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ORIGINAL ARTICLE

Dietary arginine supplementation enhances intestinal expression of SLC7A7 and SLC7A1 and ameliorates growth depression in mycotoxin-challenged pigs

Jie Yin · Wenkai Ren · Jielin Duan · Li Wu · Shuai Chen · Tiejun Li · Yulong Yin · Guoyao Wu

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Abstract This study tested the hypothesis that dietary L-arginine supplementation confers beneficial effects on growing pigs fed a mold-contaminated diet. The measured variables included: (1) the average daily weight gain and feed:gain ratio; (2) activities of total superoxide dismutase, glutathione peroxidase, diamine oxidase, as well as amino acid and D-lactate concentrations in serum; (3) intestinal morphology; (4) expression of the genes for SLC7A7 (amino acid transporter light chain, y^{+L} system, family 7, member 7), SLC7A1 (cationic amino acid transporter, y^{+} system, family 7, member 1), SLC1A1 (neuronal/epithelial high affinity glutamate transporter, system X_{AG}, member 1), SLC5A1 (sodium/glucose cotransporter, family 5, member 1) in the ileum and jejunum. Mycotoxins in feedstuffs resulted in an enlarged small intestine mass, oxidative injury in tissues, and reduced growth performance in pigs. Dietary arginine supplementation enhanced ($P < 0.05$) expression of jejunal SLC7A7 and ileal SLC7A1, in comparison with the control and mycotoxin groups. In addition, supplementing 1 % L-arginine to the mycotoxin-contaminated feed had the following beneficial effects ($P < 0.05$): (1) alleviating the imbalance of the antioxidant system in the body; (2) ameliorating intestinal abnormalities; and (3) attenuating whole-body growth depression, compared with the mycotoxin group without arginine treatment. Collectively, these results indicate that dietary supplementation with L-arginine exerts a protective role in pigs fed mold-contaminated foods. The findings may have important nutritional implications for humans and other mammals.

Keywords Arginine · Mycotoxin · Small intestine · Swine · Nutrition

J. Yin and W. Ren contributed equally to the present study.

J. Yin · W. Ren · J. Duan · L. Wu · S. Chen · T. Li (✉) · Y. Yin · G. Wu
Scientific Observing and Experimental Station of Animal Nutrition and Feed Science in South-Central China, Ministry of Agriculture, Hunan Provincial Engineering Research Center of Healthy Livestock, Key Laboratory of Agro-ecological Processes in Subtropical Region, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha 410125, Hunan, China
e-mail: tjli@isa.ac.cn

J. Yin
e-mail: yinjie2014@126.com

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

J. Yin · W. Ren · J. Duan · L. Wu
University of Chinese Academy of Sciences, Beijing 100039, China

G. Wu
State Key Laboratory of Animal Nutrition, China Agricultural University, Beijing 100193, China

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

Introduction

Mycotoxins are secondary metabolites produced by a wide variety of fungal species (Rezaei et al. 2013a, b). They are commonly found in moldy feedstuffs and raw feed materials. Mycotoxins are considered to be the major toxic contaminants, including aflatoxin (AF), deoxynivalenol (DON), zearalenone (ZEA), ochratoxins (OCH), fumonisins (FB) and T-2 (Binder 2007; Richard et al. 2007). Many studies have shown that the ingestion of these mycotoxins

Table 1 Mycotoxin content in contaminated and non-contaminated feed mixtures

Catalogue	AFB1 (μg/kg)	ZEN (mg/kg)	OCH (μg/kg)	DON (mg/kg)	FB1 (mg/kg)	T-2 (mg/kg)
Limit of detection	0.05	0.01	0.5	0.1	0.05	0.1
Normal feed	ND	0.821	3.6	1	0.6	ND
Contaminated feed	0.62	0.573	11.39	3	2	ND

The content of mycotoxins in the diet was determined by liquid chromatograph (Beijing Taileqi, Beijing, China)

AFB1 aflatoxin B1, *ZEN* zearalenone, *OCH* ochratoxins, *DON* deoxynivalenol, *FB1* fumonisins, *ND* non-detected

may induce feed refusal, a decrease in animal productivity, organ damage, and increased incidence of disease due to immune suppression (Chaytor et al. 2011). Therefore, dietary mycotoxins can greatly affect the health, growth, and reproduction of humans and other animals, including pigs (Fokunang et al. 2006). Although many studies have reported the toxic effects of individual mycotoxins in various animal species, exposure to only one mycotoxin is unlikely to occur under normal feeding conditions in the livestock industry. Indeed, during the feed-manufacturing process, various batches of different raw materials are mixed together, leading to an increase in the risk for mycotoxin-mixed contamination (Binder et al. 2007). Thus, animals are usually exposed to synergistic interactions among multiple mycotoxins.

With regard to the alleviation of mycotoxicosis, most studies have focused on methods of physical and chemical degradation of mycotoxins (Park et al. 2007; Young et al. 1986), as well as the use of their adsorbents, such as aluminosilicates (Liu et al. 2011) and esterified glucomannan (Aravind et al. 2003). However, the results of some in vivo studies (Liu et al. 2011) have shown that these methods are not always effective against all chemically diverse mycotoxins. At present, little is known about the use of nutritional treatment to ameliorate intestinal abnormalities and growth retardation induced by the ingestion of mycotoxins.

