai et al. 2010, 2012a, b). Furthermore, because weaning stress causes intestinal atrophy and dysfunction (Gu et al. 2002; Hampson 1986; Wang et al. 2009a), lysine nutrition plays a key role in maintaining gut integrity in weaned piglets (Wu et al. 2009). Thus, results of the current study provide an additional line of evidence that dietary lysine is necessary for maintaining intestinal mucosal integrity, particularly under stressful conditions such as weaning. Additionally, our findings also support that notion that a proper balance among AA (including both nutritionally essential and non-essential AA) in diets crucial for maximizing efficiency of animal production (Ren et al. 2012; Tan et al. 2012; Wang et al. 2013; Wu 2013a, b).

Nutrient content of a diet, especially AA, can affect AA transport by animal tissues (Stein et al. 1987; Wolfram et al. 1984). Dietary AA is absorbed via their transporter systems on the mucosal side of the small intestine. Several recent reports have suggested that diets with different lysine levels have different effects on the expression of basic AA transporters in the small intestinal mucosa of weanling piglets (Chen and Liou 2012; Deng et al. 2010; Zhi et al. 2010). Results of the present study indicate that the abundance of $b^{0,+}$-AT, y^+LAT-1, and CAT-1 mRNA in the jejunum was influenced by dietary content of lysine. For example, in pigs fed the Zein + LYS diet, the abundance of $b^{0,+}$-AT and y^+LAT-1 was highest, suggesting that dietary intake of lysine affects intestinal expression of these AA transporters. Interestingly, intestinal CAT1, expression was reduced in pigs fed the Zein + LYS diet. Thus, $b^{0,+}$-AT and y^+LAT-1 are expressed in the intestinal mucosa of young pigs, especially in the jejunum, but CAT1 mRNA is largely reduced in response to dietary lysine deficiency. Under nutritionally stress conditions, lysine deficiency may lead to an increase in expression of some AA transporters. Likewise, feeding a diet with imbalanced AA or a N-free diet, abundance of some AA transporters may be enhanced. It is evident that nutrient deficiency may activate the body's own protection system to maintain animal normal growth. For example, Stein et al. (1987) reported that dietary supplementation of lysine could improve absorptive capacity for basic AA. Furthermore, substrate concentration can modulate transport of nutrients in the gastrointestinal tract (Zhi et al. 2010). In general, feeding diets with high protein or AA content may increase transport capacity for some AA in the small intestine. This improvement is mainly due to an increase in the relative levels of mRNA abundance for AA transporters (Furuya and Graciano 2012; Garcia-Villalobos and Morales-Trejo 2012; Hernández et al. 2012).

Absorption and transport of basic AA is a complex physiological process that is influenced by many factors, including diet and hormones. However, the factors that play key roles in these processes are not yet well understood. Nonetheless, results of the current study reveal that a diet deficient in lysine has different effects on CAT transporters in the piglet small intestine. Dietary content of lysine differentially affects intestinal CAT expression to modulate absorption of lysine and other AA. Thus, transport of these nutrients is a key regulatory step in utilization of dietary protein by growing pigs. Whereas the findings of this study could help advance our knowledge of AA nutrition, further research will be required to elucidate the underlying mechanisms.

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Conflict of interest The authors declare that they have no conflict of interest.

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