L-Arginine is a conditionally essential amino acid for pigs (Wu 2010a, b). It is not only synthesized from glutamine, glutamate, and proline by enterocytes of the pig small intestine (Wu et al. 1994; Wu and Knabe 1995; Wu et al. 1996a), but is also degraded by both intestinal mucosal cells (Wu et al. 1996b) and luminal bacteria (Dai et al. 2012a, b, 2013a). In addition, concentrations of arginine in the blood circulation are reduced under stress conditions, including weaning (Wu et al. 2010), lactation (Lei et al. 2012), starvation (Wu and Morris 1998), and infection (Li et al. 2007). Results of recent studies have demonstrated that dietary supplementation with arginine can promote (1) intestinal cell proliferation (Tan et al. 2010), (2) reduction of white adipose tissue in obese rats (Wu et al. 2007a) and pigs (Tan et al. 2009), (3) expression of antioxidative genes (Jobgen et al. 2009), and (4) healing wounds (Stechmiller et al. 2005) via mechanisms involving the synthesis of nitric oxide (NO) (Wu et al. 2009). Furthermore, arginine can enhance growth performance (Kim and Wu 2004), improve immune function (Li et al. 2007; Ren et al. 2012), beneficially alter the metabolic profile in animals (Tan et al. 2012a), stimulate the mammalian (mechanistic) target of rapamycin (mTOR) signaling pathway and protein synthesis (Kong et al. 2012), affect intestinal morphology and production of inflammatory cytokines (Zhou et al. 2012), and up-regulate expression of vascular endothelial growth factor in the gut (Yao et al. 2011). Based on the foregoing, the present study was conducted to test the hypothesis that dietary supplementation with L-arginine may confer beneficial effects on growing pigs fed a mold-contaminated diet.

Materials and methods

Preparation of a mold-contaminated diet

A mold-contaminated diet was prepared as described by Liu et al. (2011). In brief, water was added to a non-contaminated basal diet until it reached 20 % moisture. The wet feed was then cultured under ambient conditions (temperature 23–28 °C, humidity 68–85 %) until mildew was clearly observed (Liu et al. 2011). Finally, the mold-contaminated diet was naturally air-dried, mixed, and sampled for detection of mycotoxins. Their content in the mold-contaminated diet was determined by liquid chromatography (Beijing Taileqi, Beijing, China) (Table 1).

Experimental design

The experiment was carried out in accordance with the Chinese guidelines for animal welfare. Experimental protocol and approved by the Animal Care and Use Committee of the Chinese Academy of Sciences.

Fifteen growing pigs (Landrace × Large White) (Zheng Hong Co., China) with a mean body weight (BW) of 55 kg were randomly assigned into one of three treatment groups ($n = 5$/group): (1) the control group in which pigs were fed the uncontaminated diet; (2) the mycotoxin group in which pigs were fed the mold-contaminated diet; and (3) the

Table 2 Composition and nutrient level of basal diet

Ingredient	Content (%)	Nutrient	Content (%)
Corn	67.22	Crude protein	16
Soybean meal	21.8	Ca	0.6
Wheat bran	7.95	P	0.5
Limestone	1.03	Salt	0.35
$CaHPO_4$	0.69	Lys	0.771
Salt	0.31	Met+Cys	0.584
Additive premix	1		

Premix provided the following per kilogram of the diet: Sepiolite, 8.072 g; vitamin mixture, 750 mg; $FeSO_4 \cdot H_2O$, 317 mg; $CuSO_4 \cdot 5H_2O$, 294 mg; antioxidants, 200 mg; $MnSO_4 \cdot H_2O$, 172 mg; $ZnSO_4 \cdot H_2O$, 153 mg; KI, 24 mg; Na_2SeSO_3, 18 mg

arginine group in which pigs were fed the mold-contaminated diet supplemented with 1.0 % L-arginine (purity >99 %, Beijing Chemclin Biotech, Beijing, China). The basal diet (Table 2) was prepared from corn, soybean meal, wheat bran, limestone, $CaHPO_4$, NaCl, and additive premix to meet or exceed the nutritional requirements of growing pigs as recommended by the NRC (1998). This diet contained 0.99 % L-arginine, as determined by Li et al. (2011).

Pigs had free access to drinking water and their respective diets throughout the experimental period and were weighed individually at days 0 and 60. The feed consumption of pigs was measured weekly. The average daily weight gain (ADG; g/day) and feed:gain ratios of pigs were then calculated. All pigs were food deprived for 12 h at the end of the experimental period before blood samples were obtained from the jugular vein (Rezaei et al. 2013b) for the determination of total superoxide dismutase (T-SOD), glutathione peroxidase (GSH-Px), diamine oxidase (DAO) activities, D-lactate, and amino acid levels in serum (Hou et al. 2012; Yao et al. 2012). After pigs were anesthetized by intravenous administration of sodium pentobarbital (50 mg/kg body weight) and then euthanized by exsanguination (Deng et al. 2009), the small intestine was obtained, its luminal content was carefully removed, and the lumen was washed three times with saline (Wang et al. 2008). Then, two samples were taken from both the mid-jejunum and mid-ileum. One of the intestinal samples (3 cm) was placed in 10 % neutral buffered formalin for morphological analysis (Wu et al. 1996a), and the other (approximately 2 g) was immediately frozen in liquid nitrogen and stored at −70 °C for subsequent gene expression analysis (Wang et al. 2009).

Analyses T-SOD, GSH-Px, and DAO activities, D-lactate, and amino acid profile

Serum activities of T-SOD and GSH-Px were measured using spectrophotometric kits in accordance with the manufacturer's instructions (Nanjing Jiangcheng Biotechnology Institute, China). Serum D-lactate was determined using an assay kit in accordance with the manufacturer's instructions (Biovision Inc., USA). DAO was measured according to a previous report (Hou et al. 2012, 2013). Amino acids in serum were determined by LC–MS/MS (HPLC Ultimate3000 and 3200 Q TRAP LC–MS/MS) using standards from Sigma Chemicals (St. Louis, MO, USA) (Ruan et al. 2013).

Determination of intestinal morphology

One piece of the jejunal and ileal segments (3 cm) was maintained in 4 % neutral buffered 10 % formalin, processed using routine histological methods, and mounted in paraffin blocks (Wang et al. 2008). Six-micrometer-thick sections were cut and stained with the Masson's trichrome solution (Zhang et al. 2013b). All specimens were examined under a light microscope (Nikon, Japan). Villus height and crypt depth were measured using an image-analysis system.

Quantification of mRNA by real-time PCR analysis

Total RNA was isolated from liquid nitrogen-pulverized tissues with TRIzol regent (Invitrogen, USA) and then treated with DNase I (Invitrogen, USA) according to the manufacturer's instructions (Liu et al. 2012). Synthesis of the first strand (cDNA) was performed with oligo (dT) 20 and Superscript II reverse transcriptase (Invitrogen, USA) (Zhang et al. 2013a). Primers were designed with Primer 5.0 according to the gene sequence of pig to produce an amplification product (Table 3). β-Actin was used as a housekeeping gene to normalize target gene transcript levels. Real-time PCR was performed as described by Ren et al. (2011). In brief, 1-μl cDNA template was added to a total volume of 10 μl containing 5 μl SYBR Green mix, 0.2 μl Rox, 3 μl H_2O, and 0.4 μmol/l each of forward and reverse primers. The following protocol applied: (1) predenaturation (10 s at 95 °C); (2) amplification and quantification 40 cycles (5 s at 95 °C, 20 s at 60 °C); and (3) melting curve program (60–99 °C with a heating rate of 0.1 °C S-1 and fluorescence measurement). The relative levels of genes were expressed as a ratio of the target gene to the control gene using the formula $2^{-(\Delta\Delta C}t)$, where $\Delta\Delta C_t = (C_{tTarget} - C_{t\beta\text{-actin}})_{treatment} - (C_{tTarget} - C_{t\beta\text{-actin}})_{control}$ (Fu et al. 2010). The relative expression of genes in the mycotoxin and arginine groups is given in comparison to those in the control group and is reported as the fold change from the control value (He et al. 2013a).

Statistical analysis

Statistical analyses were performed with the SPSS17.0 software (Chicago, IL, USA). The normality and constant

Table 3 Primer pairs used in the RT-PCR

Target gene	Accession no.	Nucleotide sequence of primers (5′–3′)
β-Actin	NM_001172909.1	F: 5′-CTGCGGCATCCACGAAACT-3′
		R: 5′-AGGGCCGTGATCTCCTTCTG-3′
SLC7A1	NM_001012613.1	F:5′-TGCCCATACTTCCCGTCC-3′
		R:5′-GGTCCAGGTTACCGTCAG-3′
SLC7A7	NM_001253680.1	F:5′-TTTGTTATGCGGAACTGG-3′
		R:5′-AAAGGTGATGGCAATGAC-3′
SLC1A1	NM_001164649.1	F:5′-ATAGAAGTTGAAGACTGGGAAAT-3′
		S:5′-GTGTTGCTGAACTGGAGGAG-3′
SLC5A1	NM_001164021.1	F:5′-GGCTGGACGAAGTATGGTGT-3′
		S:5′-ACAACCACCCAAATCAGAGC-3′

All these primer sequences were designed based on the accession numbers described above

SLC7A1 solute carrier family 7 (cationic amino acid transporter, y^+ system), member 1; *SLC7A7* solute carrier family 7 (amino acid transporter light chain, y^{+L} system), member 7; *SLC1A1* solute carrier family 1 (neuronal/epithelial high-affinity glutamate transporter, system X_{AG}), member 1; *SLC5A1* solute carrier family 5 (sodium/glucose cotransporter), member 1

Table 4 Effects of dietary supplementation with arginine on growth performance of pigs fed a mold-contaminated diet

Variable	Control	Mycotoxin	Arginine
Initial weight (kg)	56.1 ± 1.7	56.7 ± 1.8	56.2 ± 1.7
Final weight (kg)	90.2 ± 1.8	86.7 ± 1.8	86.9 ± 1.9
ADG (kg/day)	0.71 ± 0.02[a]	0.58 ± 0.02[b]	0.61 ± 0.02[b]
F:G (g feed/g gain)	3.22 ± 0.11[b]	3.66 ± 0.04[a]	3.38 ± 0.01[b]

Data are presented as mean ± SEM, $n = 5$. Control group: Pigs were fed a uncontaminated diet. Mycotoxin group: pigs were fed a mold-contaminated diet. Arginine group: pigs were fed a mold-contaminated diet supplemented with 1 % arginine

ADG average daily weight gain (kg/day), *F:G* feed:gain ratio

[a, b] Within a row, means with different superscripts differ ($P < 0.05$)

variance for data were tested by the Levene's test (Wei et al. 2012). Data were subjected to one-way analysis of variance followed by the Duncan's multiple comparisons test. Values are expressed as the mean ± standard error of the mean (SEM).

Results

Growth performance

Data on pig growth performance are summarized in Table 4. Compared to the control group, ADG was 18.3 % lower ($P < 0.05$) and the feed:gain ratio was 13.7 % higher ($P < 0.05$) in the mycotoxin group. Interestingly, dietary supplementation with 1 % arginine to pigs fed the mold-contaminated feedstuffs improved ($P < 0.05$) feed efficiency (as indicated by a reduced feed:gain ratio) by 8.5 %, in comparison with the mycotoxin group.

Analysis of serum T-SOD and GSH-Px activity

The activity of serum total SOD in the mycotoxin group was 49.4 % lower ($P < 0.05$) than that in the control group (Fig. 1). However, there was no difference in total SOD activity between the mycotoxin and the arginine groups. The activity of serum glutathione peroxidase did not differ among the control and mycotoxin groups, but was slightly higher ($P < 0.05$) in the arginine group.

Serum D-lactate and DAO

After pigs were fed the mold-contaminated feed, the concentration of D-lactate in serum was increased ($P < 0.05$), in comparison with the control group (Fig. 2). However, there was no difference in serum D-lactate concentrations between the mycotoxin and the arginine groups. The activity of serum DAO did not differ among the three groups of pigs.

Intestinal morphology

No abnormal morphology was observed for the small intestine in the control group (Fig. 3). In contrast, villus height in both the jejunum and the ileum of the mycotoxin-challenged pigs showed strong scattering and desquamation. Interestingly, villus height in the jejunum and the ileum was decreased ($P < 0.05$) by dietary arginine supplementation, compared with pigs fed the mold-contaminated diet without arginine treatment. An increase in crypt depth was also observed ($P < 0.05$) in both the ileum and jejunum after exposure to the mold-contaminated diet (Table 5). Compared with the mycotoxin group, dietary supplementation with 1 % arginine decreased ($P < 0.05$)

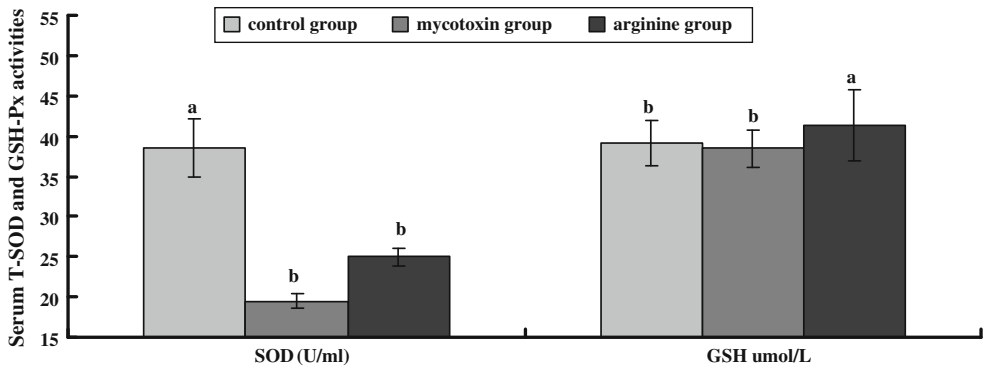

Fig. 1 Effects of dietary supplementation with arginine on serum T-SOD and GSH-Px activities in growing pigs fed a mold-contaminated feed. Data are mean ± SEM, $n = 5$. Control group: pigs were fed an uncontaminated diet. Mycotoxin group: pigs were fed a mold-contaminated diet. Arginine group: pigs were fed a mold-contaminated diet supplemented with 1 % arginine. *T-SOD* superoxide dismutase (U/ml), *GSH* glutathione peroxidase (×10 μU/l). For enzymatic activity, 1 U/ml is defined as the amount of SOD in 1 ml plasma required for catalyzing the conversion of 1 μmol superoxide anion (O_2^-) into its product per min at 25 °C. *a*, *b* For each variable, means with different letters differ ($P < 0.05$)

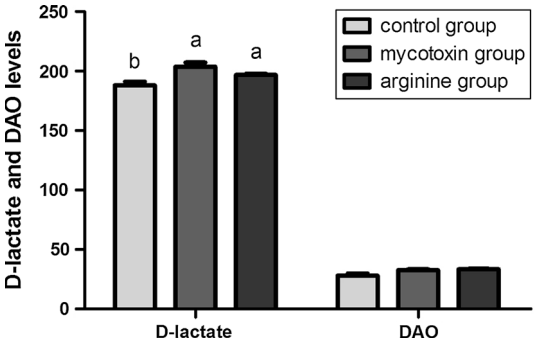

Fig. 2 Effects of dietary supplementation with arginine on DAO activity (U/ml) and D-lactate levels (μmol/l) in the serum of growing pigs fed a mold-contaminated diet. Data are mean ± SEM, $n = 5$. See Fig. 1 for explanation of legends. *DAO* diamine oxidase. *a*, *b* For each variable, means with different letters differ ($P < 0.05$)

ileal crypt depth and tended to reduce ($P > 0.05$) jejunal crypt depth.

Concentrations of amino acids in serum

Concentrations of amino acids and related metabolites in serum were determined by LC–MS/MS. In response to mycotoxin challenge, most of the amino acids exhibited decreases in the blood circulation (Table 6). For example, serum concentrations of glycine, L-tyrosine, L-glutamine, 1-methyl-L-histidine, and L-proline were decreased ($P < 0.05$) by the mold-contaminated diet, compared to the control group. In contrast, L-citrulline showed an increase in the serum ($P < 0.05$), likely as a result of increased production of this amino acid by intestinal microbes and fungi. Dietary supplementation with 1 % L-arginine reduced ($P < 0.05$) the circulating levels of citrulline but increased ($P < 0.05$) those for L-histidine and 3-methyl-L-histidine in pigs fed the mycotoxin-treated diet. As expected from the pharmacokinetics of arginine in animals (Wu et al. 2007b), the concentration of arginine in the serum of 12-h fasted pigs did not differ among the three treatment groups (Table 6).

Expression of genes for intestinal amino acid transporters

Dietary arginine supplementation up-regulated SLC7A7 expression ($P < 0.05$) in the jejunum (the major site for absorption of amino acids), compared to the mycotoxin group without arginine treatment (Fig. 4). Similar results were obtained for the ileal SLC7A1 gene. mRNA levels for SLC7A7, SLC1A1, and SLC5A1 in the ileum of pigs fed the mycotoxin-contaminated diet without arginine supplementation did not differ from those in the control group (Fig. 4).

Discussion

Among various mycotoxins, AF, DON, ZEA, OCH, FUM, and T-2 are often encountered in feedstuffs at high concentrations worldwide (Binder et al. 2007; Guan et al. 2011). Decreased feed intake and the subsequent suppression of growth are characteristics of mycotoxicosis (Andretta et al. 2012; Rezaei et al. 2013a; Swamy et al. 2003). Similar to previous reports (Rezaei et al. 2013a), results of the current study indicated that mold-contaminated feed substantially decreased growth performance in pigs. Importantly, dietary supplementation with arginine ameliorated intestinal abnormalities (Table 5) and growth depression (Table 4) in mycotoxin-challenged pigs.

Arginine regulates intestinal gene expression, growth and mucosal integrity, nutrient absorption, and metabolic

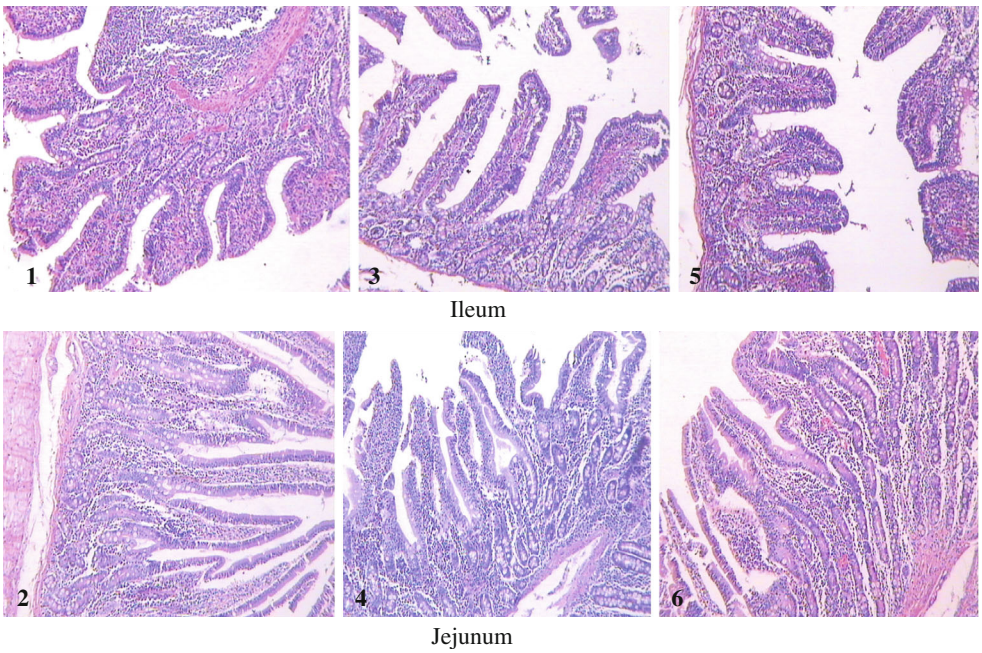

Fig. 3 Effects of dietary supplementation with arginine on small intestinal morphology (HE ×400) in growing pigs fed a mold-contaminated diet. Pigs in the control group (Panels *1* and *2*) were fed a uncontaminated diet. Pigs in the mycotoxin group (Panels *3* and *4*) were fed a mold-contaminated diet. Pigs in the arginine group (Panels *5* and *6*) were fed a mold-contaminated diet supplemented with 1 % L-arginine. There was no histological damage in the small intestine of the control pigs. In the mycotoxin group, villus was scattered and desquamated seriously in the jejunum and ileum. Higher villus height in the jejunum and ileum was observed in the arginine group, compared with the mycotoxin group

Table 5 Effects of dietary supplementation with arginine on the small intestinal morphology of pigs fed a mold-contaminated diet

Variable (μm)	Control	Mycotoxin	Arginine
Ileal villus height	255 ± 4.9^{b}	356 ± 8.1^{a}	319 ± 15^{a}
Ileal crypt depth	120 ± 1.6^{b}	217 ± 7.2^{a}	144 ± 14^{b}
Jejunal villus height	295 ± 6.4^{b}	343 ± 16^{a}	320 ± 9.7^{a,b}
Jejunal crypt depth	99 ± 3.9^{b}	132 ± 6.8^{a}	116 ± 3.8^{a,b}

Data are presented as mean ± SEM, $n = 5$. See Table 4 for explanation of treatment groups

$^{a, b}$ Within a row, means with different superscripts differ ($P < 0.05$)

pathways (Rhoads and Wu 2009; Wu et al. 2012, 2013a, b). Thus, arginine has been recognized as a conditionally essential amino acid for young mammals, including piglets (Wu 2013a). There is also growing interest in arginine in a functional amino acid in human and animal nutrition (Ren et al. 2012; Tan et al. 2012b; Wu 2013b). Notably, many reports have shown that dietary supplementation with arginine may play an important role in counteracting the suppressive effects of abnormal regulation of metabolic pathway and immunological challenges on animal growth (Yin et al. 2010). The feed:gain ratio (an indicator of the efficiency in utilization of dietary nutrients for growth) was reduced in arginine-supplemented pigs, as compared to pigs in the mycotoxin group (Table 4). This novel result suggests a potentially important role for arginine in mitigating adverse effects of food-borne mycotoxins on growing swine. Large-scale studies are required to examine this possibility under production conditions.

Antioxidant enzymes (such as SOD and GSH-Px) comprise the major defense system for preventing organ injury due to excessive quantities of reactive oxygen species that attack proteins, lipids, and DNA (Yin et al. 2013a, b). An increasing number of studies have demonstrated that some mycotoxins can contribute to oxidative stress in cells. For example, ZEA decreases the activities of SOD and GSH-Px with progressive liver and kidney injury (Jiang et al. 2011). In addition, DON induces an adapted response of the antioxidant defense system to oxidative stress in Hek-293 cells (Dinu et al. 2011). Furthermore, Mary et al. (2012) reported that the interaction of AFB1 and FB1 caused oxidative damage in spleen mononuclear cells. In this study, we found that the activity of total SOD in the pig serum was markedly decreased after exposure to mold-contaminated feedstuffs, indicating that mycotoxins resulted in oxidative stress in the whole body. Arginine stimulates NO production within physiological ranges in the small intestine and other tissues (Rhoads and Wu 2009; Wu amd Meininger 2002), and NO plays an important role in regulating the antioxidant defense system (Dai et al. 2013b). Thus, dietary supplementation with arginine helps

Table 6 Effects of dietary supplementation with arginine on serum amino acid concentrations in growing pigs fed a mold-contaminated diet

Substance	Control	Mycotoxin	Arginine
L-Arginine	23.3 ± 0.32	20.8 ± 2.82	19.0 ± 0.92
L-Histidine	32.7 ± 3.5^{b}	32.8 ± 2.7^{b}	45.4 ± 2.6^{a}
L-Isoleucine	10.9 ± 0.98	10.7 ± 0.79	11.2 ± 0.90
L-Leucine	22.5 ± 0.21	22.6 ± 0.89	24.3 ± 1.6
L-Lysine	16.7 ± 1.4	12.7 ± 1.7	13.7 ± 1.5
L-Phenylalanine	12.2 ± 0.87	11.0 ± 1.5	11.7 ± 0.47
L-Methionine	32.9 ± 0.90	31.2 ± 5.4	45.5 ± 2.0
L-Threonine	10.2 ± 0.34	10.1 ± 0.94	9.28 ± 0.58
L-Tryptophan	6.87 ± 0.36	6.31 ± 0.45	5.70 ± 0.13
L-Valine	20.3 ± 1.8	22.2 ± 1.7	20.7 ± 1.1
Glycine	87.8 ± 3.3^{a}	67.0 ± 3.1^{b}	71.5 ± 7.0^{b}
L-Serine	10.1 ± 1.7	10.2 ± 0.55	10.2 ± 0.64
L-Tyrosine	23.3 ± 1.2^{a}	17.6 ± 0.86^{b}	16.7 ± 1.2^{b}
L-Asparagine	2.84 ± 0.28	3.26 ± 0.40	3.59 ± 0.23
L-Aspartate	1.72 ± 0.28	1.67 ± 0.09	2.73 ± 0.18
L-Citrulline	10.1 ± 0.91^{b}	17.7 ± 1.09^{a}	10.6 ± 0.76^{b}
L-Glutamate	57.3 ± 3.1	49.3 ± 2.9	46.1 + 1.1
L-Glutamine	7.20 ± 0.67^{a}	3.90 ± 0.30^{b}	3.66 ± 0.48^{b}
L-Ornithine	13.1 ± 0.92	11.0 ± 1.21	12.6 ± 2.71
L-Cysteine (free)	0.49 ± 0.12	0.32 ± 0.06	0.31 ± 0.09
L-Homocysteine	0.18 ± 0.01^{a,b}	0.15 ± 0.02^{b}	0.22 ± 0.01^{a}
L-Alanine	44.0 ± 6.7	44.7 ± 3.7	45.5 ± 7.0
L-Carnosine	0.76 ± 0.18	0.69 ± 0.09	0.69 ± 0.19
1-Methyl-L-histidine	0.70 ± 0.05^{a}	0.49 ± 0.03^{b}	0.56 ± 0.03^{a,b}
3-Methyl-L-histidine	1.64 ± 0.10^{a,b}	1.46 ± 0.08^{b}	1.8 ± 0.09^{a}
L-Proline	46.1 ± 0.33^{a}	20.4 ± 0.36^{b}	22.8 ± 0.81^{b}

Values are mg/l. Serum amino acid levels were determined by HPLC Ultimate 3000 and 3200 Q TRAP LC–MS/MS. Data are presented as mean ± SEM, $n = 5$. See Table 4 for explanation of treatment groups

$^{a, b}$ Within a row, means with different superscripts differ ($P < 0.05$)

scavenge the excess reactive oxygen species induced by moldy feeds, thereby improving the balance between the production of reactive oxygen species (e.g., superoxide anion, hydrogen peroxide, and hydroxyl radical) and the biological defense against the toxicity of these oxidants.

D-Lactate, a byproduct of bacterial metabolism, is neither produced nor metabolized by mammalian cells (He et al. 2012). Under normal conditions, production of D-lactate by the small intestine is insignificant, and thus blood has a very low or negligible level of D-lactate (Dai et al. 2011). However, if the intestinal segment is infected and the mucosal barrier of the gut is degraded, D-lactate can cross the mucosal barrier in large quantities (Packer et al. 2012). Therefore, abnormally high serum concentrations of D-lactate are considered to be indicators of intestinal injury. After pigs were exposed to mold-contaminated feeds, serum D-lactate concentrations were increased, indicating that mold-contaminated feeds resulted in D-lactate production and damage to the gut. Meanwhile, the results of the macroscopic observation and histological evaluation of the small intestine (e.g., an enlarged mucosa) further demonstrated that mycotoxins induced intestinal abnormalities (Rezaei et al. 2013b). Catabolism of amino acids (e.g., arginine and methionine) by intestinal microbes to generate polyamines and other metabolites may contribute to the enlargement of the intestinal mucosa in pigs fed mold-contaminated feedstuffs. Similarly, Yunus et al. (2011) and Applegate et al. (2009) found that the weight of the small intestine and crypt depth were affected by dietary AFB1. In addition, Awad et al. (2006) found that the small intestinal morphology in the duodenum and jejunum of broilers was altered by the feeding of DON-contaminated diets at 10 mg/kg. Taken together, these findings indicate that the small intestine is one of the most important targets of mycotoxins in animals. Notably, dietary supplementation with 1 % arginine had a significant effect on ileal crypt depth and protected villi from scattering and desquamation. While the mechanisms of these positive effects are still unclear, we postulate that arginine stimulates the intestinal production of NO to inhibit the growth of luminal bacteria and fungi (Koppelmann et al. 2012). NO is an important signaling molecule involved in neurotransmission, vascular homeostasis, immune regulation, and host defense (Anggard 1994; Tan et al. 2010; Wu et al. 2009). Furthermore, arginine and NO are also critical for the normal physiology of the gastrointestinal tract and for maintaining the mucosal integrity of the intestine by enhancing growth and development of enterocytes (Rhoads et al. 2008), stimulating intestinal cell migration and intestinal protein synthesis through a focal adhesion kinase-dependent mechanism (Yao et al. 2011), and regulating expression of the bcl-2 family of proteins (Koppelmann et al. 2012).

Amino acids play important roles as metabolic intermediates in nutrition, immune response, and growth performance (Wu 2009). In the current work, we observed that concentrations of glycine, L-tyrosine, and L-glutamine in serum were decreased by the exposure to mycotoxins in feedstuffs. It is likely that degradation of dietary glycine, glutamine, and proline by the small intestine is increased by a mold-contaminated food, resulting in their deficiencies in the animals. Emerging evidence shows that these three amino acids are very important for tissue protein synthesis and metabolic regulation (Wang et al. 2013; Wu et al. 2011a, b). It still remains to be determined whether dietary supplementation with glycine, glutamine, and proline could overcome adverse effects of mycotoxins on pigs.

The absorption of amino acids mainly depends on their transporters on the membrane of the enterocyte (Wu 2013a). Although there were no differences in mRNA levels for SLC7A7, SLC7A1, SLC1A1, and SLC5A1

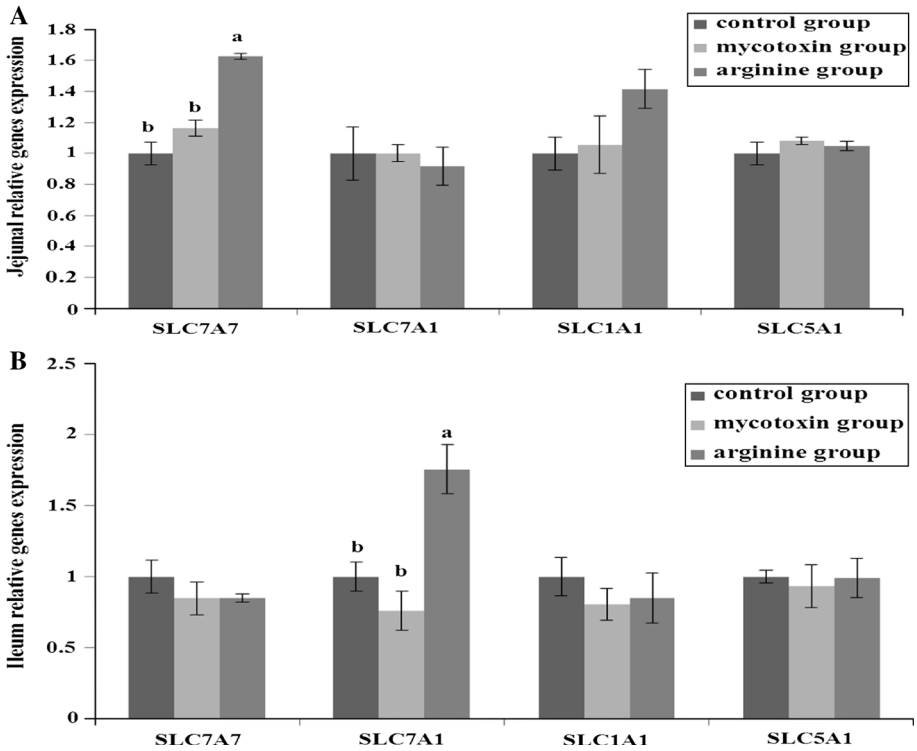

Fig. 4 Effects of dietary supplementation with arginine on gene expression in the jejunum (**a**) and the ileum of growing pigs (**b**). See Fig. 1 for explanation of legends. Data are presented as mean ± SEM, $n = 5$. *SLC7A1* solute carrier family 7 (cationic amino acid transporter, y^+ system; member 1), *SLC7A7* solute carrier family 7 (amino acid transporter light chain, y^{+L} system, member 7), *SLC1A1* solute carrier family 1 (neuronal/epithelial high affinity glutamate transporter, system X_{AG}, member 1), and *SLC5A1* solute carrier family 5 (sodium/glucose cotransporter, member 1). *a*, *b* For each variable, means with different letters differ ($P < 0.05$)

between the control and mycotoxin groups, intestinal protein levels for these transporters need to be quantified using western blot techniques (Zhang et al. 2013a). Likewise, rates of amino acid transport by the small intestinal mucosa isolated from pigs treated with or without mycotoxins can be measured with the use of Ussing Chambers (He et al. 2013b). Indeed, Maresca et al. (2002) reported that mycotoxins modulate the activities of intestinal nutrient transporters in a concentration-dependent manner. Importantly, we found that arginine enhanced expression of jejunal SLC7A7 and ileal SLC7A1 in mycotoxin-challenged pigs (Fig. 4). An increase in the entry of amino acids (e.g., arginine, lysine, and histidine) from the lumen of the small intestine into the enterocyte can enhance tissue protein synthesis and improve the efficiency of utilization of dietary nutrients, including proteins (Table 4).

In conclusion, mycotoxins in feedstuffs result in an enlarged small intestine mass, oxidative injury in tissues, and reduced growth performance in pigs. Dietary supplementation with 1 % arginine enhances expression of jejunal SLC7A7 and ileal SLC7A1, augments whole-body antioxidant capacity, and ameliorates intestinal abnormalities and growth depression in swine fed mold-contaminated feedstuffs. Thus, arginine exerts a protective role against mycotoxicosis in pigs. These results may also have important nutritional implications for humans and other mammals.

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

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